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Design of an Industrial Process for Enzymatic Cannabidiol Conversion

MSc Research Project

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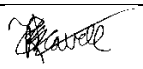
1	Erin Reece Flavell	1843629	

Executive Summary

This work aims to model a theoretical enzymatic bioreactor and all the necessary surrounding processes required to facilitate the bioremediation of THC into CBD, to produce a CBD product with THC levels below the legal concentration limits (0.001%). The primary purpose is to explore whether further research into the potential biochemical remediation of THC into CBD would be worth pursuing in terms of both functionality and profitability within the CBD industry.

Two primary process designs were modelled using SuperPro Designer, one producing a CBD isolate, and another producing a full spectrum CBD blend containing other cannabinoids beyond CBD, as well as other compounds like flavonoids and terpenes.

The CBD isolate model is composed of four parts: the extraction of the crude oil (including the pre-extraction process); the upstream processing of the oil; the reaction of THC into CBD; and the downstream processing of the oil. The full spectrum CBD model is similarly structured but with a different fourth process stage (downstream processing).

Cultivating one's own cannabis was calculated to be more economical than purchasing it from a third-party supplier, and thus a drip-based irrigation system of 1.2108 hectares was used, requiring capital costs of $ 24 641.90 and a yearly cultivation cost of $ 7629.76.

Both processes begin with milling to increase the surface area of the cannabis, followed by passing through two consecutive cold ethanol mixer-settler extraction units. Next, the oil-plant matter mixture passes through a plate-and-frame filtering system and then a decarboxylation oven, which will convert the cannabinoids into their neutral forms, producing CBD from CBDA and THC from THCA, and releasing CO_2 as a co-product.

The oil then passes into a PFR, where CLEAs catalyse the reaction of THC into CBD. Due to the theoretical nature of the as-yet-unknown enzyme, conversion was assumed to be 85 %, where 37.46 kg of the enzyme was calculated to be required per year, assuming replacement is required after seven days of operation. The possibility of either producing or purchasing the enzyme was considered, but producing the enzyme worked out more economically viable, at a yearly cost of $ 2.84. This is where the full spectrum process halts.

In the isolate process, the oil is then mixed with an acetonitrile-TBME stream before entering the CPC, alongside a heptane stream. Most of the CBD passes into the heptane, which moves

to a distillation column after exiting the CPC process, with CBD isolate emerging from the bottom stream.

The acetonitrile-TBME stream exiting the CPC will contain other cannabinoids and remaining cannabis compounds and will flow into a separate distillation. The bottom stream of the distillation column provides other valuable cannabinoid isolates, forming ancillary products (CBG, CBN, and THC isolates).

In the full spectrum CBD model, the oil flows directly into a series of three consecutive distillation columns upon exiting the PFR, designed to reduce the THC concentrations in the oil to acceptable levels.

The CBD oil emerging from both processes must then be incorporated into an MCT carrier oil. CBD isolates were assumed to be sold for $ 39.00 per 30 ml, with each unit containing 600 mg of CBD. Full spectrum CBD oil was assumed to be sold for $ 40.00 per 30 ml, where every 30 ml contains 1500 mg of cannabis oil.

Once the costs of the MCT oil were deducted from the theoretical revenue values, the net revenue values came to $ 64 089.93 per kilogram for CBD isolate and $ 26 302.64 per kilogram for the full spectrum CBD oil. Collectively, the ancillary cannabinoid products (CBG, CBN, and THC) yielded a net revenue value (less the cost of the required MCT oil) of $ 17749.48 /kg.

CBD isolate was produced at a rate of 637 kg/year, at 99.47 % purity, with ancillary cannabinoid products being produced at 494 kg/year by the isolate process. The full spectrum CBD blend was produced at a rate of 656 kg/year and did not contain any solvent residues.

The isolate process was found to have a gross margin of 83.86%, an ROI of 101.58%, a payback time of 0.98 years, and an NPV of $ 190 458 000. The full spectrum blend has a gross margin of 50.89%, an ROI of 21.75%, a payback time of 4.60 years, and an NPV of $ 14 199 000. Thus, the isolate process was deemed the more economically viable of the two processes.

An additional CBD isolate design involving supercritical CO_2 extraction was also modelled for comparison. In this variation, the cannabis buds undergo milling before passing through the supercritical CO_2 extraction unit. The CO_2-ethanol solvent feed enters a CO_2 storage unit wherein is pressurised to achieve supercritical conditions before entering the extraction unit alongside the cannabis stream.

The bottom stream from the extraction unit then passes into a plate-and-frame filtration system, which removes the plant matter from the stream; the recovered cannabis oil is then reunited with the top stream from the extraction unit. The combined oil stream then undergoes winterisation, in 24-hour cycles, before moving into a distillation column which removes any remaining solvent. The bottom stream from the column then enters the decarboxylation oven; the remainder of the process continues in the same manner as the original, cold ethanol extraction isolate process.

The CO_2 extraction process produced CBD isolate at a rate of 600.54 kg/year. CBD purity of 99.29 % was achieved. An economic analysis produced project indices of a gross margin of 84.07%, an ROI of 95.28%, a payback time of 1.05 years, and an NPV of $ 173 404 000. Thus, with gross margin being the sole exception, all project indices indicate the cold ethanol process being the process with greater potential profitability to produce CBD isolates.

Because the isolate process proved the most profitable of the alternatives, its potential profitability when scaled up to industrial size was also assessed. The process feed rate was increased to 79 200 kg of cannabis buds per year, solvent input streams were proportionally scaled up, and several equipment units were multiplied as required. Additionally, the quantity of the enzyme required for catalysing the reaction was recalculated based on the increased plant material in the process, coming to a yearly mass of 576.25 kg, for $ 345 750.

The scaled-up process produced a CBD isolate product with a purity of 99.47% and a production rate equivalent to 9800 kg per year and the ancillary cannabinoid product at a rate of 7587 kg per year. The NPV of the scaled-up process came to $ 3.99 billion and a gross margin of 99 % was achieved, with an ROI of 1340 % and a payback time of 0.07 years.

Therefore, from the simulated model and the economic analyses, the production of CBD oils using THC-to-CBD bioremediation was found to be a potentially profitable, as-yet-untapped production method that would benefit from further research. It is worth noting, however, that the research is limited by its reliance on theoretical models and assumptions, which may not fully reflect real-world conditions, potentially affecting the generalisability of the findings. The lack of empirical validation and practical factors not captured by simulations, such as enzyme stability, further constrain the applicability. Future work should focus on empirical testing and exploring a wider range of parameters to improve the results' relevance and generalisability.

Keywords: Δ9-tetrahydrocannabinol; cannabinol; enzyme reactor; bioremediation; SuperPro Designer

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Nomenclature and Abbreviations

AOT: Annual operating time

API: Active pharmaceutical ingredients

CAPEX: Capital expenses

CBC: Cannabichromene ($C_{21}H_{30}O_2$)

CBD: Cannabidiol ($C_{21}H_{30}O_2$)

CBG: Cannabigerol ($C_{21}H_{32}O_2$)

CBN: Cannabinol ($C_{21}H_{26}O_2$)

CBDA: Cannabidiolic acid ($C_{22}H_{30}O_4$)

CBGA: Cannabigerolic acid ($C_{22}H_{32}O_4$)

CFC: Contractor's fee and contingency

CFR: Cash flow analysis report

CLEA: Cross-linked enzyme aggregates

CPC: Centrifugal partition chromatography

CIP: Cleaning-in-place

CSTR: Continuous stirred-tank reactor

DFC: Direct fixed capital

EER: Economic evaluation report

FBR: Fluidised bed reactor

FTIR: Fourier transform infrared

GC: Gas chromatography

GMP: Good Manufacturing Practice

HPLC: High-performance liquid chromatography

HRT: Hydraulic retention time

ICR: Itemized cost report

IRR: Internal rate of return

IQ: Intelligence quotient

MR: Membrane reactor

MS: Mass spectrometry

NMR: Nuclear magnetic resonance

NPV: Net present value

OPEX: Operating costs

PAT: Process analytical technology

PBR: Packed-bed reactor

PFR: Plug flow reactor

PHWE: Pressurized hot water extraction

P_{sat}: Component vapor pressure

R/Rmin: Reflux ratio (R) to minimum reflux ratio (Rmin)

ROI: Return on investment

RPC: Reversed-phase chromatography

SAHPRA: South African Health Products Regulatory Authority

SFE: Supercritical fluid extraction

STR: Stirred tank reactor

TCI: Total capital investment

THC: Δ9-Tetrahydrocannabinol ($C_{21}H_{30}O_2$)

THCA: Tetrahydrocannabinolic acid ($C_{22}H_{30}O_2$)

TLC: Thin-layer chromatography

TPDC: Total plant direct cost

TPIC: Total plant indirect cost

UV: Ultraviolet

VLE: Vapour-Liquid Equilibrium

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1. Introduction

1.1. Background

Cannabis oil is an extract that can derived from the cannabis plant. The composition of chemical compounds within this extract can differ substantially depending on the plant variant from which the oil was extracted. Unprocessed, cannabis oil contains an array of substances of varying physical structures and chemical effects, where the two primary active compounds present are known to be cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC).

The use of CBD has been documented to be beneficial, both therapeutically and medicinally, with the capacity to aid in the management of conditions such as anxiety, chronic pain, epilepsy, and cancer (Xiong *et al.*, 2012; Massi *et al.*, 2013; Santiago *et al.*, 2019; Zieba *et al.*, 2019). This makes the compound highly desirable when isolated. THC, on the other hand, is notorious for its psychoactive effects, triggering the 'high' commonly associated with cannabis use – an effect CBD does not share this effect (Thomas *et al.*, 2007). It is for this reason that the consumption of THC is illegal in many countries, as well as the strict restriction of cannabis and cannabis products in general, due to the co-occurrence of CBD and THC in the plant.

By the dawn of the 21st century, most countries in the world had banned the production and trade of cannabis and its related products. However, within more recent years as research continues to emerge corroborating the usefulness of CBD, more and more countries have begun legalizing cannabis for medical use, with some – such as South Africa – legalizing the plant for recreational use as well. (Parry, Myers and Caulkins, 2019; Krishna, Boyle and Kvilhaug, 2023)

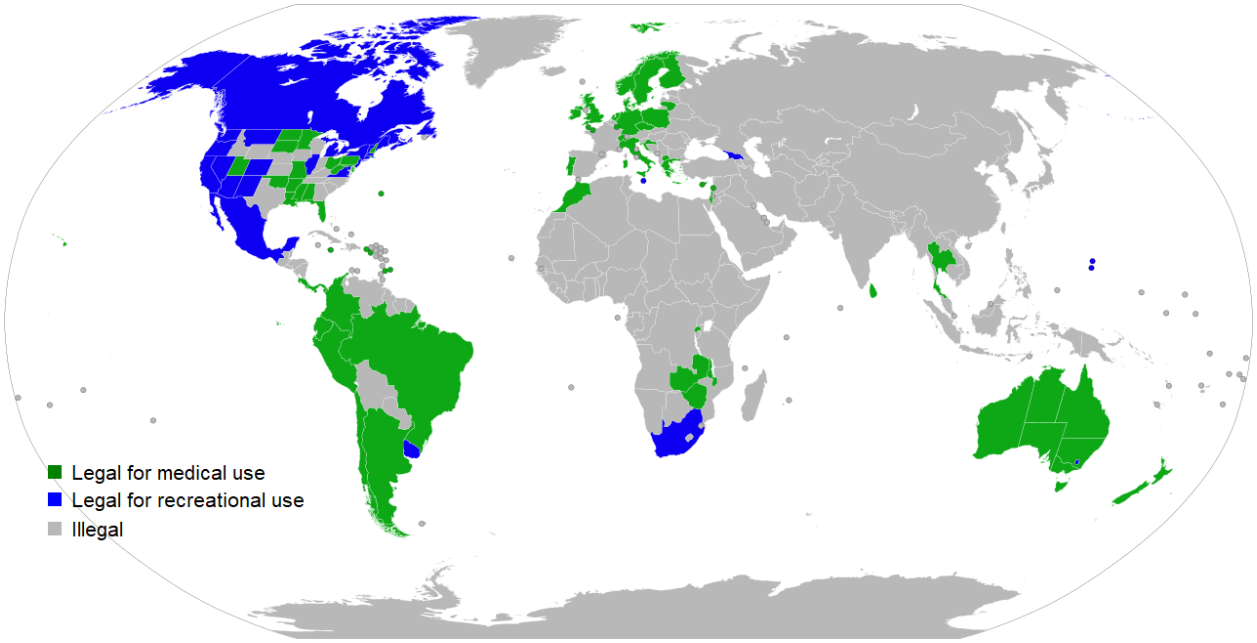


Figure 1: Legal status of Cannabis in different countries, as of April 2022 (Wikimedia Commons contributors, 2023).

The use and cultivation of marijuana and its associated THC products were ruled legal in South Africa in 2018, provided it was limited to private areas and for personal use (Parry, Myers and Caulkins, 2019). This recent legalisation stimulated a blossoming interest in cannabis, thus opening a new niche in the market for CBD products, which (unlike THC products) are legal for both commercial sale and public consumption, though on the proviso that THC is limited to trace concentrations (Rethink CBD, 2019). This increase in demand, locally and worldwide, requires increased production of CBD oils is needed to meet these demands (Grand View Research, 2022; Research and Markets, 2022). However, due to the stringent laws against the presence of THC in South African products, the creation of CBD products that fall within legal parameters (less than 0.001% THC concentrations) becomes an intensive, specialised and costly process, necessitating careful cultivation of low-THC plants and rigorous purification mechanisms (Rethink CBD, 2019).

The fact that South Africa is the fourth-largest producer of cannabis in the world (Smit, 2021), coupled with an expected national compound annual growth rate of 28.4% for CBD between 2022 and 2026 (Research and Markets, 2022), implies a simultaneous abundance of raw material (the cannabis plant) in addition to an extensive demand for the product (CBD oil). This indicates an opportunity for a profitable business venture in CBD oil production. National

estimates support this notion, approximating that over 25 000 jobs could potentially be generated within the cannabis industry, stimulating economic growth and aiding in the alleviation of unemployment and poverty (Businesstech, 2021).

This research aims to streamline this process, decrease the associated purification costs, and provide a more convenient and profitable means of producing CBD isolates through the design of a new production method – namely, one that utilizes enzymatic interconversion to transform THC into CBD. As a novel and yet unexplored process, using a theoretical enzyme as a basis, this work will investigate whether earnest research into the production of such an enzyme would be beneficial for the commercial production of CBD oil, and thus worth pursuing.

This process has the potential to reshape the landscape of cannabinoid production, particularly in the context of regulatory and market challenges. A key issue facing current South African producers is the stringent regulations around the cultivation of cannabis plants, particularly concerning the legal limits of THC. Strict restrictions on THC levels necessitate rigorous farming techniques designed to artificially select for plants with compliant cannabinoid profiles. Achieving these concentrations often involves expensive and labour-intensive processes, with high plant culling rates and reliance on artificial selection to breed strains that meet regulatory standards. (Semete-Makokotlela, 2022; Dube *et al.*, 2023)

In this context, the enzymatic conversion of THC to CBD, as explored in this research, presents an opportunity to reduce the pressures surrounding the cultivation of specific strains. The ability to efficiently convert THC into CBD could render many of these regulatory farming hurdles unnecessary, allowing producers to focus on maximizing yields without the need to discard non-compliant plants. This would not only increase usable yields but also reduce the financial and operational burden of cultivating cannabis under strict THC limitations. Additionally, this method would allow producers to utilise a wider range of cannabis strains, converting any variety with high THC levels into CBD. This could make farming less stressful and less wasteful while still adhering to the legal frameworks surrounding THC concentrations when producing cannabis oils from the plants.

From a regulatory perspective, such advances could prompt re-evaluation of current farming and production guidelines. If conversion processes like the one proposed in this research become widespread, regulations that once focused primarily on farming methods and THC thresholds could shift toward process control. Regulators may need to consider how such

processes are monitored, verified, and integrated into legal frameworks to ensure product safety and consumer transparency.

In terms of market challenges, the widespread adoption of THC-to-CBD conversion could disrupt the current CBD market, potentially reducing the costs of CBD products by increasing supply and efficiency. Producers could extract greater yields from each plant, driving down production costs and making CBD products more accessible to a broader consumer base. However, this increased availability could also create market saturation, driving prices down and increasing competition among producers.

The adoption of enzymatic conversion technologies also brings forward regulatory challenges, particularly around quality control and compliance. Good Manufacturing Practice (GMP) requirements, particularly in healthcare products, would demand rigorous documentation and control of the conversion process, including real-time monitoring of THC levels and validation of the conversion efficiency. Regulators will need to ensure that these processes are tightly controlled to prevent THC from reaching consumers in quantities that exceed legal limits, necessitating vigorous process validation and quality assurance.

Overall, while this research offers a promising solution to some of the most pressing regulatory and market challenges in cannabinoid production, its successful implementation will depend on navigating these regulatory frameworks, ensuring compliance, and managing the potential market impacts of increased CBD supply.

1.2. Problem Statement

The primary issue involved in the production of CBD oil is the reduction of THC concentrations to commercially acceptable levels. This proves difficult due to THC and CBD's shared chemical formula ($C_{21}H_{30}O_2$). Fortunately, they are structurally different enough to possess diverse physical properties, enabling separation through various techniques, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and supercritical carbon dioxide extraction (Ribeiro Grijó, Vieitez Osorio and Cardozo-Filho, 2019; Pourseyed Lazarjani *et al.*, 2020). Unfortunately, many of these processes are time-consuming, inefficient, or costly, resulting in expensive CBD products with limited commercial accessibility and thus a limited market incapable of reaching its full financial potential (Galand *et al.*, 2004; GALAK Chromatography Technology Company Ltd, 2023).

It can thereby be said that opportunity exists for the innovation of a new, efficient, and sustainable process capable of quickly and economically obtaining CBD products with acceptably low THC concentrations, allowing consumers economically feasible access to CBD products without the risk of suffering THC's psychoactive effects. Theoretically, due to similarities between the chemical formulae of the compounds, this could be achieved via enzymatic remediation of THC into CBD, or some other harmless constituent found within the cannabis oil. The humanistic and economic benefits of developing such a process could potentially be boundless, providing potential financial gain, stimulation of the economy, and the opportunity to ease the suffering of those who would benefit from CBD's medicinal and therapeutic properties.

However, current research into the development of such a process is sparse. This work will attempt to model a theoretical example of such a process, optimize it, and comment on whether it could potentially be profitable for the producers of the CBD industry.

1.3. Aim

There is a mounting demand for CBD products and a simultaneous shortage of established practices capable of efficiently and economically reducing THC concentrations to commercially acceptable levels, thereby resulting in costly separations and purifications, as well as wastage. This research endeavours to model a theoretical alternative process utilizing enzymatic conversion of THC to CBD, as well as to determine the potential profitability of such a venture and comment on whether further research would be beneficial.

1.4. Objectives

This research has the following objectives:

I. To model a theoretical enzymatic bioreactor, including upstream and downstream processing, capable of producing CBD extracts of commercially acceptable purity.

II. To perform sensitivity analyses on the modelled process and optimize the design accordingly.

III. To design the surrounding process, including extraction and purification steps, to support the primary bioremediation reaction on a commercial scale.

IV. To calculate the economic viability of the modelled process.

V. To determine if research into biochemical remediation of THC into CBD could potentially be profitable for the CBD industry and to comment on whether such research would be worth pursuing in this context.

1.5. Layout

Chapter 1: An overview of the process, including relevant background information, motivations, and the primary objectives behind the research.

Chapter 2: A literature review of subjects relevant to the proposed process, including cannabis and its history, the extraction of cannabinoids, and the use of enzymes as a means of catalysis.

Chapter 3: A technical review of potential mechanisms for the process, ranging from cultivation of the plant, pre-processing steps, means of extraction, reactor design, downstream processing steps, and purification.

Chapter 4: The modelling of the process, conducted using SuperPro, including a process flow diagram, an overview of optimized unit parameters, and technical details of the equipment chosen. This will include a primary process involving the production of CBD isolates, as well as an alternative process involving the production of full spectrum CBD blends, which include concentrations of terpenes, flavonoids, and other cannabinoids whilst still maintaining THC concentrations below the legal limits.

Chapter 5: Sensitivity analysis and optimisation of the process, as well as an economic analysis of the optimized process and profitability comparison between producing CBD isolates and full spectrum CBD blends.

Chapter 6: A discussion, comparison, and economic analysis of an alternative design capable of achieving the same results as the proposed process (i.e.: replacing ethanol extraction with supercritical CO_2 extraction).

Chapter 7: A scale-up of the primary process and a subsequent economic analysis to ascertain whether the process holds up on an industrial scale.

Chapter 8: An overview of the conclusions reached over the course of the paper and recommendations for further research on enzymatic cannabinoid interconversion, improving the design, and furthering research into the viability of the proposed mechanism.

References: A list of all literature referenced in the paper.

Appendices: A collection of relevant appendices, organised as follows:

- Appendix A: Chemical and Material Properties of Process Components
- Appendix B: Vapour-Liquid Equilibrium (VLE) Calculations
- Appendix C: Reactor Calculations
- Appendix D: Ancillary Calculations, Optimisation and Sensitivity Analysis
- Appendix E: Economic Analysis
- Appendix F: Energy Consumption and Balances

2. Literature Review

2.1. Cannabis: An Overview

Cannabis use has historical origins reaching back as far as 500 B.C.E., to North-east Asia. Its earliest applications were not only recreational and medicinal; the plant was also originally a source of fibre utilized in the creation of rope, sails, and early paper variants, while its seeds provided a source of nourishment (Li, 1974). In fact, cannabis is heralded as one of the oldest cultivated crops, lending it historical and agricultural significance in addition to its modern medicinal, therapeutic, and recreational uses. Evidence even suggests that Chinese and Siberian cultures may have cultivated cannabis specifically to increase its THC levels from as early as 500 B.C.E., in an attempt to strengthen its psychoactive effects for use in religious rituals and healing practices (Abel, 1980).

Once cannabis began its spread to Europe, it was adopted by Western physicians as a treatment for a variety of ailments. Its medicinal properties were investigated at length by Irish physician, William B. O'Shaughnessy, an Irish physician, and French psychiatrist, Jacques-Joseph Moreau, who ran a series of experiments on the plant's effectiveness at alleviating suffering wrought by rheumatism, convulsions, tetanus, rabies, cholera, and a host of mental illnesses. By the late 19th century, its use as a medical treatment had become well-established across Europe and the United States (Moreau, 1845; Mikuriya and Francisco, 1969; Fankhauser, 2022).

From the research that has since been assembled, it has been discovered that many of the intrinsic properties possessed by the cannabis plant originate from chemical compounds such as terpenoids, terpenes, flavonoids, and nitriles. Cannabis belongs to the taxological family of 'Cannabinaceae', and contains two primary genera: *cannabis indica* and *cannabis sativa*, as well as a less common third species known as *cannabis ruderalis*, containing only trace amounts of THC, though this variant is not commonly cultivated (Guy, Whittle and Robson, 2004; GRIN-Global, 2022). *Cannabis sativa* is the most popular, due to its higher THC content; however, indica strains contain greater concentrations of CBD (Atakan, 2012).

Cannabis sativa contains 421 different constituents, of 18 different chemical varieties, each contributing to the overall chemical characteristics of the plant (Barrales-Cureño *et al.*, 2020). The compounds of greatest scientific relevance are cannabinoids, which represent over 60 of the 421 constituents present. The four major cannabinoids within cannabis are cannabidiol

(CBD), cannabinol (CBN), and tetrahydrocannabinol (THC) – of which two isomers exist - namely Δ8-THC and Δ9-THC (Atakan, 2012).

In 1940, CBD was successfully isolated from cannabis oil for the first time by Adams and his team, using bis-3,5-dinitrobenzoate. Ammonolysis of the sample managed to produce a final cannabidiol product – however, due to inadequacies in the chemical analysis methods available at the time, uncertainty remained as to whether its chemical formula was $C_{21}H_{30}O_2$ or $C_{21}H_{32}O_2$ (Adams, Hunt and Clark, 1940). In 1963, over a decade later, this mystery was solved by Mechoulam and Shvo, who confirmed CBD's chemical structure to be $C_{21}H_{30}O_2$ via the application of nuclear magnetic resonance (NMR) (Mechoulam and Shvo, 1963).

Adams also managed to isomerise CBD into tetrahydrocannabinolic compounds but was stymied once again by limitations in the analytical methods available to him, and was unable to ascertain the location of the double bonds present in the chemical structure of the compounds (Adams *et al.*, 1940). In 1942, THC was isolated by Wollner and his team, but they too were unable to fully deduce its structure (Wollner *et al.*, 1942). Once again, it was Mechoulam (this time alongside Gaoni) who unveiled the final chemical structure; he determined the location of the double bond in THC in 1964, using NMR (Gaoni and Mechoulam, 1964).

Thus, by the late 20th century, the chemical structures of both compounds had been fully visualised and extraction methods existed for the acquisition of their isolates.

2.2. Medicinal Applications of Cannabidiol

Since the mid-twentieth century, further investigation into the effects of CBD on the human body has yielded reliable evidence in support of the compound's capacity to aid in the treatment of a variety of health conditions. Interestingly, studies have also shown that CBD is also able to reduce the psychoactive effects induced by THC. This antipsychotic quality supports the hypothesis that CBD holds potential as a therapeutic treatment for psychosis, especially in patients suffering from schizophrenia (Waldo Zuardi *et al.*, 2012; Boggs *et al.*, 2018).

Furthermore, cannabinoids possess pro-apoptotic and anti-proliferative properties that suppress the spread of cancer; this is due to the compounds' capacity to obstruct migration, adhesion, invasion, and metastasisation of cancerous cells within the body, as well as the impedance of neovascularization of tumours. Though this ability is possessed by CBD and

THC alike, the medicinal application of THC is restricted by its unwanted side effects, and thus CBD reigns as the favoured treatment (Massi *et al.*, 2013; Abrams, 2022).

CBD has also been observed to possess anti-convulsive effects. Because epilepsy, in particular, has proven resistant to treatment in up to a third of recorded cases, this presents an opportunity for CBD to carve out a niche as a new anti-epileptic treatment option (Farrell and Soltesz, 2019). In fact, studies have already shown that over 50 % of patients with Dravet syndrome and Lennox-Gastaut syndrome who ingested CBD experienced reduced seizures, with several of them emerging from the experiment entirely seizure-free – a promising reassurance of the compound's anti-convulsive potential (Stockings *et al.*, 2018).

In addition, CBD's anti-inflammatory and antioxidant properties lend credence to its potential as a neuroprotective drug. Early trials suggest these properties can mitigate the cognitive and pathophysiological damage experienced by those suffering from Alzheimer's disease since many of these symptoms stem from neuroinflammation, neurotoxicity, and oxidative damage in the brain, all of which CBD could potentially combat (Cheng *et al.*, 2014). These same properties also mark it as a potential treatment for those with Parkinson's disease (Rieder, 2020).

Several studies have been conducted into the compound's potential for therapeutic use in the treatment of chronic pain, anxiety, and depression (Blessing *et al.*, 2015; Corroon and Phillips, 2018; Zieba *et al.*, 2019). CBD use is also predicted to have beneficial effects on those suffering from diabetes, though studies in this field are still in the early stages of development (Santiago *et al.*, 2019).

Considering the substantial evidence in support of CBD's medicinal value, an investigation into finding a more efficient and economical means of obtaining it can be asserted to be a worthy endeavour.

2.3. *Effects of Δ9-Tetrahydrocannabinol on the Human Body*

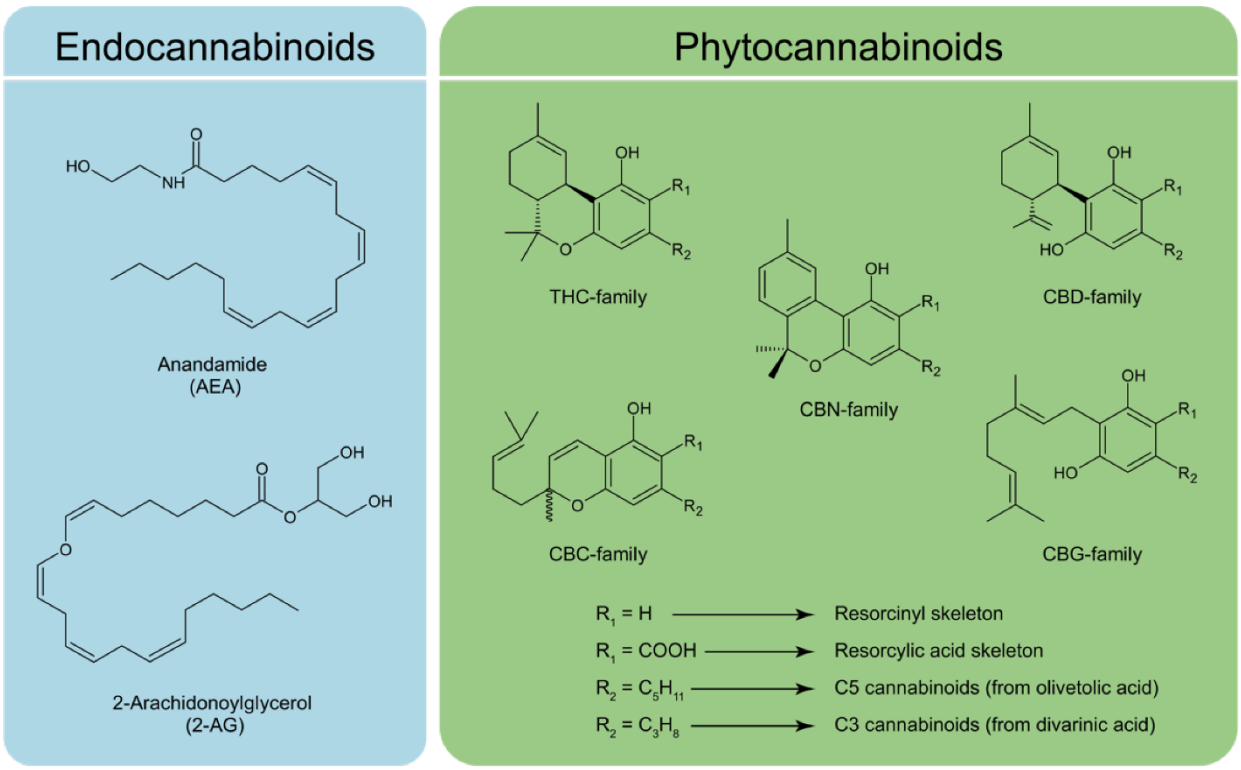


Figure 2: Molecular structure of endocannabinoids and phytocannabinoids (Favero et al., 2022).

In the brain, two cannabinoid receptors exist which, when activated, result in the inhibition of adenylyl cyclase, an enzyme that plays a key regulatory role in most cells; this is part of what is called the endocannabinoid system. It consists of three primary elements: endocannabinoids, which our bodies produce, enzymes which help break down the cannabinoids and endocannabinoids, and finally, the receptors. These receptors are activated when they interact with the cannabinoids found in plants, called phytocannabinoids (Chen, Han and Xie, 2005).

THC can bind to these receptors. Thus, when a person uses cannabis, THC will behave as a partial agonist, binding to CB1, a receptor found in the central nervous system, and inhibiting the release of neurotransmitters that would normally be modulated by endocannabinoids. This results in the psychoactive effect for which THC has become notorious. The location of these receptors within the brain is also responsible for many of the common negative side effects of THC such as poor memory, increased appetite, paranoia, nausea, retardation of motor function, and distortion of time (Bayewitch *et al.*, 1996; Thomas *et al.*, 2007).

The existence of these cannabinoid receptors was hypothesised as early as 1988, through the use of a synthetic molecule modelled after THC (Devane *et al.*, 1988). It was proven two years later, first by mapping possible binding sites via observation of brain activity in rats, and again by duplicating a receptor gene through molecular methods – this receptor site has since been termed 'CB1' (Herkenham *et al.*, 1990; Matsuda *et al.*, 1990).

In 1993, this cloning process was repeated to reveal another receptor, found primarily in the immune system, known as 'CB2' (Munro, Thomas and Abu-Shaar, 1993). It is these receptors that comprise the endocannabinoid system and it is CBD's limited affinity for said receptors that results in the absence of psychoactive effects, unlike in the case of THC.

THC can also be used medicinally, possessing many of the same benefits as CBD, such as its capacity to ease chronic pain and suppress the spread of cancerous cells (Massi *et al.*, 2013; Abrams, 2022). However, the negative side effects of THC are significant, and since CBD possesses the same benefits with none of these detriments, the positive medicinal attributes of THC are ultimately eclipsed by the adverse effects outlined above.

Additionally, THC inspires addictive behaviours due to its capacity to increase serotonin levels within the brain, leading people to crave the resultant euphoria even after it fades; this also results in an increased risk of depression amongst users, due to THC also acting to inhibit the reuptake of this serotonin. This effect can result in addiction, including a range of withdrawal symptoms such as insomnia, anxiety, and irritability (Gorelick *et al.*, 2012). Other dangers associated with long-term THC intake include decreased IQ (particularly amongst adolescent users), memory loss, and increased likelihood of developing psychosis disorders such as schizophrenia (Meier *et al.*, 2012; Zalesky *et al.*, 2012; Di Forti *et al.*, 2014).

2.4. Cannabinoid Extraction

THC and CBD are chemical isomers that share the same formula ($C_{21}H_{30}O_2$); however, due to structure variations, they have different properties that make separation possible. The structure of these compounds can be seen in Figure 3, with CBD possessing an additional double bond when compared to its THC counterpart.

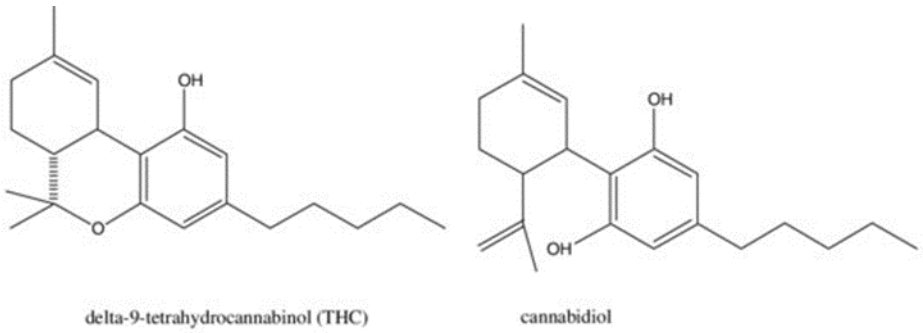


Figure 3: A visual representation of the chemical structures of CBD and THC (Russo and Guy, 2006).

There is, however, already a variety of processes available to separate THC from CBD, with chromatography reigning as the most common. Traditional column chromatography remains a reliable extraction method, though flash chromatography is employed for more rapid separation. Other options also exist, each with its own advantages and disadvantages; some of these options will each be considered in depth in Section 3.8 as part of a comprehensive technical review. Some of these alternatives include gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical carbon dioxide extraction, pressurized hot water extraction, and cold ethanol extraction.

Post extraction, regardless of the separation method, an analytical classification is required to identify and quantify different components present in the product. Common methods include mass spectrometry, flame ionization detection, diode array detection, and the application of ultraviolet spectra (Zivovinovic *et al.*, 2018; Pourseyed Lazarjani *et al.*, 2020). Due to the lack of an 'ideal' extraction method, where each potential process is inherently hindered by several noteworthy shortcomings, demand clearly exists for the innovation of new extraction processes capable of maximising CBD yields whilst avoiding the pitfalls associated with existing methods, such as inefficiency and high capital expenses (Galand *et al.*, 2004).

2.5. Remediation of Δ9-Tetrahydrocannabinol

It is known that enzymatic activity is the driving force that propels THC and CBD to diverge from their common predecessor, cannabigerolic acid (CBGA), of formula $C_{22}H_{32}O_4$, as seen in Figure 4. CBGA is converted via enzyme into Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), or cannabichromenic acid (CBCA); these are the carboxylated

forms of THC, CBD, and cannabinochromene (CBC), respectively, and will remain this way until the compounds have been decarboxylated due to exposure to light or heat. As can be seen in Figure 4, this is a long and complex process, involving a long string of intermediates and several enzymatic reactions. Post-decarboxylation, these compounds will be transformed into their neutral states – THC, CBD, and CBC. It is these neutral forms on which this research will focus (Favero *et al.*, 2022).

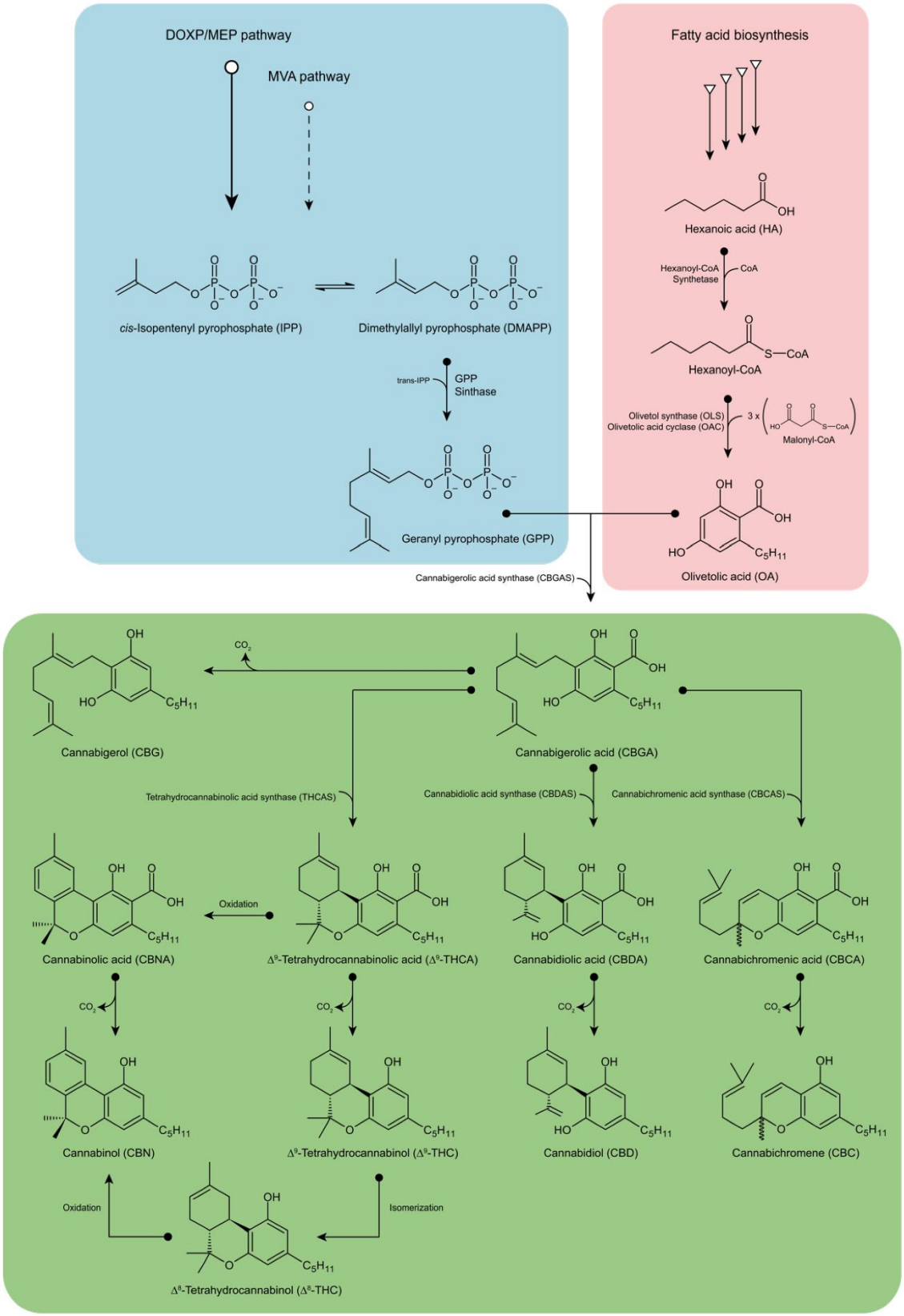


Figure 4: Biosynthesis of cannabinoids in Cannabis sativa L (Favero et al., 2022).

THC and CBD, being isomers of each other, are similar to each other in terms of physical structure, differing only in the possession of a double bond, in the case of CBD, and a change in the position of a single hydrogen atom that is attached to an oxygen atom in the case CBD and a carbon molecule in the case of THC.

It has been established that interconversion between other cannabinoids is largely controlled by enzymatic reactions. Thus, the prospect of exploiting enzyme activity to remediate THC into CBD seems theoretically feasible, though scientific exploration of this topic is currently sparse (Thomas and ElSohly, 2016).

Currently, only chemical methods of performing this conversion exist. It is known that CBD converts to Δ9-THC and Δ8-THC when treated with strong acids such as hydrochloric acid or sulphuric acid (Golombek *et al.*, 2020); however, as discussed in Section 2.6, alternative 'green' methods of catalysing industrial processes are being sought for processes such as these, which have the potential to expel harmful waste products into the environment. Regardless, the existence of such a reaction gives further credence to investigating the potential of remediating THC into CBD through enzymatic means, as it proves the reaction can occur; thus, the notion of interconversion between the two cannabinoids is not unfounded.

Thus, for the purpose of this work, a theoretical enzyme will be used. If the use of this theoretical enzyme for this purpose is profitable, it will support further investigation into the identification or creation of this enzyme, but this is beyond the scope of this work. For the sake of this investigation, and due to the isomeric nature of the compounds, the enzyme will be assumed to be – and partially modelled after – an isomerase.

It is important to reiterate that the enzyme in question is theoretical, and as such, empirical data on its characteristics, including conversion rates, is not available. All assumptions made, such as considering the enzyme as an isomerase, are therefore based on theoretical reasoning rather than experimental evidence.

Given the highly theoretical nature of this study, it is also worth noting that this review does not necessarily seek to address specific gaps in existing research, specifically regarding enzyme identification or modification. Instead, it focuses on evaluating whether further exploration of this novel topic, which currently lacks reliable coverage in literature,

is warranted. The aim is to assess the potential value of pursuing this new research further, rather than to fill existing gaps.

2.6. Enzyme Application

Enzymes have, historically, played a greater role in human history than one might realize. People have utilized them for millennia, albeit unknowingly at first; for example, the production of alcohol via fermentation has been performed using enzymes from as early as 7000 BC (McGovern *et al.*, 2004). It was thousands of years later, in 1833, that the first enzyme (diastase) was discovered and categorized, with the term 'enzyme' only being coined much later, in 1877 (Heidelburg, 1877; Armstrong, 1933). Catalysis, or the concept of a chemical facilitating a reaction without undergoing chemical change itself, was first theorized in 1836, shortly after which it was hypothesized that enzymes were, indeed, such compounds (Northrop, 1946; Robertson, 1975).

From here, research into enzymes for industrial use began in earnest, and in 1858, Louis Pasteur discovered the kinetic resolution of racemic tartaric acid using fermentation via the activity of microorganisms, propelling enzymes into the scientific spotlight (Gal, 2008). Enzyme use in organic chemical transformations then experienced a rapid increase in popularity in the later part of the 20th century, particularly in the food and pharmaceutical industries. In fact, as of now, up to 80 % of enzymes' current commercial value can be attributed to their application as process catalysts (Santi *et al.*, 2021).

Traditionally, enzyme use has been limited to hydrolytic reactions, performed after the enzyme catalyst has been dissolved in an aqueous medium; more recently, however, interest in enzyme-catalysed processes has expanded significantly due to the introduction of enzymes into the process of organic synthesis (Illanes and Altamirano, 2008). This has sparked an increased motivation to expand the application of enzyme catalysis into other, currently uncharted industrial and commercial processes.

Though the use of enzymes to remediate THC into CBD remains largely untested, the use of enzymes in the remediation of chemical compounds into less harmful alternatives is not unprecedented. The recent surge in pollution, for example, has birthed substantial research efforts into the development of processes capable of reducing pollutants in the environment –

enzymes are one such method and have been recorded detoxifying pollutants at a detectable rate (Rao *et al.*, 2010; Karigar and Rao, 2011; Gupta *et al.*, 2021). With an enzyme capable of remediating THC into CBD inserted into a bioreactor, a process capable of producing a cannabis product with maximised CBD concentrations and minimised THC concentrations could theoretically be designed to perform in the same way.

Biocatalysis experienced a rapid burst of popularity only recently, in the later part of the 20th century, due to the growing demand for sustainable, 'green' chemical processes birthed by the dawn of the environmental movement (Santi *et al.*, 2021). This can be achieved largely through the higher selectivity possessed by enzymes as opposed to most chemical processes, resulting in reduced waste and greater product yields (Haan, 2015).

The range of reactions that can be catalysed by enzymes is extremely broad, allowing enzyme catalysis to be successfully applied to most processes (Haan, 2015). They are also biodegradable, and taken from renewable resources micro-organisms, and plant matter, thus adding to their 'green' appeal (Omori *et al.*, 2016). In opposition to most chemically catalysed reactions, enzymes also work under relatively mild conditions – at moderate temperatures, atmospheric pressure, and in non-toxic and economical solvents (for example, water is commonly used), reducing the potential danger associated with such a process (Santi *et al.*, 2021).

Realistically, no solution is perfect; biocatalytic processes, whilst increasing in popularity, remain limited in their application by several notable issues; examples of this include relatively long reaction times, difficulty in successful scale-up operations, and lengthy optimization processes (Santi *et al.*, 2021). Furthermore, enzyme display instability over time and are incompatible with other reaction conditions, becoming denatured and losing functionality - this makes one-pot cascade reactions challenging, necessitating time-consuming downstream purifications processes to mitigate the issue (Wang, Nema and Teagarden, 2010; Sheldon and Brady, 2018; Sheldon, Brady and Bode, 2020; Anderson *et al.*, 2021).

Finally, the use of water as a solvent (as is common in biocatalysis) often leads to over-diluted solutions because of substrate solubility problems, risking high solvent usage, lower product concentrations, and thus lower overall process efficiency. Power usage – and thus the associated energy expenses – may also be elevated due to higher required boiling points (Sheldon and Brady, 2018).

Therefore, it can be concluded that enzymatic biocatalysis exists as a viable commercial option to produce CBD, as proposed, but will require careful optimization of parameters to maximise efficiency and limit expenses.

2.7. Similar Existing Processes

Whilst no existing process for the biological interconversion of THC into CBD currently exists, several current industrial processes can be looked to for guidance – below, processing concerning artificial cannabinoid production, as well as seemingly unrelated processes that use some of the more niche processing equipment that will be considered in this review, will be discussed.

Traditional cannabinoid extraction methods will be further explored in Section 3.5, as part of the technical review, due to their significance in the proposed process.

In terms of the artificial production of cannabinoids, chemical synthesis, and biosynthesis emerge as potential alternatives to the traditional cannabinoid production mechanisms, which focus primarily on extraction and purification. Chemical synthesis enables the production of cannabinoids through a series of chemical conversions, often starting from an unrelated chemical entity that can be manipulated into different cannabinoids. In terms of manufacturing, its scope is often limited, however, as this mechanism can generally only be operated efficiently on a small scale. Additionally, chemical synthesis is only appropriate for certain cannabinoids, with many others being limited by impossible or currently unknown synthetic reaction sequences (Edison Group, 2023).

The process proposed in this work would, while focussing on extraction and biological interconversion in place of chemical synthesis, potentially find itself in competition with such processes. Currently, Purisys is a leader in the production of cannabinoids via chemical synthesis, producing CBD containing less than 0.001 % THC. However, this level of purity comes with prices of up to $ 400 per 100 mg (Edison Group, 2023). Whilst more ‘general’ CBD products focus primarily on ensuring the THC percentages fall within legal parameters, in the case of the pharmaceutical industry, CBD isolates of significant purity are required. As the Purisys product shows, these are costly and require complex synthetic mechanisms to achieve the desired purity. A simpler, less costly alternative mechanism could prove invaluable to this industry.

An existing alternative to chemical synthesis is biosynthesis – a process capable of producing cannabinoids via microorganisms. *E. coli* and *S. cerevisiae* are popular choices in biosynthetic processes, in the form of microbial cell factories. These cell factories are lauded as environmentally friendly, reasonably economical, and more easily scalable than chemical synthetic methods, with existing large-scale equipment, such as bioreactors, showing potential to be adjusted for biosynthesis with little issue. Feedstock is also simple to obtain, with cannabinoids commonly being produced from sugar (Edison Group, 2023).

Due to the many advantages of biosynthesis, any innovation in the CBD production market, especially of isolates, would likely face biosynthesis as its primary competition. For this reason, a list of the advantages and disadvantages of the process has been compiled below.

Table 1: Advantages and disadvantages associated with biosynthesis of CBD.

Biosynthesis of CBD	
Advantages	*Disadvantages*
Due to strict legal regulations around cannabis plants, avoidance of having to resort to cultivation would be advantageous from a legal perspective.	The low water solubility of cannabinoids means that the process, that is carried out in an aqueous medium, may not achieve satisfactory production yields (Moreno-Sanz, 2023).
The ability to directly synthesize CBD would considerably reduce wastage, as well as the necessary size of the production plant per gram CBD produced, when one considers that most cannabis buds contain only 1 % CBD or less, with anything above CBD being considered 'CBD rich'. The implication here is that even obtaining 1 gram of CBD might require 100 g of the plant or more if CBD is traditionally extracted (Dutch Passion, 2022).	Biosynthesis does not allow for extracts rich in other components; this is positive for the production of very pure isolates, but more varied oil compositions are often more popular, even when higher purities are demanded. This is because it is postulated that at least some interaction molecules in plant extracts, even in small percentages, might improve the cannabinoid's medicinal value (Ferber *et al.*, 2020).
Continuous production cycles mean that CBD can be produced continuously, avoiding the seasonal production cycles linked to traditional farming methods. However, it is also worth noting that careful use of greenhouses and control of environmental parameters could avoid this issue as well.	Because CBD is often lauded as 'a natural alternative', consumers have a preference for naturally derived products, with German researchers finding 73.1 % of epilepsy patients preferring natural CBD (O'Brien, 2020).
Estimations suggest biosynthesis could be 4-to-5 times cheaper than traditional cultivation, and 30 times cheaper than conventional chemical synthesis (Moreno-Sanz, 2023).	Microorganisms capable of producing natural oils, such as algae, are more difficult to manipulate genetically and their use may require complex precursors such as cannabigerol (CBG) (Moreno-Sanz, 2023).

Whilst cultivation cannot be avoided as part of the proposed process, methods of streamlining the process and mitigating common issues associated with the process will be discussed in Section 3.2. Using these strategies, the proposed process would also share the potential biosynthesis possesses, as mentioned in Table 1, regarding continuous processes. The cost analysis in Section 5.4 will offer insight as to whether it has the potential to be profitable. Additionally, it would inherently avoid the issues commonly associated with biosynthesis, as discussed in the table above – this positions the proposed process as capable of competing with

biosynthetic mechanisms in terms of the production of CBD and supports investigation into this method.

In terms of the biocatalytic interconversion of cannabinoids, as proposed, several existing industrial processes could offer insight into some of the technical details associated with the proposed process.

Consider, for example, the production of biofuels via enzymatic reactors. The production of bioethanol from lignocellulosic materials has recently been gaining traction as a green alternative to fossil fuels. One of the primary stages of this process is enzymatic hydrolysis – this is of interest, in terms of the proposed process, because of its potential insight into effective bioreactor design. Much of the same principles apply, with enzymes taking the lead in a transformative reaction process, in both cases.

In the production of bioethanol, several limitations arise in terms of bioreactor design, with maximising mass transfer, ensuring effective mixing, and lowering shear stress emerging as key parameters in facilitating interaction between enzyme and substrate. Membrane Bioreactors (MBR) and Stirred Tank Bioreactors (STBR) are the reactors most commonly applied in bioethanol production, with enzyme recycling playing an important role in minimizing costs (Pino *et al.*, 2018).

Another existing process that could potentially offer insight into the use of enzymes for remediation, such as the one proposed, is the bioremediation of wastewater. The use of oxidoreductases are increasingly being used to remediate dangerous pollutant substances into less harmful intermediates, but much like in the case of bioethanol production, several issues have been noted in regards to this manner of enzymatic reaction – primarily, the considerable expense associated with enzymes, their limited reusability, and maintaining stability throughout the process (Marulanda, Gutierrez and Alzate, 2019; Al-Maqdi *et al.*, 2021).

A common means of overcoming some of these challenges is immobilisation on different support structures - this makes the enzymes more expensive initially as while costs of immobilisation may vary depending on the method applied, it ultimately works out to be more cost-effective, making recycling the enzymes less challenging and allowing greater stability in regards to reaction parameters such as pH and temperature (Homaei *et al.*, 2013).

Enzyme immobilisation will be explored in further detail in the technical review, Section 3.7.3, detailing the different methods and the practical effects of each on the process. What's

noteworthy regarding its incredibly common use in wastewater remediation, however, is that enzyme immobilisation can be taken as common practice for the development of a cost-effective, stable enzyme remediation process, and hence the incorporation of this practice would be paramount in the proposed process.

The final established process that bears similarity to the proposed process, from which design notes can be taken, is the production of fructose from glucose using glucose isomerase. This is the most successful industrial use of immobilized enzymes, and so can offer valuable insight into the most effective manner of incorporating immobilized enzymes into an industrial process.

In terms of this process - and indeed most immobilized enzyme-related industrial practices - continuous processing is used. In terms of glucose isomerase manufacturing, column reactors such as fixed, expanded, or fluidized bed reactors, are preferred over their stirred-tank counterparts, with packed-bed being the most used variant, for several reasons. Because of the decreased risk of damage to the enzymes, increased retention, and consistently superior predicted results for reversible Michaelis-Menten reactions, packed-bed reactors reign as the established method of producing glucose isomerase, with substrate conversions reaching 90 % of the theoretical maximum (Illanes et al., 1992).

It is worth noting that the specific type of reaction - whether reversible or irreversible, first order or otherwise - involved in the proposed process is uncertain because of the theoretical nature of the reaction. Experimentation into the minutiae of the reaction falls outside the scope of this work. However, notes can still be taken from the process involving glucose isomerase (specifically, the use of immobilized enzymes, continuous processing, and packed-bed reactors) for the design of the proposed process. A more detailed look at reactor design will be conducted in the technical review, Section 3.7.2.

A final consideration to be taken into account is the emerging trend of enzymatic processes across all fields moving towards genetic modification of the enzymes to mitigate functional issues such as limited catalyst activity, substrate specificity, and stability in different reaction conditions (Marulanda, Gutierrez and Alzate, 2019). Genetic modification is often a costly investment, although it is capable of significantly increasing the effectiveness and durability of the enzymes - they are especially useful for processes involving extreme parameters under which enzymes, unmodified, might quickly become denatured, or regarding reactions which the desired product is not favoured under the required process conditions.

The prospect of genetic modification falls beyond the scope of this work but must be mentioned for the sake of completion; due to the unknown nature of the enzyme, such modification may be required to achieve the desired characteristics and thus must be noted as a potential expense during the enzyme's production.

2.8. General Processing Consideration

In terms of larger commercial and industrial processes, it is worth considering common issues experienced in the operation of such plants and how some of these issues can be mitigated through the practice of thoughtful and conscious design.

Cleaning, for example, remains a pervasive issue, particularly in continuous processes. Fouling cycles raise problems as waste material builds up within the process, risking contamination and reducing processing efficiency by 'blocking up' the system. This is particularly prevalent in piping, heat exchangers, tanks, and other equipment (Wilson, 2003).

For continuous processes, cleaning-in-place (CIP) systems are commonly employed to mitigate the build-up of waste deposit layers. While CIP is also used for batch and fed-batch processes, its use in continuous processes allows cleaning to occur with minimal disruption to ongoing operations. Without CIP, a complete halt in the process would be required to dismantle equipment for traditional cleaning, resulting in significant downtime and loss of materials (feed, intermediates, and product alike). In contrast, CIP reduces the length of downtime by allowing cleaning to be conducted more efficiently.

If a traditional cleaning method is used instead of CIP, the equipment often be left to stew in a caustic cleaning solution, it might be scraped clean manually or be subjected to a pressure washer. However, in industries like pharmaceuticals, where more rigorous methods are required, CIP is preferred for ensuring complete decontamination with careful measurements taken to guarantee effective cleaning (Roey, 2020). Unlike batch processing, cleaning often requires considerably higher asset utilisation to minimize the impact on productivity and efficiency, so cleaning requires careful planning and consideration during the design of continuous processes (Markarian, 2021).

Measurements are commonly made to ascertain the efficacy of cleaning techniques applied. They can be localised, often with a focus on heat transfer equipment, or indirect, monitoring key parameters to ensure process performance is maintained.

Localised measurements are made at key points within the process to assure proper decontamination - for example, while temperature sensors remain the most common and are widely used per regulatory documents and ISO standards for sterilization and decontamination, alternative methods such as heat flux sensors are being increasingly applied in heat transfer systems to ensure proper decontamination. Indirect techniques focus on monitoring parameters such as detergent concentrations, temperatures, and valve openings, which are used as proxies to ensure decontamination, even though they do not directly measure contamination levels themselves. However, the primary issue with indirect techniques is how well they represent the cleaning process, as these values are often subject to measurement error and open to interpretation, with measurements often implicating events outside of the cleaning process (Wilson, 2003)**.** In terms of design and construction, the necessity of these kinds of CIP monitoring systems will need to be incorporated for the process to run optimally.

It is also worth noting that, theoretically, traditional process analytical technology (PAT), which commonly monitors system variables in existing batch processes, could be adapted for CIP monitoring. However, in cases where trace levels of contaminants may be a concern, future advancements might see the use of ultraviolet (UV) or Fourier Transform Infrared (FTIR) spectroscopy-based sensors as more sensitive options for such applications. These possibilities have been suggested in emerging research (Markarian, 2021), though they are not yet standard or approved methods in pharmaceutical regulatory practices, where conductivity meters remain the primary tool for assessing the trace levels of contamination.

Specific processing units will also require specific types of cleaning, depending on their function. For example, solvent distillation units will suffer from sludge build-up that risks contaminating the vaporized solvent. Heating units also build up sludge, and this can reduce the effectiveness of the unit, requiring more time and energy to heat the solvent to the desired temperature. Any thermal oil used will need to be replaced to avoid carbon build-up in heating units, whilst steam jackets degrade over time and could result in accumulating calcium deposits if the water used is not treated properly. All of these issues will negatively impact the effectiveness of the vessel and increase the energy requirements while impacting output rates (Roey, 2020).

Scouring and pigging are also commonly used to clean industrial processes; a newer variant of this is known as ‘ice pigging,’ in which suspensions of ice are used to purge products from equipment pieces like pipe fittings and different process units. Other common CIP techniques include high-pressure jets and spray balls, though these methods are not typically used for cleaning heat exchangers (Wilson, 2003).

In terms of design, convenience, and effectiveness of cleaning should always be a consideration. Design should aim to minimize necessary cleaning where possible, consider where measurement of parameters might be taken to monitor the efficacy of cleaning, take note of how these measurements might be performed, and incorporate necessary CIP mechanisms into the design. The detailed design of cleaning operations falls outside the scope of this work, but the cleaning mechanisms should still be noted as a common concern of industrial processes, and it is recommended that the necessary care be taken during the construction of such a process.

Enzymatic processes often introduce a host of unique challenges to the process. They are expensive and thus processes involving them are often considered economically infeasible for this reason - the most common workaround for this issue is on-site enzyme production. Whilst requiring increased capital investment, as well as greater resources to produce the enzymes - most commonly via fermentation using micro-organisms - this method is widely lauded as the more commercially feasible alternative to purchasing the enzymes. In fact, in the production of lignocellulosic ethanol, researchers found that on-site production could reduce enzyme costs from 30 % to 70 % (Pino *et al.*, 2018).

While the fermentation of these enzymes should certainly be considered for the proposed process to maximise potential profitability, the specific design details of such a plant fall outside of the scope of this work. However, the production of enzymes will be discussed in the technical review, Section 3.3, and the monetary implications thereof will be considered further in the economic analysis performed in Section 5.4.

Any industrial process will have to contend with the issue of waste: how to minimize it and how to treat it. Waste generated from industrial processes can be classified into three broad categories: solid waste, chemical waste, and toxic/hazardous waste. The kind of waste in question will determine how it will need to be treated to mitigate the harm it could cause to both the environment and public health.

Ideally, solvent recycling should be applied wherever possible to reduce waste generation. In the pharmaceutical industry, specifically, where achieving high-purity products is the paramount objective of the process, excessive use of solvents is often applied to achieve the desired product concentrations. Approximately 25 – 100 kg of waste is produced for every kilogram of product manufactured within the pharmaceutical industry - this is incredibly high when one considers the adverse effects associated with such waste (Sheldon, 2017).

Generally, this waste, depending on its nature, will be disposed of either onsite, offsite or by incineration techniques. However, this is inherently problematic, as waste solvents often leak into the surrounding areas, resulting in contamination and harming both the land and potentially the people living off it. Chemicals resulting from combustion, including gases emitted from incineration, can also be hazardous (Aboagye, Chea and Yenkie, 2021).

A sensible middle-ground between waste mitigation and high purity yields (requiring high solvent volumes) is solvent recovery, which not only reduces the risk of contamination in terms of limiting the risk of spills and stray emissions but can also often be linked to lower overall costs of the process if implemented wisely (Aboagye, Chea and Yenkie, 2021). By limiting disposal- and solvent costs, waste mitigation can be a useful tool in creating an economical yet environmentally friendly process.

However, solvent recovery systems should also be economical and efficient in their conception. Thus, careful design is required to limit capital costs, ensure solvent purity is maintained, and ensure profitability for solvent recovery to be viable.

It's also worth noting that for whatever waste cannot be avoided via recovery techniques, waste-to-energy technology could be a useful tool for producing energy and possibly recovering process materials. For example, even though (as mentioned) combustion might produce hazardous gases and could potentially be harmful, if paired with solvent recovery it could still limit the environmental damage caused by the waste in terms of spills and contamination whilst also potentially increasing the profitability of the process. As it provides a means to feed energy back into the process, it has the potential to limit costs by utilizing the power and heat generated. Some alternatives to combustion could also include pyrolysis and gasification, which allows for cost mitigation in a similar fashion - though all will require some capital expense (Kumar *et al.*, 2017).

Ideally, a circular model would be applied, in which materials would move in a closed loop, with all disposed materials being recycled for use in the process, or a sister process on the same

plant. This often is not feasible, or easily attainable, in practice - and the conception of which falls beyond the scope of this work - but it should be noted as the idealized version of a solvent- and material recovery system, as it would maximize waste generation and minimize the need for raw materials, resulting in increased revenue. All solvent- and material recovery systems should, ideally, err as close to circular as the process allows.

The design of waste-to-energy processes falls outside the scope of this work, but the concept of solvent recovery and waste minimisation will be further explored in Section 3.10.

Safety is also always a major consideration. Automated control systems, alarms, trips, and interlocks are recommended for the control of process variables like temperature, pressure, and flow rate, and the layout of the plant should always be considered with care to limit damages should an incident occur. Duplication of key equipment services should always be included, as well as safety basics such as sprinklers, blast walls, and fire-fighting equipment should fires or explosions break out (Towler and Sinnott, 2009).

All pressure vessels should include pressure relief devices to avoid parameter variation outside of the maximum working pressure - examples of this include valves and bursting discs, which are often used in conjunction with each other (valves for minor pressure overshoots, and discs for more major overpressure). Equipment should also be protected from underpressure, to avoid buckling of the vessel due to compressive stress (Towler and Sinnott, 2009).

In terms of temperature monitoring, great care must be taken to ensure the equipment can withstand the worst feasible temperature fluctuations of the process, such as the inclusion of quench systems during emergency shutdowns and through the application of additional detectors to provide localised and redundant parameter detection. Examples of this include skin thermocouples in vessel walls and thermowells (Towler and Sinnott, 2009).

Safety measures that can be taken to ensure careful handling of potentially hazardous solvents and other process materials have been listed below. It is of paramount importance that all processes (particularly those involving pressures, temperatures, solvents, and other relevant parameters and materials with the potential to cause harm) be overseen with utmost care and that all the discussed safety measures be followed.

The following safety measures should always be adhered to in industrial processes:

- Substitute hazardous or toxic process material with hazardous or toxic materials where possible.
- Design of equipment and piping with care to ensure leak-free containment.
- Provide adequate ventilation.
- Put in place careful disposal, such as using vent stacks to properly disperse vented materials and the meticulous collection and treatment of waste liquids.
- Ensure proper escape routes, rescue equipment, safety showers, eye baths, respirators, and other relevant emergency services are always readily available.

3. Technical Review

3.1. Overview of Technical Review

In the technical review of this work, each step of the proposed process will be discussed, including a comparison of known methods, available equipment, and scientific findings. From this, the superior method will be selected for each stage and the relevant equipment and parameters will be outlined. More intricate details of each piece of equipment can be found in Section 4 and Section 5.4, where the process design will be finalised.

An overview of each required stage and its associated process equipment can be found below in Figure 5 and Figure 6, where the former represents the process to produce a CBD isolate and the latter produces a full spectrum CBD blend – this variant contains other cannabinoids beyond CBD, such as CBG and CBN, as well as other compounds like terpenes and flavonoids, whilst still maintaining negligible THC concentrations. These two process set-ups and their respective products will be compared further in Sections 4 and 5.

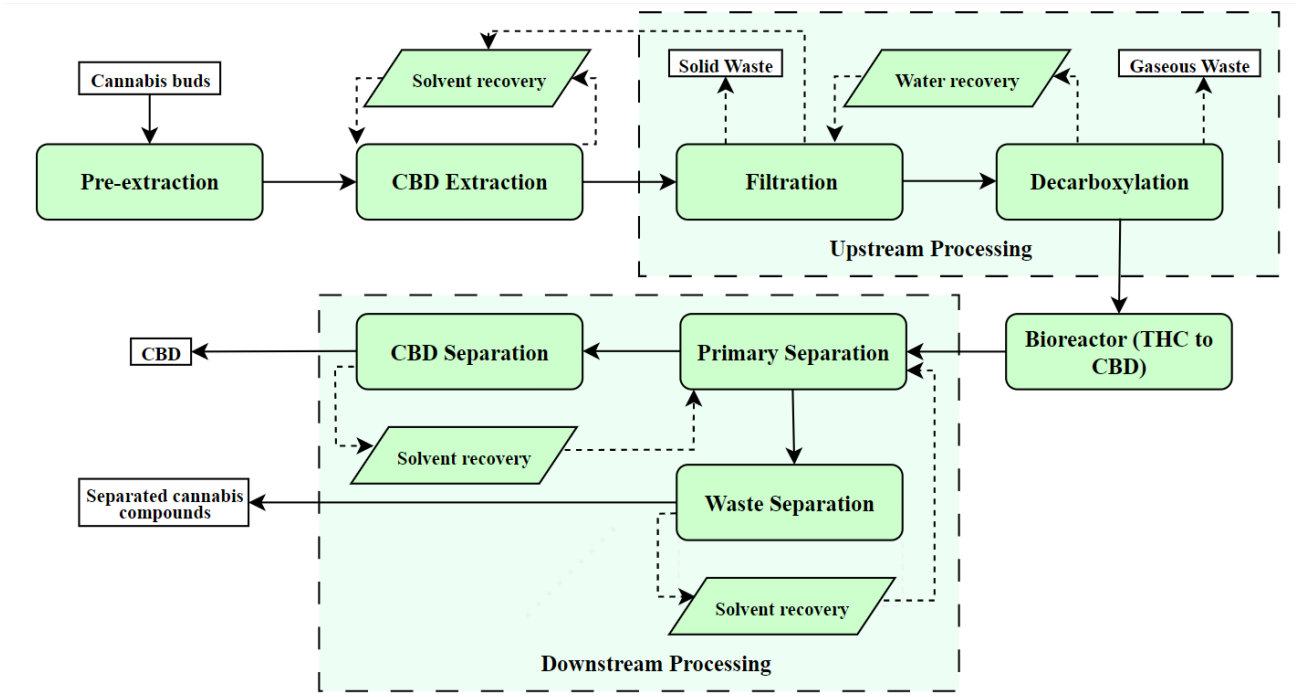


Figure 5: A process flow diagram depicting the general process for producing CBD isolates.

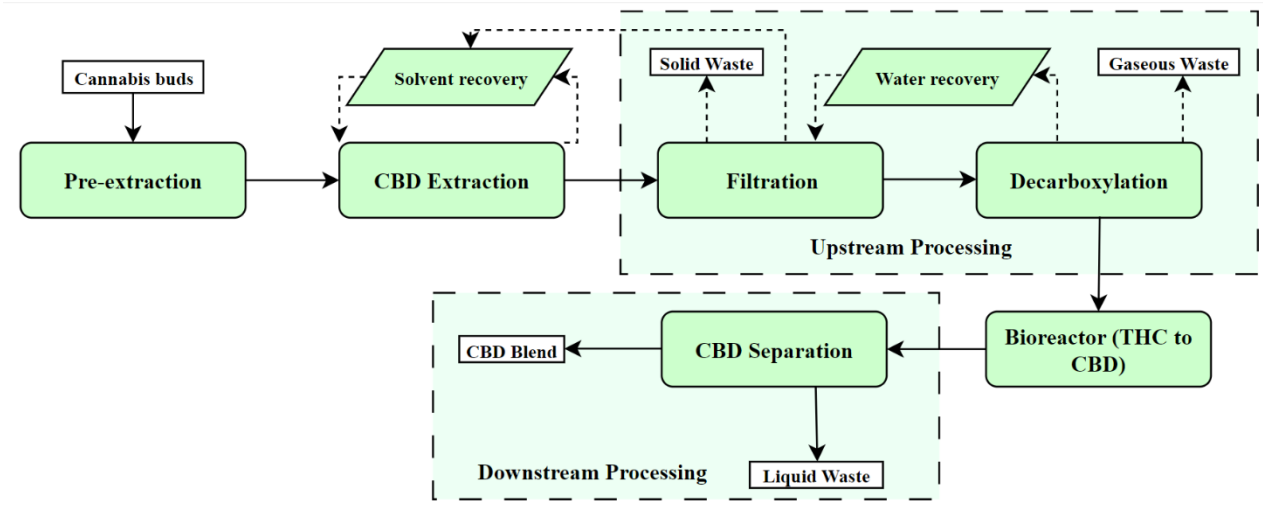


Figure 6: A process flow diagram depicting the general process for producing full spectrum CBD oil.

3.2. Cultivation of Cannabis

Figure 7: A flowering cannabis plant.

When one envisions the production of CBD products, there is one initial consideration that must be decided upon before extraction and processing can begin: procuring the plants. Whilst cannabis could theoretically be purchased for industrial use, from a licensed supplier, the substantial amounts that would need to be purchased, transported, and stored for lengthy periods would make farming one's own cannabis an alternative worthy of consideration. In addition, this allows the quality of the plants and the regulation of cultivation parameters to be assured, encouraging a more reliable product outcome.

Cultivation can affect cannabinoid concentrations significantly; for example, defoliation, defined as removing primary and secondary branches, and pruning, has been shown to aid in standardising cannabinoid profiles of the plants, which is useful in the production of a standardised cannabinoid product. Additionally, grooming the plants in this manner will allow fresh growth, and make monitoring for pests easier (Lazarjani *et al.*, 2021).

Naturally, pest populations must be controlled as tightly as possible to avoid interfering with plant growth, including rodents and insects such as aphids and mites. Rattraps, pesticides, and 'biologicals' (i.e.: harmless varieties of insects that are allowed to live amongst the plants to control undesirable pest populations) can be employed to limit infestations. It is suggested any pest control chemicals employed should be flushed before harvesting, to avoid negatively affecting the harvested buds.

Mineral concentrations have also been shown to notably impact the cannabinoid profile of a plant. For example, increasing nitrogen concentrations in the soil resulted in decreasing concentrations of most cannabinoids and terpenoids. However, limiting nitrogen concentrations to maximise cannabinoid production in the plant resulted in retarded growth, development, and function – thus, compromise becomes necessary to balance both cannabinoid concentration and physical plant growth (Saloner and Bernstein, 2021).

Alternatively, mineral concentrations (and, by extension, pH) of the 'irrigation solution' could be varied according to the life phase of the plant. Once again using nitrogen as an example, higher nitrogen levels could be introduced during incipient development phases to maximise initial growth, with lower levels of nitrogen as maturity approaches to encourage cannabinoid production. At a commercial level, an automated system can be implemented to measure the pH of the soil and introduce various concentrations of irrigation solution depending on its readings to maintain the desired mineral levels for the current life phase of the plant. This

provides an opportunity to increase the efficiency of cultivation and aid in monitoring and standardising growth parameters.

Other conditions to which the plant is exposed during growth, as well as its overall health during cultivation, also impact cannabinoid composition. As a rule, lowering relative humidity and temperature is found to result in lower rates of mould growth, but also risks inhibiting growth if restricted too severely (Mamun, 2018). Compromise will be necessary, ideally maintaining the temperature between 18 °C and 24 °C and limiting humidity to avoid mould growth and bud rot. This can also be easily controlled via automated greenhouse mechanisms.

Given the significant variation in plant quality and cannabinoid profiles based on these factors, this encourages the cultivation of one's own cannabis to produce a product that is of consistently high standard - however, this is often not financially feasible. The economic implications of cultivating one's one cannabis, as opposed to purchasing from a supplier, will be discussed as part of the economic analysis, in Section 5.2.

In terms of chemical compounds, buds will be assumed to contain 5 % CBD and 5 % THC. Ratios of CBD: THC vary considerably, ranging between 1:10 to 20:1, and sometimes higher (Cannasouth, 2021). However, the cultivation of high CBD strains requires rigorous control of growing parameters and generations of careful cross-breeding which can prove challenging, especially in the cases of cultivating one's own cannabis. (CenturionPro, 2018; Voser, 2022) This is part of the reason the conversion of THC has such potential - it has the potential to ease the laborious cultivation process by allowing the ratio to be altered during processing.

3.3. Enzyme Procurement

In the application of enzymatic biocatalysis as part of a commercial or industrial process, the procurement of the enzyme catalyst will be a significant concern. Theoretically, enzymes are not 'consumed' through their utilization as a catalyst, and can be reused over several process cycles, but over time they do tend to become denatured, damaged, or irreversibly bound to certain process constituents (Liu, Zhang and Bao, 2015). Of course, steps can be taken to mitigate enzyme consumption – some of these considerations will be discussed in Sections 3.7.4 and 3.7.5 – but regardless of precautions, regular renewal of the enzyme catalyst is a necessary part of the overall process.

In the creation of biocatalytic membranes, enzymes such as protease, peroxidase, glucose oxidase, laccase, formate dehydrogenase, alcohol dehydrogenase, formaldehyde dehydrogenase, dextranase, β-galactosidase, pectinase, and lipase are commonly utilized, and thus are often easily available via commercial sale (Luo *et al.*, 2020). Therefore, enzymes can often be purchased as necessary to maintain membrane operation. However, it is worth noting that for a novel enzyme such as that in the proposed process, it will not be easily available for purchase from an outside provider.

When enzymes are purchased from the industrial enzyme market, they often prove expensive, running the risk of sabotaging the potential profit-earning potential of the business (Liu, Zhang and Bao, 2015). A viable alternative to this issue exists in the form of on-site (or near-site) enzyme production, in which case enzymes are produced specifically for introduction into the biocatalytic process.

When on-site enzyme production is blended into the production process, running expenses will slightly increase due to greater utility costs, and additional capital investment will also be required to install the necessary apparatus for enzyme cultivation. Overall, however, it is commonly shown that this proves to be less expensive long-term when compared to the procurement of enzymes via an outside supplier, especially with optimization methods such as improving hydrolysis conditions and increasing the performance of the loading material (Liu, Zhang and Bao, 2015; Luo *et al.*, 2020). Thus, the production of one's own enzymes is generally recommended. It is important to note, however, that for pharmaceutical applications, adherence to GMP practices introduces additional complexity and expense, including requirements for cleanrooms, process validation, release assays, and QC/QA measures. These factors can significantly impact the overall cost and feasibility of in-house enzyme production in such contexts.

The precise process design required falls beyond the scope of this work, but commonly utilized strategies will be discussed briefly. Microorganisms such as fungi, mould, and bacteria are commonly used to produce enzymes. The enzyme production process would involve microorganism propagation, recovery of the enzyme, enzyme purification, and finally, product formulation. Recovery of the enzyme generally involves solid-liquid separation mechanisms, the product formulation will require purification strategies and enzyme stabilisation. Beyond this, the remaining medium will often be concentration or cells will need to be disrupted and

the debris removed (Lambert, Meers and Best, 1983). An example of such a process can be seen in Figure 8.

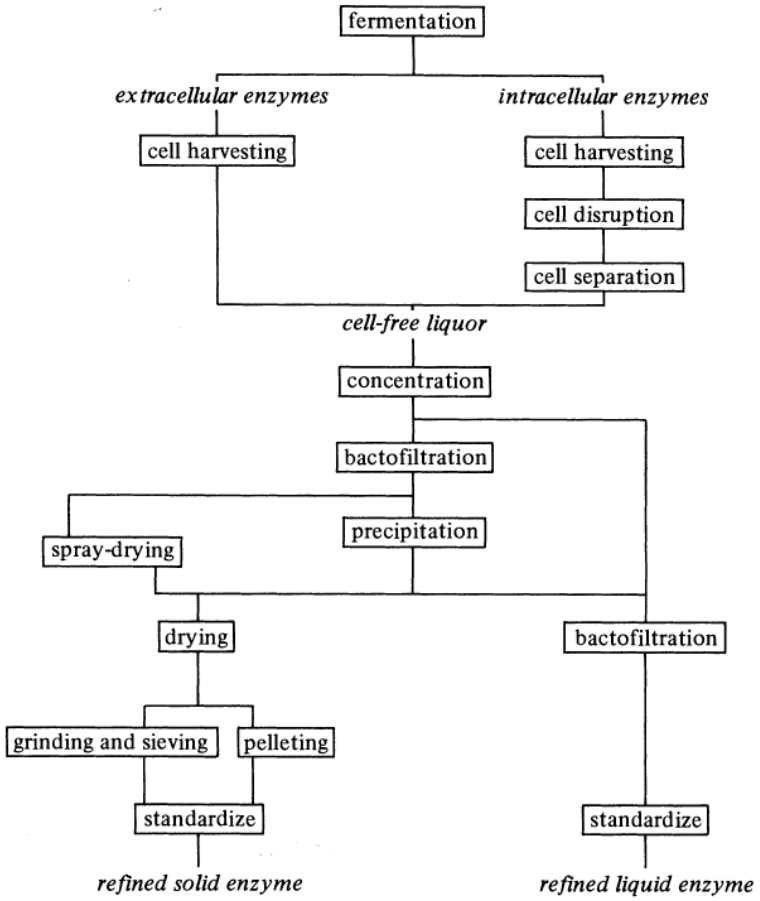


Figure 8: Example of commercial enzyme production process (Illanes, 2019).

The generation of one's own enzyme is a process in and of itself and will require significant capital expense that must be justified against the cost of an externally purchased enzyme. Whilst these externally purchased enzymes are costly, the production cost of one's own enzyme has the potential to be exorbitant depending on the mass of enzyme required for the process. The profitability of producing one's own enzyme as opposed to purchasing the necessary enzymes will be further explored in Section 5.2.

In brief, microbes are commonly used to produce enzymes via fermentation strategies, requiring a liquid medium, nutrients, and a lignocellulose substrate to facilitate enzyme production. Stirred tank reactors are most commonly used to maintain parameters like temperature, aeration, and pH (Sakhuja *et al.*, 2021).

If we operate on the assumption that the enzyme involved in this process would be structurally similar to glucose isomerase - an enzyme used for remediation and transformation processes of a similar nature to the proposed reaction - we can gleam some insight as to how the theoretical enzyme might be cultivated based on the process involved in the production of glucose isomerase.

As an example, *streptomyces sp.* CH7 is a bacterial strain with the capacity to produce this enzyme when grown on xylan or residues from agricultural processes, using xylose as a nutrient source (Chanitnun and Pinphanichakarn, 2012). *BaciUlus sp.* (NCIM 59), another bacterial strain, was also found to be able to produce extracellular xylose isomerase, requiring xylose or wheat bran as a source of carbon (Chauthaiwale and Rao, 1994).

Note that these processes to produce isomerase enzymes require considerable care in inoculating, purifying, and separating the enzyme from the medium, including processes such as centrifugation, gel filtration, lyophilisation, precipitation, gel electrophoresis, adsorption, and ion exchange chromatography (Chauthaiwale and Rao, 1994; Chanitnun and Pinphanichakarn, 2012). The precise processes involved are not germane to this work, but it is worth noting the intensive - potentially in terms of time and resources - involved.

In addition, it is worth noting that the production of such a niche enzyme, as is required by the proposed process, could potentially require enzyme modification that would require considerable scientific knowledge and access to a variety of highly specialised equipment, potentially necessitating the production of the enzyme be outsourced, at least in part.

Recent advancements in enzyme engineering, such as directed evolution and rational design, could be incredibly useful in the creation and optimisation of an enzyme capable of converting THC to CBD. Directed evolution involves generating a library of enzyme variants through random mutations and selecting those with improved performance (for example, to approach to enhance the thermostability and activity of enzymes), while rational design uses structural insights to create more targeted modifications (Arnold, 2018). For instance, enhancing the enzyme’s stability and activity under various conditions through these methods (such as temperature and pH) could increase the efficiency of an enzyme converting THC to CBD.

Moreover, improving substrate specificity is crucial. Enzyme engineering can enhance an enzyme's ability to preferentially bind and convert specific substrates. By altering the active site of the enzyme, it could be designed to preferentially bind THC and catalyse its conversion to CBD effectively. Recent developments in computational tools such as molecular docking

and dynamics simulations can facilitate this process by predicting how structural changes will affect enzyme function (Peluso and Chankvetadze, 2024).

Finally, enzyme immobilization techniques, as discussed in Section 3.7.3, can also play a role in developing a practical process for THC to CBD conversion. Enhanced immobilization methods can stabilize the enzyme, allowing for its reuse and improving process efficiency. Enzymes can be stabilized on many diverse types of supports, allowing for easier separation and reuse in continuous processes. Improved immobilization methods can enhance enzyme longevity and activity, which is crucial for scalable industrial applications (Cao, 2005; Illanes and Altamirano, 2008). All these methods will require significant research and experimentation, which falls beyond the scope of this research, but will be pivotal to the creation of an enzyme capable of facilitating this process.

3.4. Pre-extraction

Once the cannabis plants, cultivated as described in Section 3.2, are at a suitable stage of maturity, they can be harvested and cleaned of dirt and residues. Cannabinoids are housed predominantly in trichomes outside the buds, so it is recommended to trim around the buds, ridding the plants of superfluous leaves and branches which ultimately make negligible contributions to the final extract. In smaller semi-commercial operations, this can reasonably be performed by hand. On a commercial or industrial scale, however, the help of automatic sterilizers and bucking machines become pertinent to achieving greater levels of productivity; bucking machines are pieces of specialised equipment also called de-budders and/or de-stemmers.

Cannabis plants are often dried before further processing. This aids in the prevention of microorganism growth that would otherwise cause the decomposition of plant tissue and mitigates the risk of fungal infection, thereby facilitating longer storage periods whilst maintaining all the desired efficacy, potency, medicinal purposes, and existing CBD/THC concentrations (Lazarjani *et al.*, 2021).

Drying the plant to a moisture content of 0 – 15 % by mass is considered the standard. Drying options are vast and include ‘slow drying’ for 5-to-6 days in a dark room of specific humidity and temperature, with the plants hung upside down or spread on drying sheets. This is not suggested, as it can lead to uneven drying and mould growth. Humidifiers can be used to assist

in this regard. This method requires large available spaces and lengthy time periods. Scaled-up processes commonly replace this process with microwaves or ovens, for the sake of efficiency - however, this often results in the alteration and/or evaporation of certain compounds, which can prove undesirable (López-Olmos *et al.*, 2022).

Freeze-drying has also proven useful for the extraction of cannabinoids. In this process, the plant material is reduced to powder form and operated under a vacuum, breaking apart the cells to release the desired compounds. It boasts the lowest possible loss of valuable compounds, resulting in dried plant stuff rich in both cannabinoids and terpenes. The major issue with this method, however, is the fact that it is very expensive, with a high energy expenditure, costing 4 - 10 times more than traditional drying methods (Challa, Misra and Martynenko, 2020).

It is also worth mentioning that the drying process can be forgone entirely, and indeed often is when the co-extraction of volatile terpenes is desired. Extraction from fresh cannabis is less expensive as it does not consume energy during the drying process, and it also prevents the degradation of desirable compounds. Unfortunately, the fresh plants take up considerably more space than their dried counterparts, requiring large enclosed containers to preserve their freshness before extraction (Jan and Koumans, 2019).

Decarboxylation (which co-occurs during heating) can also be carried post-extraction when using this method, which also avoids alternating of the cannabis's compounds, as well as minimizing loss of terpenes, should this be desirable.

Decarboxylation being carried out later in the process also allows smaller ovens to be used for decarboxylation, which is useful insofar as saving on equipment capital and energy expenditure (López-Olmos *et al.*, 2022). Decarboxylation - i.e.: the removal of a carboxyl ground from acidic cannabinoids, creating their neutral form - is essential to produce CBD and THC from CBDA and THCA, respectively, so this process should not be neglected when performing extraction on fresh cannabis or cannabis which has been dried through methods which do not involve heating.

Next, the flower surface area should be increased before extraction, particularly if solvent extraction is to be employed. Because the desired cannabinoids are housed inside the bud trichomes, the concentration gradient between the solvent and the trichomes' surface will be the primary obstacle to the extraction, with large particles often hindering the process and increasing the required extraction time.

Milling is commonly applied, especially in large-scale set-ups, due to it being a simple and inexpensive method, albeit slow. Very forceful crushing of the buds is not recommended, as this increases the risk of inadvertently increasing concentrations of undesirable compounds substances in the resultant extract as well – thus it becomes important to strike a balance between maximizing the dissolution of cannabinoids, whilst also minimising undesirable dissolution (Aladić *et al.*, 2015; Valizadehderakhshan *et al.*, 2021). The suggested particle size is reported as 0.5 mm or slightly smaller - too small risks clogging the mechanism and too-high pressures which negatively impact extraction performance (López-Olmos *et al.*, 2022).

Additionally, a simplified distribution of constituents will be assumed for the sake of the simulation as shown in Table 2, with 'solid impurities' referring to the solid plant matter – i.e.: the physical cannabis buds. 'Undesirable compounds' refer to non-CBD constituents such as chlorophyll, lipids, other cannabinoids, and terpenes, which will be separated out by the proposed process and have no place in the CBD isolate product (but will remain in conservative concentrations in the full spectrum CBD product). More information on the amount of water in the plant can be found in the discussion on decarboxylation in Section 3.6, whilst more context for the values given for the cannabinoids can be found in Section 3.2.

Table 2: Simplified mass composition of the extraction feed upon exiting pre-extraction.

Compound	Assumed Mass Composition (%)
CBD	5
THC	5
Solid Impurities	60
Undesirable Compounds	10
Water	20

3.5. Extraction

3.5.1. Extraction Overview

Before any discussion into the extraction of cannabinoids, it must be noted that there is a notable lack of literature regarding the recovery and purification of CBD, with only 6 % of relevant published literature revolving around extraction protocols, and the majority of this small portion detailing 'total extracts' instead of CBD, limiting their usefulness (Marzorati *et al.*, 2020). Thus, assumptions must be made to determine the changes in concentration that might result from the extraction involved in the theoretical process - this will be expounded upon as it becomes relevant in the following section.

The cannabis feed, at this point, contains a range of compounds beyond merely the simply desired CBD. It also contains other cannabinoids, terpenoids, amides, flavonoids, phenols, alkaloids, waxes, and chlorophyll, amongst others (Al Ubeed *et al.*, 2022). The extraction process involves removing these components from the feed to produce cannabis oils can be rigorous, depending on desired purity levels, and thus extraction and purification become pivotal components in the production of cannabis oils.

In industry, extraction techniques are often applied to the process to concentrate certain components from a raw material, which can then be further processed as part of product development. Estimates show that between 30 – 60 % of cannabinoids in the buds are lost during extraction and purification processes involved in industrial cannabis manufacturing, often due to poor optimisation of the process. It can therefore be asserted that careful selection and optimization of extraction and processing methods will significantly impact the efficiency and profitability of the process, where the efficiency of extraction will be heavily determined by parameters such as feed particle size, agitation, temperature, and extraction times (Valizadehderakhshan *et al.*, 2021).

Several different forms of extraction have proven effective and are currently in operation as part of commercial and industrial procedures worldwide. However, solventless extraction methods such as dry-sieving and water extractions are applicable only for the removal of the trichomes from plant matter, entirely disregarding extraction of the cannabinoids from the trichomes themselves – these methods are therefore not applicable to the proposed process.

Because of this, solvent-based methods are much more common on a commercial and industrial scale. Examples of established methods include maceration, Soxhlet extraction, ultrasonic-assisted extraction, and microwave-assisted extraction, often employing solvents such as ethanol, alkane gases, ethers, carbon dioxide, and olive oil (Lazarjani *et al.*, 2021).

3.5.2. Traditional Solvent Extraction Methods

Traditional solvent extraction methods involve immersing the cannabis trichomes in some form of organic solvent media to facilitate the mass transfer of the cannabinoid mixture from the biomass into the solvent carrier, followed by agitation. They are also known as 'maceration' or 'immersion' extraction methods and can be performed as batch, semi-batch, or continuous processes (Valizadehderakhshan *et al.*, 2021). The length and temperature of the process will vary but is commonly heralded as an effective, popular, and economical means of extraction (Azmir *et al.*, 2013).

A major deciding factor behind the effectiveness of different solvents for the extraction of different compounds is the solubility of the compound in question (in this case, the cannabinoids); though of course, solubility, affinity, mass transfer, toxicity, environmental safety, and temperature profile of potential solvents are also considered to maximize suitability of the set-up (Azmir *et al.*, 2013; Lazarjani *et al.*, 2021).

It is worth noting, however, that regardless of measures taken to maximise extraction effectiveness, solvents are generally not selective enough to directly extract pure cannabinoid mixtures – terpenes, moisture, volatiles, fatty acids, pigments, heavy metals, and other compounds will also be drawn from the plants as co-extracts. At higher temperatures, this mixture of undesirables will often thicken into a black, viscous tar-like substance called wax which must be removed before further processing, lest it spoil the thermodynamics necessary for separation (Valizadehderakhshan et al., 2021).

Ethanol, as a rule, is often regarded as the superior solvent of choice for several reasons, which will be discussed. However, the use of vegetable oils such as olive oil are capable of extracting higher amounts of terpenes from the trichomes of cannabis plants (which is not particularly relevant for this particular venture), but as a non-volatile substance, it proves difficult to separate from the extract (Hazekamp and Romano, 2013).

Low molecular weight hydrocarbon gases such as butane and propane have been used for extraction purposes, as well as halogen-substituted hydrocarbon variants. These solvent gases are cooled or pressurized into liquid form and passed through the plant material, drawing the cannabinoids into the solvent; this extract can then be collected as the solvent is re-evaporated, returning the alkanes to their initial gaseous form (Lazarjani *et al.*, 2021). Being nonpolar in nature, these gases are adept at facilitating the dissolution of similarly nonpolar compounds; this is useful for the extraction of cannabinoids, as they are predominantly composed of carbon and hydrogen and thus fall into this category.

However, the presence of some alcohol and acid groups introduces the need for some solvent polarity as well. Thus, a mixture of polar and non-polar solvents achieves the highest cannabinoid yield. Care must be taken in choosing the ratio and identity of the solvent, as this determines extraction effectiveness, as well as evaporation time after extraction, which should be minimized to maximise process efficiency (Namdar *et al.*, 2018). This allows versatility, where the ratio can be varied according to the needs of the process.

Unfortunately, pressurizing such flammable (and often explosive) gases poses considerable safety risks, and these gases also oftentimes contain impurities that could result in a contaminated extract. Even the solvents themselves run the risk of becoming residue in the extract potentially jeopardizing product integrity (Lazarjani et al., 2021). Additionally, when the recovery of acidic cannabinoids, in particular, was tested, ethanol had a considerably higher yield, outperforming the hydrocarbon gases in terms of extraction (Fathordoobady *et al.*, 2019).

Unfortunately, using ethanol as a solvent can result in chlorophyll being extracted with the cannabinoids, lending an undesirable flavour and green colour to the extract as well as with any GC-MS analysis. While treating the extract with activated charcoal managed to successfully remove the chlorophyll, it also reduced cannabinoid concentrations by up to 50 % (Lazarjani et al., 2021). Despite this, however, ethanol still proves to be the superior extraction method for non-psychoactive cannabinoids, including CBD. Brighenti and company (2017) found that dynamic maceration using ethanol for 45 min at room temperature managed to extract greater cannabinoid concentrations than even newer, more elaborate extraction methods (which will be discussed later in the section) (Brighenti *et al.*, 2017).

Furthermore, Valizadehderakhshan and company (2021) concluded that even with additional considerations such as relative costs, toxicities, and boiling points (imperative for maximising solvent recovery and mitigating process running costs), ethanol soundly trumped competitors such as propanol, butanol, and methanol, emerging as the superior solvent choice for cannabinoid extractions.

Therefore, ethanol has been selected for use in the extraction process proposed in this dissertation.

Supercritical Fluid Extraction

With the current growing awareness of climate change and pollution pushing the world towards 'greener' production approaches in industrial processes, supercritical fluid extraction (SFE) has emerged as a viable alternative to conventional extraction methods, with the application of supercritical fluids reducing toxic residue contaminating product extracts, lessening high production costs, and mitigating environmental damage inflicted by the process (Aladić *et al.*, 2015; Valizadehderakhshan *et al.*, 2021).

During SFE, temperature and pressure are elevated to move the solvent into its critical state (i.e.: the point at which the solvent cannot be transformed into liquid via a further increase in pressure), thereby elevating the density of the solvent and thus affecting solvent power. The biomass of interest can then be solubilized within the selected solvent; common examples of such solvents include water, ethanol, methanol, carbon dioxide, ethene, nitrous oxide, and pentane, amongst others (Valizadehderakhshan *et al.*, 2021). The control this method allows over solvent power enables very selective extractions and the ease of transition between liquid and gas phases facilitates superior rates of mass transfer, thus increasing the efficiency of the process (Perrotin-Brunel, 2011).

Because supercritical fluids are gaseous at room temperature, this allows for simple and effective solvent recovery via evaporation, thus reducing wastage (Lazarjani *et al.*, 2021). Lower process temperatures also mean that energy consumption will be reduced, lessening running expenses, and compounds at risk of deterioration at higher temperatures can be easily extracted where traditional extraction methods might present difficulties; cannabinoids are an example of such a thermosensitive component and thus would benefit from SFE (Aladić *et al.*, 2015).

When designing an SFE process, parameters such as pressure and temperature, moisture, the solvent: feed ratio, selected means of solvent recovery, pre-treatment of the solid matrix, etc. This is important for maximising extract yields, naturally, but also mitigating potential safety concerns and lessening high capital costs; for this reason, as well as saving on labour expenses, these processes are generally automated (Valizadehderakhshan *et al.*, 2021).

This does make the necessary equipment extremely expensive, however, deterring investors who do not possess the necessary funding despite the benefits. It is also worth noting that the existing protocols for scaling are severely lacking, with trial-and-error remaining the sole manner of optimization – this makes process design difficult and time-consuming, in addition to the considerable capital expenses.

Supercritical Carbon Dioxide

An example of a commonly used solvent for SFE is carbon dioxide gas (CO_2). Though CO_2 is a polar gas, supercritical CO_2 behaves as a non-polar solvent; it is relatively inert in nature, non-flammable and non-toxic, renewable and readily available, easy to remove and recycle, low-cost, and forms at near room temperature (Di Forti *et al.*, 2014; Moslavac *et al.*, 2014).

Additional advantages of using CO_2 as a solvent include easy evaporation from the extract, requiring only the simple adjustment of pressure and temperature with no additional power usage, and the capacity to fully recycle all of the recycled solvent for reuse due to its enduring purity (Valizadehderakhshan *et al.*, 2021). Thereby, supercritical carbon dioxide extraction is seen as superior to extraction by organic solvents in terms of environmental concerns and operational efficiency.

Collectively, these advantages make CO_2 a very attractive option for SFE; however, because supercritical CO_2 may not always be polar enough to extract certain compounds, polarity modifiers such as acids, alcohols, and water are often introduced as polarity modifiers or ‘co-solvents’ (Lazarjani *et al.*, 2021). An example of such a setup can be seen in Figure 9.

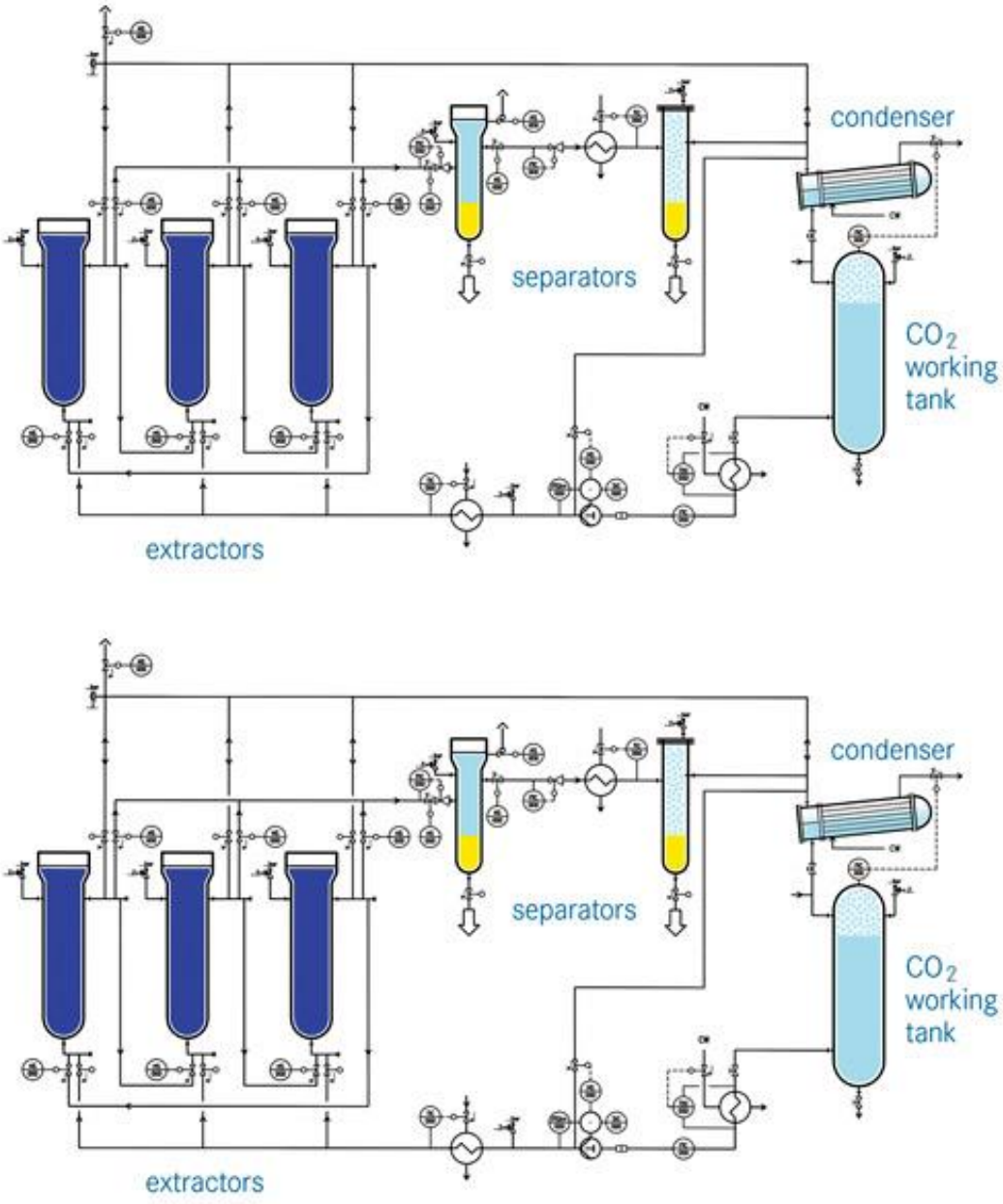


Figure 9: An example of supercritical CO_2 extraction (NATEX, 2023).

However, it is worth noting that supercritical CO_2 has a lower theoretical extraction potential for cannabinoids than pure ethanol, which has a wider solubility sphere – i.e.: a greater range of solubility. This supports the use of ethanol as a solvent for the given process. Interestingly, the addition of alcohol to supercritical CO_2 in the form of a co-solvent, whilst not strictly necessary for successful extraction, has been shown to increase cannabinoid solubility of the solvent, thereby reducing the flow of CO_2 required for the process to progress successfully and increasing product yields – ethanol is one such alcohol (Lazarjani *et al.*, 2021;

Valizadehderakhshan *et al.*, 2021). The addition of ethanol to the process provides an opportunity to capitalize simultaneously on supercritical CO_2's minimal post-extraction requirements as well as ethanol's vast solubility range, paving the way for greater extraction capacity.

On the downside, this may also enhance the extraction of unwanted components, lowering product purity – this is not desirable (Valizadehderakhshan et al., 2021). Research does suggest, however, that increasing pressure will increase solvation power and lower selectivity, so pressure can ideally be optimized to achieve an acceptable compromise between solvation power and product purity, whilst simultaneously injecting ethanol in pulses to increase cannabinoid extraction rates (Lazarjani *et al.*, 2021).

In Table 3, a comparative overview of CBD product purity, process yields, and solvent-to-plant ratios for ethanol extraction and supercritical CO_2 extraction can be found, weighing the performances of both methods.

Table 3: Comparative analysis of supercritical CO2 extraction and ethanol extraction

Method	Percentage Purity Achieved	Yield	Solvent-to-Plant Ratio	Additional Notes	Source
Ethanol Extraction	52.7 % - 99 % Percentage purity proved to be highly variable. Lower purities (52.7 % and 68 %) tend only to occur when no downstream processing is applied. Winterisation alone raised percentage purity to 88 %, with more rigorous downstream processing such as distillation, liquid/liquid chromatography or crystallisation consistently achieving >95 % in all sources investigated.	5.8 % - 28 % The yields achieved by the process are once again quite broad. It is also worth noting that these yields are defined as CBD extracted over the mass of the original total extract; thus, this is inherently variable based on inconsistency in the CBD mass percentages in the original extracts. Based on the lack of yields reported in relevant literature, this is difficult to truly analyse.	5-44 L/kg	Supercritical CO_2 offers advantages such as easy solvent recyclability, lack of solvent residue in the extract, as well no solvent flammability and toxicity comparative to many other, more volatile solvents. However, there are also intrinsic disadvantages associated with the use of supercritical CO_2. CO_2 has considerably longer extraction times (3–6 hours, compared to the maximum of 3 hours in the cases of other solvents such as hexane, butane and ethanol), elevated power consumption, and expensive and complex equipment necessary to operate at elevated temperatures and pressures, especially at the more extreme extraction conditions required to maximise purity and yields.	(Roura, 2017; Eyal and Zeitouni, 2018; Oroskar *et al.*, 2019; Moreno-Sanz *et al.*, 2020; López-Olmos *et al.*, 2022)
Supercritical CO_2	54 % - >99 % A wide range was similarly present for the percentage purities of CBD isolates produced from Supercritical CO_2 extraction. It is noted that lower values such as 54 % and 58 % where achieved downstream processing steps such as winterisation, as well as a purification step such as thin-film evaporation, flash chromatography, or crystallization, was omitted. Higher percentage purities (>91 %) were achieved at more extreme extraction conditions (e.g.: 60 °C and 300 bar) and when ethanol was used as a modifier (e.g.: 2 %).	8.4 % - 91 % It must be noted that yields reported for supercritical CO_2 extraction of CBD were scarce, with the reported values found only in the research performed by Whittle and company (Whittle *et al.*, 2002). Lower values (8.4 %) were present only at very mild conditions, while more extreme supercritical conditions (60 °C and 300 bar) instantly increased yield up to 78.5 %, and an ethanol modifier increased this up to 91 %.	Up to 192 L/kg		(Whittle *et al.*, 2002; Mueller, 2014; Eiroa Martinez *et al.*, 2016; López-Olmos *et al.*, 2022)

3.5.3. Recent Breakthroughs

In addition to the previously discussed traditional organic solvent extraction and supercritical fluid extraction methods, several innovative and environmentally friendly methods have recently been employed for cannabis extractions – an example of one such method is supercritical hot water extraction.

Water possesses similar solvent properties to ethanol when pressurised into a supercritical state and has proven capable of extracting several cannabinoids, including CBD and THC, with

particular selectivity towards non-psychotic compounds such as CBD (Nuapia *et al.*, 2020). Though this method is quicker than the established solvent methods, water also has a much higher critical temperature and critical pressure than CO_2. (Lang and Wai, 2001) It can thereby be asserted that subjecting water to supercritical conditions is considerably riskier, more expensive, and consumes greater amounts of power than its CO_2 alternative, in addition to being less suitable for thermosensitive extractions. Therefore, for the current research, supercritical hot water extraction is outperformed by supercritical CO_2.

Another innovative new method worth mentioning is microwave-assisted extraction (MAE), during which the biomass is bombarded with microwaves that are absorbed by the plant matrix, disintegrating it to improve mass transfer rates. Rezvankhah and associates (2019) researched the capacity of this method to extract fatty acids from hemp seed oil; comparing their results to those achieved by Soxhlet extraction revealed that MAE achieved lower extract yields but higher oxidation stability. MAE also required shorter extraction times and eased pre-extraction requirements by rendering drying the biomass unnecessary (Valizadehderakhshan *et al.*, 2021).

Thus, MAE is a useful pre-extraction treatment for many processes, but in the case of cannabinoids, concerns exist regarding the compounds' temperature sensitivity; though energy entering the plant matrix is mechanical in nature, this is ultimately converted into heat, thereby risking decarboxylation of the desired cannabinoids and jeopardizing the quality of the extract (Valizadehderakhshan *et al.*, 2021). MAE is therefore not an option for the proposed process.

The final method to be discussed is hydrodynamic cannabis extraction, which has recently been developed from within the cannabis industry. The plant is placed into water, frozen, and exposed to ultrasonication, thus converting it into a nanoemulsion. The hydrodynamic forces of the water can then break cell walls within the emulsion, facilitating the liquid-liquid solvent extraction which follows. Next, a centrifuge is used to separate the extract, which can then be dried (Lazarjani et al., 2021).

This method theoretically exceeds most traditional methods of organic solvent extraction due to its milder required temperatures and reduced organic solvent consumption, but there is incredibly limited research on this method, with no official scientific publications exploring it. Before integration into a process such as the one proposed, further research is required.

3.5.4. Proposed Extraction Method

Taking into consideration all of the aforementioned extraction methods, traditional ethanol maceration has been repeatedly been reported to outperform many competing processes – including the new and innovative methods discussed in Section 3.5.3 – in terms of cannabis extraction yields, particularly non-psychoactive cannabinoids such as CBD (Brighenti *et al.*, 2017; Fathordoobady *et al.*, 2019).

Supercritical CO_2 has been established as ethanol extraction's closest competition in terms of cannabinoid extraction, as per Section 3.5.2, and thus selected as an alternative design to be explored in Section 6; however, the demanding capital requirements and the uncertainty associated with the design and optimization of the supercritical CO_2 extraction processes have led to dynamic maceration using ethanol being selected as the optimal method for this research.

Findings suggest that longer extraction times tend to extract greater concentrations of compounds, overall, due to basic mass transfer principles. These extraction times can be reduced without greatly impacting the total concentrations of extraction metabolites by increasing extraction temperatures, but care must be taken - increasing temperature in this way also decreases compound stability due to heat sensitivity (Brighenti *et al.*, 2017).

Cannabis is composed of a host of different constituents, with Pieracci and associates (2021) reporting that cannabinoids - the constituents of interest in this work - accounted for only 54.4 % of the total dry mass of the plant. Unwanted substances such as ketones, nitrogen-based compounds, amino acids, sugars, aldehydes, alcohols, glycosides, flavonoids, vitamins, and pigments should - ideally - be left behind in the extraction. To maximise the concentrations of desirable cannabinoids in the extract whilst minimizing the presence of other constituents, a middle-ground between temperature and extraction time must be found.

The selection of a solvent of suitable polarity also goes a long way in achieving this, though no solvent is known to achieve a 'perfect' CBD extraction. Non-polar solvents tend to remove lipid-soluble material such as fats and waxes from the plant material, whilst polar solvents - like ethanol - tend to absorb chlorophyll, alkaloids, and terpenes (López-Olmos *et al.*, 2022). However, some concentration of these undesirable compounds in the final extract is inevitable and further processing will always be required.

Szalata and associates (2022) made several findings with their research into ethanol-based cannabinoid extraction which can be used to inform one of the expected CBD extracted at

specific parameters - this report will lean on many of these values to create a basis atop which the proposed process may be designed.

In terms of initial mass percentages, it was found that CBD made up between 2.26 % and 3.63 % of the dry masses of the samples used by Szalata (2022) - these samples came from three separate varieties, namely Futura 75, KC Dora, and Tygra, with two samples of each variety grown in different conditions. THC varied between 0.50 % and 0.15 %. To give an idea of the percentage of these compounds extracted, data was taken for the extractions performed in 80 % ethanol using dynamic maceration for 3 days at 100 rpm. The findings for each sample variety can be found below in Table 4.

Table 4: Mass percentages of CBD and THC found within samples taken from 3 different cannabis strains before and after ethanol extraction. (Szalata et al., 2022)

Sample Variety	Initial CBD	CBD in extract	Initial THC	THC in extract
Futura 75	2.48	1.31	0.06	0.05
	2.43	1.39	0.05	0.05
KC Dora	3.60	1.26	0.09	0.06
	3.63	1.16	0.10	0.03
Tygra	2.26	0.10	0.12	0.05
	2.58	0.95	0.15	0.04

For the sake of developing a reasonable simulation of such an extraction, averages were taken of the initial CBD- and THC mass percentages, as well as the percentages of the relevant cannabinoids in the extracts. From these values, a percentage of the mass of each compound that could be feasibly extracted during the simulated extraction can be predicted. The calculated values can be found below, in Table 5. These estimations must be used as a baseline for the simulation.

Table 5: Average percentage mass of CBD and THC extracted from cannabis by ethanol extraction (Szalata et al., 2022).

ETHANOL EXTRACTION	
Change in Cannabinoid Content	
Initial Masses per 100g Plant (%)	
Average CBD	2.83
Average THC	0.095
Extraction Output Stream %	
Average CBD	1.17967
Average THC	0.04283
Mass 'Lost'	
CBD	1.65033
THC	0.05217
Percentage Mass Extracted	
CBD %	58.31567
THC %	54.91228

It is important to note that these values will not be strictly accurate to any extraction performed, as variations in cannabis variety, extraction method, and parameters will inevitably have an effect. However, with a notable shortage in literature with usable extraction values, it will serve as a basis for what output CBD and THC one might expect when performing such an extraction.

From Szalata (2022), one can also glean that greater percentages of ethanol (when using an ethanol-water mixture) will result in greater extract concentrations of both CBD and THC. Brighenti and company (2017) found the optimal parameters of such an extraction - using ethanol, at room temperature - for an extraction time of 45 minutes. Both studies agreed that ethanol proved to be the most efficient solvent for the extraction of CBD.

Two extraction stages will be performed to increase the concentration of cannabinoids in the extract, as is commonplace in such processes.

It is also worth noting that, in terms of cannabinoids, non-decarboxylated - i.e.: acidic - cannabinoids tend to be more soluble in polar solvents such as ethanol. Neutral cannabinoids, such as those obtained post-decarboxylation, tend to have considerably lower solubility in polar solvents - and indeed, overall (López-Olmos *et al.*, 2022). It is for this reason that it is suggested decarboxylation be conducted after the ethanol extraction, to maximise CBD concentrations in

the extract. Thus this will be a part of the ‘upstream processing’ (Section 3.6) part of the proposed process rather than the ‘pre-extraction’ (Section 3.4) segment, where it is often performed in CBD extraction processes.

Another pivotal part of ethanol-based CBD extraction processes is the winterization step. Due to the solubility of lipids in ethanol-based solvents, the resulting extract is often frozen at temperatures between -10 °C and -30 °C, often for one-to-several days, to solidify the waxes and enable their removal in the form of sediment (Hazekamp, 2018; Marzorati *et al.*, 2020; Valizadehderakhshan *et al.*, 2021). It is, however, possible to avoid this step by using cold ethanol extraction instead of traditional room temperature methods.

Cold ethanol extraction, carried out at temperatures between 5 °C and -80 °C, enables the extraction of cannabinoids while limiting the co-extraction of waxes, triglycerides, and chlorophyll (Caulkins, 2010). While more power will be required for cold ethanol extraction as opposed to traditional room temperature methods, this method increases efficiency of the process by avoiding the winterization process, which would draw out the process by an additional 24 - 48 hours in which the extracts would need to be kept at temperature ranging from -20 °C to -80 °C (Hazekamp, 2018; Valizadehderakhshan *et al.*, 2021; López-Olmos *et al.*, 2022). Thus, cold ethanol extraction has been selected for the proposed process.

Literature containing solid numerical data on such an extraction process is limited, as is the literature on CBD extraction processes in general. In Table 6, some values have been compiled regarding similar processes, some at room temperature with winterization occurring post-extraction, about the amount of ethanol required for the process as well as the percentage of CBD extracted. One particular extraction mechanism, proposed by Castillo (2020) is a cold ethanol extraction that involves decarboxylation occurring post-extraction, thus erring very closely to the proposed process and offering useful insight into optimal ethanol ratios and the expected extraction yield.

Table 6: Numerical data for ethanol extractions in which winterization occurs post-extraction.

Source	Number of Extractions	Total Ethanol Ratio	Ethanol Per Extraction	Final CBD Yield	Additional Notes
(Castillo, 2020)	1	44 L/kg	40 L per every 900 g processed (i.e.: per extraction).	-	▪ Cold ethanol extraction ▪ Plant matter chilled to 0 – 10 °C for cryogenic grinding. ▪ Vertical cylindrical drum rotating at 40 rpm. ▪ Extraction period of 10 min. ▪ Ethanol pre-chilled to −30 °C.
(Roura, 2017)	Continuous mode	19.3 L/kg	1 L/min; 25 + 25 L	▪ 16.4 % CBD extraction yield. ▪ CBDA + CBD content of 52.7 %. ▪ Extraction efficiency of 83.1 % for CBDA + CBD.	▪ Room temperature ▪ A CBDA-rich variety used contained 10.39 % total CBDA + CBD
(Moreno-Sanz *et al.*, 2020)	3	25 L/kg	1 + 0.75 + 0.75 L (2.5 L ethanol used in total)	▪ 20.1 % extraction yield. ▪ 67.14 % CBD after decarboxylation.	▪ Room temperature ▪ 100 g of CBD-rich cannabis containing 7 – 10.5 % CBD.

In the cold ethanol extraction process proposed by Castillo (2020) - parts of which will be used as a template for cold extraction to be instituted in the proposed process - cryogenic grinding is carried out at 0 °C – 10 °C to minimize decarboxylation and loss of compounds. Ethanol is chilled to −30 °C to minimize the co-extraction of waxes, lipids, and chlorophyll. The extract is then filtered to remove any lingering plant material, producing a particulate waste fraction, a colourless cake recovered from the filter, and a filtrate - i.e.: the final extract - which can then undergo decarboxylation. The waste fraction contains waxes and lipids, carbohydrates, and plant material, which will need to be disposed of.

In general, cold ethanol extraction minimises undesirable co-extraction, increasing selectivity at the cost of a slightly lower yield than achieved by extraction at room temperature. However, with purification mechanisms such as distillation, chromatography, or crystallization isolates of purities exceeding 95 % can be produced irrespective of whether cold ethanol extraction or room temperature extraction was applied (López-Olmos *et al.*, 2022).

In regards to solvent feed, according to the data compiled by López-Olmos and associates (2022), which compared multiple extraction processes and the operational details thereof, ethanol-to-plant ratios tended to vary greatly, ranging between 5 - 44.4 L/kg. However, due to the lack of concrete numerical data, extraction performances cannot be compared or directly correlated with ethanol ratios in any meaningful way.

The density of ethanol (measured at room temperature, as it is assumed this measurement range was taken) has been taken as 789 kg/m^3 (Louisiana State University, 2023). To build a basis for the simulation, an ethanol-to-plant ratio within the 5 – 44 L/kg range should be applied.

For the proposed process, a total extraction time of 55 minutes at -35 °C will be applied. After the conclusion of the solvent extraction process, once the ethanol has been evaporated from the extract, the solvent can be condensed and recycled back into the process. Minimising solvent use (and thus solvent waste) will be further discussed in Section 3.10. Recovered wax sold for use in lubricant production, candle wax, or other unrelated products (Valizadehderakhshan et al., 2021).

3.6. Upstream Processing

After undergoing two consecutive cold ethanol extractions, the extract will need to be passed through a filtering system to remove the waste fraction - or solid 'cake' made up of waxes, chlorophyll, plant matter, lipids, and other undesirable components. The extract can then pass through to be decarboxylated, while the waste fraction can be disposed of, sold, or repurposed as mentioned at the end of Section 3.5.4.

Filtering plays a vital role in ensuring the removal of solid particles which would otherwise negatively impact the quality of the final product and the efficiency of further processing units. Thus, before the extract can proceed through the proposed process, it must pass through some sort of filtering system.

For small, batch filtering systems Nutsche filters or Buchner funnels might suffice, but for cannabis extracts demanding great purity and efficiency of production, more meticulous filtering systems are demanded, often requiring multiple steps. For larger batches, automated plate and frame filtering systems are often recommended, often incorporating activated carbon into the system to facilitate chlorophyll removal. For this particular process, however, the use

of cold ethanol extraction results in the majority of the chlorophyll being solidified and removed as part of the waste ‘cake’ stream, rendering this unnecessary (Filtrox AG, 2019; López-Olmos *et al.*, 2022; ErtelAlsop, 2023).

Plate and frame filters generally suffice for the filtration of similar processes if a series of vertical filters are paired with pressurised gas (López-Olmos *et al.*, 2022). Even more ideally, this process would be automated. The fewest number of filters possible should be used to remove the solid cannabis plant residues whilst achieving the required purity, as López-Olmos (2022) asserts that multi-step processes tend to decrease product yield due to compound losses, and thus these processes should be kept as short and simple as possible without compromising the quality of the product.

Aside from the series of plate and frame filters, centrifuges, other rotary filters, and continuous moving simulated bed processes have also been effectively used in cannabis extract purification processes (López-Olmos *et al.*, 2022). In Table 7, some potential filtration methods are described.

Table 7: List of additional potential filtration methods (Towler and Sinnott, 2009).

Filtration Method	Description
Rotary Drum Filters	• Generally vacuum operated. • They can manage large throughputs and are widely used for free-filtering slurries. • Filters consist of a hollow drum with a filter medium fitted around it. The drum is partly submerged in a slurry trough, resulting in filtrate rising through the filter medium, drawn upwards by the pressure differential caused by the vacuum within the drum. • Wash water is sprayed onto the surface of the drum, which is multi-compartmental in nature to avoid mixing between the wash water and the filtrate. • Continuous operation.
Disc Filters	• Can be either pressure- or vacuum-operated. • Similar to rotary filters in general principle, but using thin discs mounted to a shaft rather than a drum, given a larger filtering area per given floor area. • Application is limited due to their incongruity with pre-coating and wash water.
Belt Filters	• Vacuum operated. • A reinforced rubber belt with drainage holes lining its centre passes over a stationary suction box which draws the filtrate into it via a vacuum. • Slurry and wash water can be sprayed onto the top of the belt.
Centrifugal Filters	• Centrifugal force drives the filtrate through the filter cake. • Classified into two classes: 1. Sedimentation centrifuges: separation depends on variations in density between the solid and liquid phases. 2. Filtration centrifuges: the centrifuge basket has porous walls which filter out the liquid, leaving a solid cake.

A series of multiple filters is generally advised regardless of the chosen filtration mechanism to maximise filtrate purity. Cold extraction aids in the efficacy of cannabis extract filtration by solidifying many of the undesirable components. This lessens the rigour required during the filtration process, providing a mostly solid mass to be separated from the extract instead of a series of smaller particles, which would inevitably be more difficult to separate while in liquid form. (López-Olmos *et al.*, 2022).

To determine the most suitable filtration method for separating crude oil from the plant mass, a quantitative analysis was conducted on three commonly used techniques: plate and frame filtration, centrifugal filtration, and vacuum filtration. Considerations including recovery,

power utilization, cost estimations, technological complexity, cleanability, and processing time, and were compared across the three methods.

Plate and frame filters are the most cost-effective and efficient choice. They manage to offer respectable recovery rates with comparatively low power consumption, capital and operating costs, as well as simpler maintenance and cleaning compared to centrifuges and vacuum filters. Centrifuges, due to the additional energy and operational complexity, make them less than ideal – they also tend to produce wetter filter cakes than plate and frame filters, risking additional loss of the liquid stream. Vacuum filters offer excellent performance but do not compare to plate and frame filters in terms of energy efficiency and processing times. In addition, plate and frame filters are better suited to more viscous, 'sludgy' liquids than its competitors, which makes it better equipped to handle crude oil. (Longzhong Machinery, 2015; Latham International, 2020; Hawach Scientific, 2023)

Thus, for the sake of the proposed process, plate and frame filters will be used, operating under pressure, consisting of a series of three screens of decreasing pore size. After filtration, the extract will undergo decarboxylation. The importance of decarboxylation in cannabis processing lies in the fact that neutral cannabinoids such as CBD and THC are present only in small concentrations within the plant. The concept of neutral and acidic forms of cannabinoids has been introduced previously, in Section 3.5.4, but it will be explored in considerably more detail in the section to follow. Within the cannabis buds, cannabinoids exist in their acidic - or carboxylic - forms; in practice, this means CBD exists as cannabidiolic acid (CBDA) and THC exists as tetrahydrocannabinolic acid (THCA), both of which are derived from CBGA (Valizadehderakhshan *et al.*, 2021). (Figure 10)

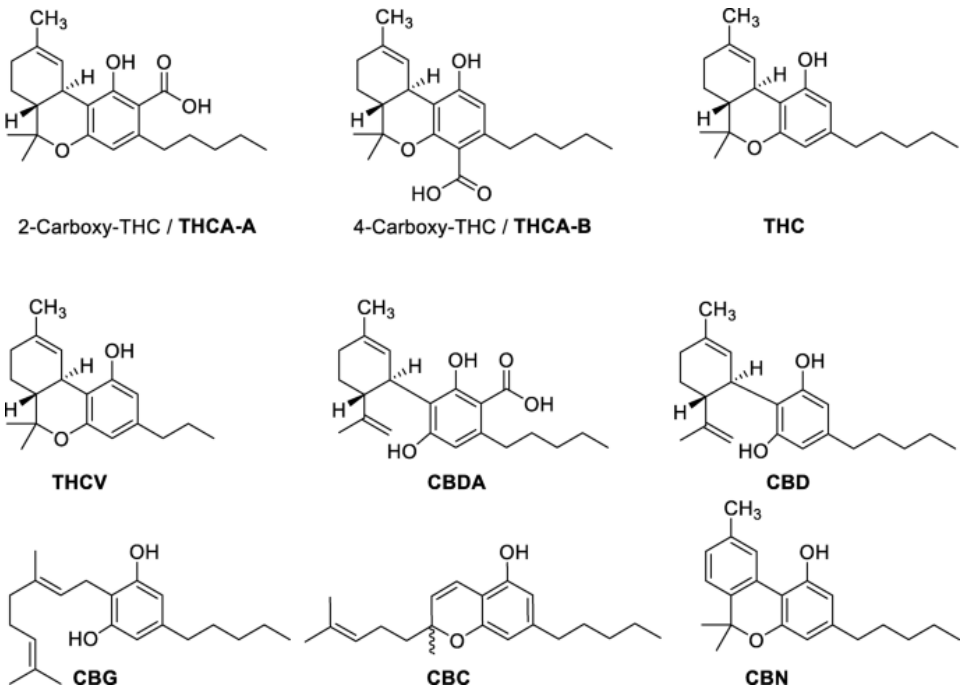


Figure 10: Structures of different cannabinoids and their acidic precursors (Tahir et al., 2021).

In fact, in fresh cannabis plants, 95 % of the THC and CBD that could potentially be extracted exist in the forms of THCA and CBDA, respectively – however this varies greatly, and many of the commercial forms of cannabis have comparatively greater amounts of THC and CBD, with smaller masses of their acidic counterparts (Tahir *et al.*, 2021). Decarboxylation (or the removal of CO_2) is thereby crucial to converting these acids into their neutral forms to produce CBD oil (Tahir et al., 2021; Valizadehderakhshan et al, 2021). (Figure 11)

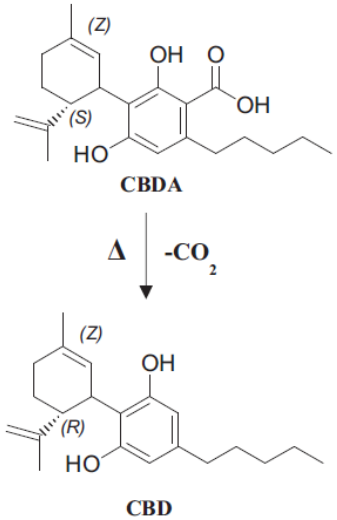


Figure 11: Chemical change involved in the decarboxylation of CBDA to CBD (Brighenti et al., 2017).

In the cases of small-scale processes, some lesser-known pre-treatment alternatives also exist, such as high-pressure homogenization, a fast and efficient pre-treatment, as well as ultrasonication, which is also incredibly fast, but costly (Lazarjani *et al.*, 2021). Generally, however, the simple, cheap, and effective method of a decarboxylation oven is applied, though really all that is required is a temperature rise high enough to facilitate decarboxylation but not so high as to degrade the resulting neutral cannabinoids in any significant way.

Wang and associates (2016) used a vacuum oven to investigate the decarboxylation conditions for *cannabis sativa* extracts at temperatures of 80 °C, 95 °C, 110 °C, 130 °C, and 145 °C for different time periods. Their findings indicated that a temperature of 130 °C, for a decarboxylation period of 30 minutes, yielded the greatest concentrations of both CBD and THC, with both cannabinoids displaying slightly decreased concentrations at 145 °C, where before this point concentrations of both compounds had been increasing with decarboxylation temperatures.

Hence, 130 °C has been taken as the optimal decarboxylation temperature for maximising both CBD and THC concentrations - which for the proposed process is ideal, as THC will later be converted into CBD and thus maximising the concentrations of both compounds is desirable. The relevant concentrations for Wang's experiment for temperatures 130 °C and 145 °C after 30 minutes of decarboxylation can be found in Table 8.

Table 8: CBD and THC concentrations pre- and post-decarboxylation (Wang et al., 2016).

Drying Time (min)	CBD (mM)	THC (mM)
130 °C		
0	0.153	0.284
30	0.749	0.639
145 °C		
0	0.153	0.284
30	0.748	0.632

Decarboxylation, on average, results in a 12.3 % loss of mass (Troiani, 2017). Drying generally results in a 10 % loss of mass (Leafly, 2023). Thus, if fresh cannabis is used, a collective loss of 22.3 % might be expected - however, if we assume that the average decarboxylation occurs when the cannabis has been dried to 10 % moisture, as is common practice, this implies 10 %

of this value being water (20 % of the total mass) and 2.3 % being the carbon dioxide let off during decarboxylation (Sartorius, 2023).

Buffers are often added before entry into the reactor to achieve optimum pH for the process. A major obstacle for this sort of theoretical process, however, is the fact that optimal solvent concentrations and pH's would generally need to be found experimentally. As the required enzyme for the proposed process is entirely unknown, parameters such as solvent concentrations and buffer ratios - and the associated mechanisms - cannot be functionally included in the simulation. However, they are still worth noting and should be kept in mind for further research or any attempts at practical application.

For the sake of the proposed process, we will assume no buffers will be added. The density of the oil will be greater without buffers, allowing it to move slowly through the decarboxylation unit, which will be designed as a cylindrical heat exchanger, and thus adhere to the continuous process flow rather than requiring set batch operation.

3.7. Bioreactor

3.7.1. Batch-Wise Versus Continuous

The capacity of enzymatic bioreactors to host biochemical transformations and reactions has been thoroughly documented throughout the last several decades, and their use is widespread across the food processing, pharmaceutical processing, and biosensor industries, amongst others (Zhang & Xing, 2011).

Bioreactors are particularly useful to produce small-molecule active pharmaceutical ingredients (APIs). Their production is complex, and their production is stringently regulated, demanding precision and high-quality products. Thus the high selectivity, limited by-products, and mild conditions make enzymes a popular choice (Lindeque and Woodley, 2019).

Enzymatic bioreactors are comprised of a vessel or series of vessels in which an enzyme catalyst facilitates the conversion of reactants entering the process, with the fine-tuning operational conditions to facilitate the optimal transference of heat, mass, and momentum between phases being paramount to the design of an efficient system.

Whilst enzymes are often sensitive to variation in parameters, an optimally designed biocatalyst process should be stable, environmentally friendly, able to withstand harsh reaction conditions, and economical. Some methods to achieve this, as well as an overview of potential design choices, will be provided in the section below.

Bioreactors are capable of operating batch-wise or continuously. Batch processes are commonly used because of their fast implementation and flexible production planning, as well as their improved product traceability – however, they also require significant investment in chemical storage facilities, solvents, and process intermediates. Scalability also often proves difficult, with heat and mass transfer proving to be a challenge (Basso and Serban, 2019).

Batch processes also face several other drawbacks when it comes to enzymatic processes. Dissolved enzymes are relatively unstable and prove difficult to recover for reuse, thus resulting in elevated running costs and lowered productivity levels; additional expenses are also incurred due to the required investment in chemical storage facilities, solvents, and process intermediates (Ó'Fágáin, 2003; Basso and Serban, 2019). Whilst process optimization and careful enzyme stabilization can mitigate many of these concerns, they remain a disadvantage of batch systems (Guisan, 2006). Batch operations also suffer from prolonged start-up and shutdown periods that repeat with each reaction (Lindeque and Woodley, 2019).

While biocatalysis is well-known to increase sustainability and reaction specificity, low productivity is commonly associated with them – the combination of biocatalysis and flow facilities has been prescribed as a means of mitigating this issue, as opposed to batch operations (Basso and Serban, 2019).

Flow biocatalysis has been found to increase productivity through improved parameter control and heightened mass transfer, as well as minimising waste production and power consumption. By avoiding the stirring commonly associated with batch reactors, the lifespan of the enzyme is also prolonged, reducing costs associated with their replacement (Basso and Serban, 2019).

Continuous flow also facilitates automation and reduces the need for manual interference, as well as providing easier scale-up alternatives in the form of connecting continuous reactors in series or parallel, thus avoiding the heat and mass transfer issues associated with traditional scale-up methods. They are also generally safer (even under intense conditions that could not be safety applied in batch systems) and increase sustainability and cost-efficiency through feedback loops and process intensification (i.e. increasing sustainability and economy by minimising waste, power usage, and equipment size) (Basso and Serban, 2019).

Despite these advantages, however, many industries remain reluctant to adopt continuous bioreactors due to the additional expenses associated with amending conventional procedures, as well as managing mass transfer limitations and potential yield losses during the immobilisation process (Illanes & Altamirano, 2008). However, with continuous processes currently gaining traction due to the emergence of new methods centred on the optimization of biocatalytic stability through innovative enzyme immobilisation strategies, this hesitance seems set to dissipate in the future, opening the door for further incorporation of continuous bioreactor applications (D'Souza 1999).

Common advantages associated with flow biocatalysis have been listed below (Lindeque and Woodley, 2019; Santi *et al.*, 2021).

- Reduced inhibition
- Improved total turnover
- Shortened reaction times
- Improved enzyme stability
- Enables cascade reactions
- Enables easy co-factor regeneration
- Facilitates multiphasic reactions
- Enables chemoenzymatic processes
- Increases sustainability
- Enables easy automation and incorporation of process analytical technologies (PAT), thus reducing downtime and aiding in ensuring product quality
- Reduced variability
- Enabled in-line purification

While biocatalysis offers many advantages (as listed above), some notable challenges should be considered. In the pharmaceutical industry, regulatory requirements present a hurdle, particularly for continuous processes. Maintaining proper batch records can be difficult, as it is essential to define the start and end of each batch to meet quality and regulatory standards. If product quality does not meet specifications, the entire batch may be lost due to the lack of process flexibility, increasing the risk of waste if careful precautions are not taken (Olanrewaju, 2023).

Additionally, the supporting systems required to ensure compliance, such as validation processes, quality control protocols, and documentation, can become more complex and

tedious in continuous processes. Additionally, scaling biocatalytic processes to industrial levels while maintaining enzyme activity, stability, and reproducibility can prove challenging, which could limit the practical application of biocatalysis in certain scenarios (Tufvesson *et al.*, 2010; Hauer, 2020).

So, while biocatalysis has been selected for the processes (and, indeed, is necessitated by the topic) and will be incorporated in the form of a continuous process based on the advantages explored in this section, it is also important to acknowledge the difficulties biocatalysis (specifically flow biocatalysis) present, alongside its advantages.

3.7.2. Reactor Types

Catalysts play a pivotal role in many chemical reactions. Many reactors utilize enzymes to increase the reaction rate and selectivity of the reaction occurring inside it, thus reducing energy consumption and improving the yield of the desired product (Fogler, 2016).

There are four basic types of reactors – batch reactors, CSTRs (continuous stirred-tank reactors), PFRs (plug flow reactors), and semi-batch reactors, though specialised variations and innovative alternative designs also exist. For enzymatic processes specifically, some commonly used variants include MRs (membrane reactors), PBRs (packed bed reactors), continuous flow STRs, and FBRs (fluidised bed reactors) (Zhang and Xing, 2011; Fogler, 2016).

In batch operation mode, the enzyme and the substrate molecules will have the same residence time within the reactor and, in the ideal case, will have a homogeneous continuous phase of uniform composition and temperature. In the continuous operation mode, however, the substrate is continuously fed into the reactor whilst products are withdrawn, maintaining constant solution volume (Zhang and Xing, 2011).

Several factors should be considered when deciding on the best type of reactor for a given process, some of which will be outlined in Table 9. Due to the theoretical nature of the reaction, many of the considerations often made regarding reactor choice will not prove especially helpful due to the lack of information about the reactor requirements, but it is still worthy of deliberation.

Table 9: Factors in choosing a reactor type (Zhang and Xing, 2011).

Consideration	Explanation
The nature of the enzyme	This includes the kinetics of the reaction, the chemical and physical properties of any immobilisation support used (e.g.: whether it is fibrous, particulate, or membranous), its compressibility, density, particle size, and regenerability.
Physical properties of the substrates	Any reactor can be used in the case of soluble substrates, but reactors operated in batch mode are more suitable for substrates of low solubilities or solutions of low substrate concentrations.
Requirements of reaction operation	The scale of operation, temperature and pH control, addition and removal of gases, and the stability of substrate, products, and the enzymes themselves must all be considered. For example, FBR operates well for reactions where a gas supply is required STR allows frequent adjustment of pH.
Enzyme stability	Loss of enzyme activity in reactors can be caused by denaturisation and the disruption or disconnection of the enzyme carrier. CSTRs are most likely to result in this kind of activity loss and thus require greater enzyme stability than the other types mentioned.

Another consideration would be the probability of multienzyme reactions – this is certainly a possibility for the proposed process, especially if one considers the probability of remediation of THC into CBD via their shared predecessor CBG. This might require two enzymes – one, a theoretical enzyme, to reverse the reaction performed by THCA synthase within the plant (i.e. to convert THCA back into CBGA) and another, CBDA synthase, to convert CBDA into CBGA. However, due to the assumption of a singular theoretical enzyme, we will assume a singular reaction for the sake of the proposed process.

Below, several potential reactor types with the potential to operate using enzymes will be considered in more depth:

- Fluidised bed reactors
- Membrane reactors
- Plug flow reactors
- Stirred tank reactors

Fluidised-Bed Reactors

Fluidised-bed reactors involve a bed of enzymes, fluidized using the upward movement of the substrate stream. Immobilisation is crucial in restricting the enzymes and binding them in small particles that move with the fluid successfully achieves this (Zhang and Xing, 2011).

FBRs are a combination of CSTRs and PBRs, behaving as an intermediate between these reactors. Depending on the design, it can behave more similarly to either a CSTR or PBR. FBRs are commonly applied when, for example, the substrate is colloidal, significant heat is generated, gas supply is required, or significant pH changes are involved (Zhang and Xing, 2011). None of this is particularly relevant to the proposed process.

FBRs require a large power input, risk significant loss of catalyst due to high flow rates and have been found to cause destruction or decomposition of the enzymes; thus, fluidised bed reactors will not be applied in the proposed process.

Membrane Reactors

Membrane reactors (MRs) rely on a semipermeable membrane that allows the product stream to pass through freely whilst retaining the enzyme molecules. It can be used in batch or continuous mode and allows easy separation of the product stream and the enzymes.

If substrate molecules can pass through the membrane, they can be introduced on either side of the membrane, but otherwise will need to be added into the same compartment as the enzyme – this will make effective separation significantly more difficult. Due to the unknown nature of the enzyme, however, it is impossible to determine whether the size of the reactant stream molecules will be small enough comparative to the unknown enzyme to facilitate a membrane through which the substrate can freely pass.

The kinetics of MRs are similar to CSTRs when used in batch mode, or BSTRs when used in batch mode. MRs are commonly used with free enzymes, though deviations can be made in the case where this might not be suitable. They are also commonly used on a small scale, especially in the cases of multienzyme systems, where significant regeneration of coenzymes is required, for use alongside labile enzymes (i.e. enzymes that are more stable at 0 °C than at room temperature), or for use in biphasic reactions. Disadvantages of MRs are the risk of

membrane fouling and product inhibition, as well as the costs of the membrane and the necessity of regular changes of the membrane (Zhang and Xing, 2011).

Based on the assumption of a simple single-enzyme, single-phase reaction, without the need for coenzymes or the use of labile enzymes, MRs do not hold much relevance to the process. Thus, a membrane reactor will not be applied.

Stirred-Tank Reactors

Stirred-tank reactors (STRs) are the most common type of enzyme reactors; substrates and enzymes are fed into a tank fitted with baffles that stir the mixture, resulting in even concentrations throughout the reactor.

Control of temperature and pH are necessary in STRs to ensure the optimal conditions for the enzymes to function effectively. The enzyme and the substrate are mixed inside the reactor, minimising diffusion resistance – thus, STRs facilitate the treatment of high-viscosity substrates well, as well as those with poor solubility. However, STRs also have relatively low reaction efficiency and introduce the need to separate the enzymes from the product stream post-reaction; additionally, high power input is required for stirring (Zhang and Xing, 2011).

STRs can be operated continuously or in back mode in the form of a CSTR or a BSTR, respectively. Materials are continuously fed into and withdrawn from CSTRs, keeping the working volume of the reactor constant. Thus, enzymes must either be fed continuously into the reactor to compensate for the enzymes being lost in the product stream (requiring large enzyme masses and thus increased enzyme expenses) or retained inside the reactor by immobilisation or a semi-permeable membrane.

The reaction rate will remain constant with respect to time, though because of the constant removal and addition of substrate, the product will always contain some substrate – thus, full conversion is impossible in CSTRs (Lindeque and Woodley, 2019). This is not desirable, as the complete conversion from THC into CBD is the primary purpose of the proposed process – thus CSTRs would not be ideal.

BSTRs differ from CSTRs in that substrate and enzyme are fed into the reactor at the beginning of the reaction and no material is removed until it is halted. In BSTRs, the substrate is consumed rapidly during the initial stage of the reaction, but the reaction rate slows as the substrate

concentration decreases. However, if given enough time in the reactor and ensuring favourable equilibrium conditions, complete conversion can be achieved, unlike in the case of CSTRs (Lindeque and Woodley, 2019).

BSTRs are commonly applied in biocatalytic processes and thus are worthy of consideration for application in a THC remediation process – however, due to the continuous nature of the proposed process, a batch-wise reactor would not be ideal. This would disrupt the process flow of an otherwise continuous process.

Thus, due to its continuous nature, high substrate activation, and successful mitigation of inhibition and reversible reactions, a PBR has been selected for the proposed process. Due to the assumption that the theoretical enzyme will operate under temperatures similar to those facilitating the activity of enzymes like THCA synthase and CBDA synthase within the plant, the reaction will be conducted at room temperature. Additionally, due to the uncertainty regarding optimal pH due to the theoretical nature of the reaction, the addition of buffers will be disregarded for the sake of the review.

Plug Flow Reactors

Plug flow reactors are tubular in shape, with the mixture continuously flowing through its length. If a heterogeneous catalyst in used – for example, if the enzyme is immobilised onto a fixed surface – the PFR must have a packed bed (i.e. a PBR). Particle diameter, packing porosity, and catalyst loading must all be considered in this case (Lindeque and Woodley, 2019). If a homogenous catalyst is used, a method of either separating the product or limiting the movement of the enzyme to the confines of the reactor must be incorporated into the design.

PBRs are packed with enzymes, with the substrate stream entering at one end of a cylindrical reactor and the product stream exiting from the other end. Biocatalysts are often immobilized onto the reactor wall or carrier particles, which are then packed into a cylindrical tube (Lindeque and Woodley, 2019).

To produce an ideal plug flow, a turbulent flow is preferable to a laminar flow, resulting in improved mixing and heat transfer, as well as reduced back-mixing. High concentrations of enzymes can be used in PBRs, with the enzyme immobilized in the reactor bed. The product can be continuously collected as effluent from the reactor.

Advantageously, any required degree of reaction can be achieved using an ideal PBR with a suitable length; therefore, if the reactor is long enough, the substrate could theoretically be fully converted. PBRs have also been found to outperform CSTRs and BSTRs in cases involving product inhibition and reversible reactions, as well as boasting superior substrate activation capacity (Zhang and Xing, 2011; Lindeque and Woodley, 2019).

PBRs also have several disadvantages, however. The lack of a stirring device results in variation of the stream in the longitudinal direction, as well as the radial direction to a lesser extent. They also do not allow for easy pH- or temperature control, immobilized enzymes are easily fouled by precipitates, and unless care is taken, PBRs run the risk of increased back pressure, leading to particle deformation and restricted flow (Zhang and Xing, 2011).

The proposed process would be assumed to operate at room temperature and at a constant, close-to-neutral pH without the production of precipitates, with the theoretical enzyme operating similarly to those found within the plant. Because of this, these disadvantages should not prove particularly problematic.

For the proposed process, either a PFR or PBR will be selected, depending on the immobilisation method chosen – this will be discussed in Section 3.7.3.

3.7.3. Enzyme Immobilisation

Enzyme immobilisation is the process of attaching or confining enzymes to a solid support or matrix. In 1916, it was discovered that invertase retained its enzymatic activity whilst adsorbed onto solids like charcoal or aluminium hydroxide at the base of a reaction vessel and this paved the way for the development of new and innovative enzyme immobilisation methods. Since then, hundreds of enzymes have been immobilized, and over a dozen such immobilized enzymes—including *penicillin G acylase*, invertase, lipases, and proteases—have been harnessed as catalysts in large-scale processes (Homaei *et al.*, 2013).

Unlike batch-wise reactors, continuous processes are inherently associated with such immobilisation methods because continuous bioreactor operations necessitate elevated levels of enzyme stability (Illanes & Altamirano, 2008). Thus, in the case of PFRs or PBRs, immobilisation of the enzyme catalyst is required.

While free enzymes are large enough to fall into the category of heterogenous catalysts, they behave much more similarly to homogenous catalysts thanks to their soluble nature. This is not the case for immobilised enzymes, however, which are truly heterogenous in nature, with the particle size of the catalyst, as a whole, always eclipsing the size of the enzymes themselves (Haan, 2015).

Aside from stability, immobilized enzymes offer many other advantages over free enzymes, some of which can be found below. However, immobilisation can also potentially result in lower activity levels compared to free enzymes and greater difficulties in facilitating substrate-enzyme contact (Homaei et al., 2013).

The benefits of enzyme immobilisation have been listed below (Zhang and Xing, 2011; Homaei *et al.*, 2013; Basso and Serban, 2019).

- Free enzymes retain some activity post-reaction, which cannot be recovered for reuse. Immobilised enzymes, on the other hand, can be reused as they typically are large enough to be retained in the reactor. This decreases the cost of enzyme replacement.
- Free enzymes contaminate the product unless they are removed from the stream, adding to the process separation costs. Immobilised enzymes eliminate this issue, as they can be easily retained within the reactor.
- Allows for selectively altered chemical or physical properties, often making immobilised enzymes more stable than free enzymes.
- Can achieve high volumetric productivity and decreased capital costs, as well as saving in enzymes, overhead costs, and labour.
- Increased stability against temperature, pH, and organic solvents.
- Improved reaction rates.
- Allows access to a wider array of bioreactor types.

Immobilisation does come with some potential issues, however. The potential financial benefits of immobilised enzymes rely on the reaction kinetics and specificity of the enzymes, as well as their reuse cycles. Whilst it is well documented that immobilisation of enzymes often increases the overall profitability of enzyme-catalysed processes (Zhang and Xing, 2011; Homaei *et al.*, 2013), the main issue with upscaling such processes stems from considerations like resin expenses, downstream processing costs, reactor cost, disposal costs of immobilized enzyme post-use, and the carriers' regeneration expenses (Basso and Serban, 2019).

Enzyme immobilisation can use either reversible or irreversible interactions. Irreversible immobilisation techniques offer higher stability and mitigate enzyme leaching in flow reactors, lessening the need for enzyme replacement by lengthening their lifespans. Unfortunately, irreversible methods of immobilisation also cause difficulties when it comes to the recyclability of the enzymes when activity falls (Basso and Serban, 2019).

Different methods of immobilisation yield different advantages and disadvantages for the process, as explored in Table 10; a summary of each method can also be found in Table 10. When selecting a method of immobilisation, one should consider how the enzyme interacts with the structure supporting it. These considerations include:

- The cost of the enzyme and its support structure.
- Stability of the enzyme and the speed with which its activity falls when paired with different supports.
- The lifetime of the enzyme and the regenerability of its carrier.
- The way the support (i.e., its density, particle size, shape, porosity, pore size, etc.) will affect the activity and kinetics of the enzyme.

Table 10: Overview of enzyme immobilisation methods (Zhang and Xing, 2011; Britton, Majumdar and Weiss, 2018; Blanco-Llamero, García-García and Señoráns, 2021).

Immobilisation method	Explanation	Examples
Adsorption	Salt bridge, van der Waals, hydrophobic, and hydrogen bonding interactions are harnessed to facilitate the adsorption of the biocatalyst onto the support or carrier.	Cellulose; coconut fibres; kaolin; micro- and mesoporous materials.
Covalent immobilisation	Immobilisation occurs due to covalent bond formation between non-essential amino acids present on the enzymes' surface and the solid support structure. A variation of covalent immobilisation also exists in which enzymes are cross-linked to each other, forming a network of enzymes that can exist either with or without a support structure.	Peptide-modified surfaces for controlled protein orientation; silica; epoxides; nanowires; chitosan; Sepharose; cyanogen bromine-infused agarose; glutaraldehyde-modified glass surfaces.
Cross-linked enzyme aggregates	Cross-linked enzyme aggregates (CLEAs) are insoluble, cross-linked networks of physical enzyme aggregates. A single type of enzyme is generally used, but methods of combining different enzymes into aggregates have also been developed (combi-CLEA's).	Many kinds of lyases, hydrolases, and oxidoreductases.
Entrapment immobilisation	The biocatalyst is caged within a network of polymers forming gels or fibres, linked using either covalent or non-covalent interactions. An immobilisation support is required for this immobilisation method, whilst covalent bonds may or may not be formed between the enzymes and the matrix.	Nanowires; chitosan; mesoporous silica; hybrids between alginate and gelatin; calix[n]arene polymers; κ-carrageenan.

Table 11: Advantages and disadvantages of different enzyme immobilisation methods (Zhang and Xing, 2011; Zhao et al., 2015; Blanco-Llamero, García-García and Señoráns, 2021; Sampaio et al., 2022).

Advantages	Disadvantages
Adsorption	
❖ Easy and inexpensive to achieve. ❖ Avoids enzyme denaturation by minimising distortion to the protein. ❖ Enzymes can be separated and purified even whilst being immobilised. ❖ Removal and replacement of enzymes is possible due to adsorption immobilisation's reversible nature. ❖ In the case of adsorption to matrices (i.e., hydrogels, polymers, etc.) rather than solid supports, high enzyme loading is also possible.	❖ Desorption is common, limiting efficiency. ❖ Sensitive to temperature, pH, and ionic strength, shortening enzyme lifespans. ❖ Weaker interactive forces result in lower enzyme loading.
Covalent immobilisation	
❖ Increased enzyme lifetime because of reduced leaching. ❖ Strong binding forces decrease enzyme losses.	❖ Regeneration of enzymes is impossible. ❖ Covalent binding through multiple sites on the enzyme may diminish enzyme activity because of alterations to or distortion of the active sites. ❖ Costly reagents increase the expenses involved in immobilising the enzymes. ❖ Low enzyme loading.
Cross-linked enzyme aggregates	
❖ Simple to prepare, especially due to the opportunity to use crude enzyme extracts without jeopardising efficacy. ❖ Does not necessarily require a support. ❖ Very stable ❖ Inexpensive ❖ Reusable ❖ Applicable to almost all enzymes	❖ Depending on the enzyme's amino terminals, intense intermolecular cross-linking can be difficult, resulting in low mechanical strength. ❖ Limited to reactors that do not require high mechanical resistance. o This can be mitigated by binding the CLEAs to solid supports with a higher mechanic resistance, such as silica. ❖ Diffusion may be hampered on the path for the substrate to reach the enzyme molecules.
Entrapment immobilisation	
❖ Because there is no chemical modification involved, the inherent properties of the enzyme remain intact. ❖ A broad variety of enzymes can be immobilised with this method as it does not rely on chemical reactions. ❖ Many of the issues posed by this method (seen under the disadvantage column) can be mitigated through optimisation and design alterations. ❖ Many kinds of matrices can be used for encapsulating the enzymes, allowing flexibility in optimising this method for specific processes. Fewer limitations to diffusion than some other methods.	❖ Gel formation can deactivate enzymes. ❖ Substrates with high molecular weights face difficulties in diffusing through the matrices. ❖ Substrates face more obstacles while diffusing toward the active site, though decreasing the particle sizes of the capsules or matrices can help mitigate this. ❖ Enzymes are at risk of leaking into the solution due to breakages in the capsules or gel, though reducing the solid matrices' pore size or the molecular weight limit of the membrane can help mitigate this. ❖ Lack of environmental control within matrices diminishes enzyme stability and activity, though this can be mitigated by altering process parameters, altering the relevant matrices, and reducing capsule/particle sizes.

Alongside the different immobilisation methods, several kinds of immobilisation supports exist, each with its own advantages and disadvantages. These supports must also be considered alongside the immobilisation techniques to formulate an optimal method of immobilisation. Inorganic supports, synthetic polymers, biopolymers, hydrogels, and membranes will all be considered.

Solid enzyme immobilisation supports defend the enzymes from reaction conditions, enhancing their reusability. They should always be nontoxic and inert, as well as safe for the environment. An array of inorganic solids can be used as solid support structures, examples of which include silica, alumina, titanium, and zeolites, where silica supports are heralded as the best matrices for industrial and research purposes (Homaei *et al.*, 2013; Al-Maqdi *et al.*, 2021).

Silica gels boast greater mechanical strength, excellent overall surface area, and increased flexibility under different operating pressures when compared to soft gels, and can be chemically modified with many different functional groups to allow attachment of different ligands; it is also chemically inert, and therefore environmentally- and solvent friendly (Homaei *et al.*, 2013). Commonly, they are immobilised using simple adsorption.

Polymers can also be used as immobilisation supports, including synthetic polymers and biopolymers.

Synthetic polymers such as acrylic resins and microporous copolymers can be used as well. They are mechanically- and chemically stable over a wide pH range – however, as the proposed process will occur at pHs close to neutral, this is not particularly relevant. Covalent attachment to acrylic resins is the most common industrial use of this kind of support. However, limitations on substrate diffusion prove an issue for synthetic polymers, especially in kinetically controlled processes (Homaei *et al.*, 2013; Lyu *et al.*, 2021).

Most biopolymers used for enzyme immobilisation are matrices of water-insoluble polysaccharides like chitosan, agarose, carrageenans, starch, and cellulose, forming inert aqueous gels. These polymers have high mechanical strength and, due to their ease of activation, can bind to enzymes reversibly or irreversibly, showing considerable versatility in application (Homaei *et al.*, 2013; Lyu *et al.*, 2021).

An alternative kind of gel – hydrogels – can also be applied. They can be natural, synthetic, or a variant called cryogels, which are formed via a freeze-thaw method. This is more commonly

used for whole cells rather than enzymes, which are small enough to diffuse out of the gel matrix (Homaei *et al.*, 2013).

Membranes can also be used to immobilise enzymes. Confining enzymes within a membrane is a specific form of entrapment, in which enzymes in an aqueous solution are contained within microscopic hollow spheres located within a porous, semi-permeable membrane (Zhang and Xing, 2011). The enzyme is retained within the membrane, effectively combining catalysis and separation into a single unit.

Because of the rapid product removal, these membranes can reduce product inhibition and avoid side-reactions occurring, increasing conversion efficiency in cases where these issues may otherwise be problematic; membrane immobilisation also increases stability and facilitates reuse (Luo *et al.*, 2020).

Adsorption and affinity methods are often used to facilitate reuse through easy elution and reloading; when enzyme leakages occur in the cases of adsorption, these enzymes can simply be recaptured by the ligands found on the membrane, allowing for an inexpensive immobilisation method (adsorption) that does not result in the enzyme losses associated with other supports to which enzymes are commonly adsorbed (Zhang and Xing, 2011).

However, in the case of biocatalytic membranes, there is the additional issue of fouling, which interferes with the separation process, as well as enzyme inactivation. However, this can often be mitigated by altering the membrane material to reduce adsorption, increasing pore size to reduce blocking, or changing the reaction pathway. When a membrane has been fouled to the point of a noticeable drop in process performance, it is recommended to clean the membrane to recovery enzyme activity. However, due to most cleaning agents negatively affecting enzyme activity and configuration, reloading fresh enzymes onto the membrane has become a common alternative (Zhao *et al.*, 2015).

Although the enzyme discussed in this project is theoretical and the choice of immobilization method may ultimately depend on available technology and practical constraints, it is still useful to consider the potential advantages and disadvantages of different methods. Immobilization plays a critical role in enzyme stability, reusability, and overall process efficiency, and this analysis is intended to provide insight into the merits and limitations of existing immobilization techniques, with the understanding that the final selection would be based on the enzyme's specific properties and the technological options available.

Thus, for the sake of the proposed process, cross-linked enzyme aggregates (CLEAs) will be used. They can be loaded onto the walls of a PFR using a silica base and reused for several cycles without significant activity loss. The reusability, preparative simplicity, and stability make this an inexpensive and effective option. The lower mechanical strength of the CLEAs also will not prove an issue in a plug flow reactor, as the lack of any vigorous mechanical mixing means it does not require enzymes of particularly high mechanical resistance. Therefore, CLEAs are a sensible choice.

3.7.4. Enzyme Theory

Enzyme catalysts are essential in many biochemical reactions and their use has significant implications in the fields of medicine, biotechnology, and food processing. They are specific and highly efficient, with reaction rates that are often millions of times faster than non-catalysed reactions, and they are capable of producing specific products with high yields and purities (Basso and Serban, 2019).

Enzymes are composed of L-amino acids held together by covalent bonds in an intricate, coiled arrangement. Only a few specific amino acids will directly bind to substrate molecules; these make up the enzyme's active site, which will be characteristic of the particular type of enzyme that it is (Haan, 2015).

Catalysis is achieved by lowering the activation energy required for the reaction to occur by stabilizing the transition state of the reaction. Enzymes achieve this by binding to the substrate via the active site and manoeuvring it in a specific manner to facilitate the reaction (Heinzle, Biwer and Cooney, 2007). In doing this, an alternative reaction mechanism is provided, providing a transition state with lower free energy.

Three phases occur in biocatalysis – the enzyme will first combine with substrate molecules. In the second phase, the enzymes and substrate molecules reach a dynamic equilibrium that continues as long as fresh substrate molecules are available, with maximum enzyme activity being achieved. In the third stage of the reaction, the substrate concentration has fallen significantly and the rate of reaction falls asymptotically (Haan, 2015).

Enzyme activity can also be maximised via the optimisation of key parameters such as pH, temperature, and substrate concentration. Concentration and its effect on inhibition are discussed further in Table 12.

Four common types of inhibition are recognized, one of which is irreversible and three of which are reversible. A summary of the various kinds of inhibition can be found in Table 12.

Table 12: Overview of the different kinds of inhibition affecting enzyme-catalysed reactions (Haan, 2015; Yoshino and Murakami, 2015).

Competitive inhibition (reversible)	➢ Substrate molecules are prevented from binding with the enzyme active site by a structurally similar molecule – thus the molecules 'compete' for access to the enzymes. ➢ Inhibition can be reduced by increasing substrate concentration.
Non-competitive inhibition (reversible)	➢ An inhibitor molecule binds to an allosteric site (i.e. a site other than the active site), resulting in decreased enzyme activity. ➢ This cannot be reduced by varying substrate concentration.
Excess substrate inhibition (reversible)	➢ Two separate substrate molecules bind to the enzyme, one at the active site and one at an inhibitory, allosteric site. ➢ Thus, excess substrate inhibits the reaction rather than reaching steady-state equilibrium at the maximum reaction speed. An optimal substrate concentration must be thereby found which limits excess substrate.
Irreversible inhibition	➢ Inhibitory molecules combine with the enzymes irreversibly, chemically altering their structure and permanently reducing their catalytic activity.

Whether or not inhibition would occur in the context of the theoretical enzyme – and whether substrate concentration would affect this – cannot be known. Thus, in the case of the proposed process, the crude oil will enter the reactor post-extraction without the addition of buffers or dilutant solvents, operating under the assumption that no inhibition occurs. Naturally, if the theoretical enzyme were to be found or produced and these characteristics were to be discovered, such considerations would be essential before the development and implementation of the proposed process.

Enzymes are grouped into six categories, depending on the kind of reaction they catalyse. An overview of these categories can be found in Table 13.

Table 13: Categorisation of different kinds of enzymes (Haan, 2015).

Type of Catalyst	Catalysed Reaction
Oxidoreductases	Oxidation-reduction reactions, specifically those involving oxygenation.
Transferases	Transfer of groups such as aldehyde, ketone, or acyl between molecules.
Hydrolases	Acts on hydrolysable groups such as esters, anhydrides, amides, peptides, and glycosides.
Lyases	Addition of groups to double bonds or the reverse reaction.
Isomerases	Transfer of groups within molecules to yield isomers of the initial molecules.
Ligases	The formation of carbon-sulphur, carbon-nitrogen, or carbon-oxygen bonds.

Isomerases are a class of enzymes used in several industrial processes, such as the production of sugar, high-fructose corn syrup, and ethanol (Hilterhaus and Liese, 2012). They are also involved in the metabolism of many living organisms and are pivotal to many processes involving organic synthesis and drug discovery (Asano and Hölsch, 2012). These enzymes catalyse the conversion of one isomer into another without changing its overall chemical formula – in the proposed process, the theoretical enzyme will operate equivalently, transforming THC into its chemical isomer, CBD. Thus, our theoretical enzyme will be an isomerase.

There are several types of isomerases such as racemases, epimerases, and cis-trans isomerases. Racemases catalyse the conversion of one optical isomer into its mirror image, epimerases catalyse conversion between epimers (carbohydrates that have identical chemical formulas, but with variation in the position of the -OH group), and cis-trans isomerases, which catalyse the conversion of peptide bonds between cis- and trans isomeric variants (Asano and Hölsch, 2012; Ha and Bhagavan, 2023).

Additionally, it should be noted that although enzymes are large enough to be considered heterogeneous catalysts, they are generally classed as homogeneous due to their solubility. However, some immobilised enzymes may be considered heterogenous – such as when they are fixed on or within a solid surface (Homaei *et al.*, 2013).

It is also worth noting that many industrial enzymes are produced via the evolution of native enzymes to increase their efficiency and specificity – this is done by protein engineering, tailoring the enzymes to a specific industrial process. While it is likely that the theoretical enzyme for the proposed process will require this manner of modification, the precise mechanism for doing so falls outside of the scope of this review.

Many enzymes are not capable of operating alone, requiring the presence of cofactors to conduct the necessary catalysis. Much like the substrate molecules, these cofactors also undergo chemical alterations during the reaction and thus must be continuously converted back into their original state for the reaction to continue.

This regeneration of the cofactors can occasionally occur spontaneously by oxidation or hydrolysis if the cofactors are in aqueous, aerobic conditions through hydrolysis or oxidation reactions. Usually, however, it will require direct coupling with the oxidation of a high-energy substrate molecule. Examples of such cofactors include NAD+, NADP+, ATP, folic acid, and coenzyme A (Haan, 2015).

The enzymes found within the cannabis plants vary as to whether they require cofactors. THCA synthase, for example, requires a *flavin adenine dinucleotide* (FAD) cofactor to convert CBGA into THCA, whilst CBDA synthase does not require a cofactor to function (Sirikantaramas *et al.*, 2004; Taura *et al.*, 2007). Whether or not the theoretical enzyme would require a cofactor to function is unclear and could only be determined after the enzyme is known. Thus, it will be assumed that the reaction will occur without a cofactor during the proposed process.

3.7.5. Additional Design Considerations

A well-designed enzyme reactor should optimise the reaction occurring inside it. To achieve this, operating parameters within the reactor such as substrate feed, product removal temperature, pH, and agitation will need to be carefully monitored and controlled.

Ideally, reactor design should optimize the strengths of the reactor type while counteracting its disadvantages to achieve quality products, an efficient process, and conservative costs. The primary goal is to achieve the greatest product concentration at the lowest possible cost.

Hydraulic retention time (HRT) and loading rate are also significant parameters, capable of affecting a continuous process. While a faster loading rate generally leads to a faster but less efficient process, HRT – the average length of time substrate remains in the reactor – is related to both conversion and kinetics (Eibes *et al.*, 2007).

Longer HRTs provide more opportunities for substrate molecules to encounter the enzyme, leading to higher conversion rates and reaction efficiencies. HRT also plays a role in determining the size and configuration of the reactor and influences the flow rate of substrate solution through the reactor. Thus, adjusting the HRT can help regulate the flow and ensure

the substrate spends enough time in the reactor for sufficient enzyme-substrate interaction, allowing for more complete conversion and minimising waste generation (Eibes *et al.*, 2007).

It is also important to assess whether the reaction or downstream purification is likely to be the more expensive operating cost of the process. If an expensive enzyme is used, then it is logical to design the reactor to make the most effective use of the enzyme kinetics. If productive isolation is significantly more expensive than the enzymes, the enzyme kinetics become less of a priority, and using greater enzyme concentrations to simplify the required downstream processing will prove more advantageous. A PFR with a well-defined residence time will aid in maximising substrate conversion in such a case, to avoid cumbersome separation processes (Lindeque and Woodley, 2019).

In the case of enzyme inhibition close to the desired feed concentration, a lower substrate concentration can be fed into the PFR at several points along the reactor's length to mitigate the inhibition (potentially risking difficulty in radial diffusion in larger reactors), or a series of CPFR's with a feed between each can be implemented (Lindeque and Woodley, 2019). However, as we are assuming an uninhibited reaction, the proposed process will use a single reactor.

Heat transfer should also be considered in a reactor design. Heat transfer capacity is determined, in large part, by the inner diameter of the reactor, which establishes the heat transfer area. Smaller diameters have higher heat transfer capacity but also lower flow capacity and greater drops in pressure, as well as an increased risk of blockages (Fogler, 2017). Thus, a trade-off must be made between heat transfer and flow capacity, alongside heat transfer and pressure drop.

Concerning heat transfer, temperature control is another key consideration, as poor temperature control can notably affect product quality and yield, as well as potentially resulting in freezing, boiling or overpressure, halting operation entirely. Plug flow reactors implement simple feedback control of the product temperature, gathering data from sensors and adjusting the temperature accordingly to maintain it around a set-point value (Fogler, 2016). This mechanism of feedback control is also often used to monitor parameters like pressure and concentration.

The final consideration is mixing. Continuous processes rely either on diffusion or implement mechanical agitation, though the latter is applied primarily in the case of CSTRs. PFRs generally rely on diffusion, the effectiveness of which hinges upon both the inner diameter of the reactor and the concentration or temperature gradients within the product. This approach is

more effective with reactors possessing smaller diameters where heat can be transferred to and from the heat transfer surface via conduction. In large reactors, especially those containing immiscible fluids, this is not always practical (Hafner, Wolff and Roeder, 2022).

In the case of immobilised enzymes, it is also worth noting that kinetic parameters can be affected by limitations placed upon the diffusion of the substrate molecules due to the support surface used for immobilisation (Bhatia, Naidu and Kamaruddin, 1999). For substrate molecules to encounter the enzyme surface, diffusion must be twofold – the substrate must first diffuse through the reactor to the immobilised enzyme, and then diffuse through the pores of the support structure. Pores do not surround the enzyme entirely; some of the cross-section would be the solid portion of the support, and the geometry of the pores is often complex (Bhatia, Naidu and Kamaruddin, 1999). Thus, the effectiveness of diffusion may vary from what is expected from free enzymes.

Due to the theoretical nature of the enzyme and its unknown properties, only a simplified version of the reactor can be simulated for this process; however, the complexities involved in the in-depth design of such a reactor were the enzyme known and research initiated in earnest, must be noted.

3.8. Downstream Processing

Once the extract exits the bioreactor, downstream processing becomes incredibly important. Any legal cannabis product must have below 0.001% THC to comply with legal requirements (Rethink CBD, 2019) but beyond this, it is also important to concentrate the CBD within the extract to achieve the desired composition. If an isolate is sought, all other terpenes, cannabinoids, and other compounds must be removed, producing a CBD isolate of 95 % or greater. If a more full-spectrum approach is desired, and other components are allowed to co-exist with CBD in the extract, much of this downstream processing could be cut.

For clarity, CBD isolate is a pure form of CBD, often up to 99 %. Full-spectrum CBD is a cannabis extract containing primarily CBD, as well as other cannabis compounds like terpenes and other cannabinoids. For the sake of this review, the full spectrum blend will still need to meet legal requirements and thus contain less than 0.001 % THC.

However, for this section of the review, the focus will be on the production of CBD isolate and rigorous downstream processing will be assumed; the potential mechanisms for such processing will be investigated. The difference between the processing for a CBD isolate versus that of a CBD full spectrum blend will be touched upon in this section but will be fully compared in terms of profitability in Section 5.

3.8.1. An Overview of Downstream Processing

In terms of downstream processing, there will always be a compromise between yield and purity. The bioreactor itself plays into this as well - the greater the concentration of the desired compound exiting the bioreactor, the less rigorous the downstream processing to follow will be. This is always desirable, as every addition downstream process increases the required capital, operating cost, and the amount of product lost.

Thus, it is ideal for the fewest amount of downstream process steps to be applied to meet the product requirements. Each process step should also ideally be selected carefully based on the product purity it achieves, its cost, yield, required parameters, and possible product denaturisation.

CBD is known to be hydrophobic and not particularly volatile in nature, with a boiling point between 160 °C and 180 °C, though this is also commonly cited as 180 °C (Rocky Moutain Regents; Ethos Cannabis, 2023). This must be considered when deciding upon the most applicable downstream processes for the proposed process.

Due to the uncertainty surrounding the process, being highly theoretical in nature, it will be assumed that - due to the lack of inclusion of buffers and required carrying solvents - there will be no need for concentration of the extract. Practically, since cannabis oil is reported as having a density of approximately 980 kg/m^3 (literature on this varies as different concentrations of different constituents will affect density, but values tend to oscillate within the range of 900 – 980 kg/m^3), viscosity should not be a major barrier and the oil should be able to move through the process without the need of a carrier (Salas-Guerrero, Buendia-Atencio and Orozco, 2023).

Thus, the downstream process will consist of separation and purification to remove any lingering impurities, such as terpenes and other cannabinoids - though, as mentioned, much of

this will be rendered unnecessary when considering a full-spectrum product. For now, it will be assumed the process will be geared toward the production of a CBD isolate.

The most commonly utilized methods in the production of CBD isolates tend to be chromatography and distillation, which will be discussed in further detail in Sections 3.8.2 and 3.8.3 respectively. Beyond this, it is not uncommon for crystallization to be applied as a form of final product processing. However, for the sake of this work, it will be assumed the desired product is a CBD oil instead of CBD crystals. Thus, the topic of crystallization will fall outside the scope of this review.

While CBD oils are sold both with and without a carrier, CBD is commonly sold dissolved in an oil carrier, which increases the bioavailability of the cannabinoid due to its lipophilic nature and increases the stability of the compound when exposed to light and/or heat (Grifoni *et al.*, 2022). An exploration into the economic implications of using a carrier oil will be conducted in the economic analysis found in Section 5.2.

Once the relevant separation processes have been performed, it is also important to note that residue from any solvents used in these processes would compromise the quality of the product. If we consider pharmaceutical limits, the ICH Guideline for drug products limits hexane residues, for example, to be 290 ppm, while ethanol is limited at 5000, due to it being a lesser risk for human health (International Council for Harmonisation, 2020). Thus it becomes pertinent to keep these limits in mind for all solvents used and to ensure they are adhered to - common methods for removing these solvent residues post-process include rotary evaporators or distillation units (López-Olmos *et al.*, 2022).

3.8.2. Chromatography

Chromatography operates by selective retardation of different solutions to resolve mixtures. As a rule, a mobile phase (the solvent) will flow through a particle bed which acts as a ‘stationary phase’ with the solutes travelling at different speeds depending on their affinity for the stationary phase. In this way, the output stream will contain the desired product, with most of the impurities having been removed.

Chromatography comes in several forms and can be operated in a packed bed or expanded bed column, with elution being carried out isocratically (using an elution buffer of constant

composition) or via gradient elution (in which the composition is changing, either continuously or in a step-wise manner) to improve solute fractionation (Heinzle, Biwer and Cooney, 2007).

Several forms of chromatography exist, each geared towards removing a different kind of solute. For example, gel chromatography is based on molecular sieving which separates solutes according to the size of their molecules and is commonly used in protein purification. Ion-exchange chromatography uses electrostatic attraction to achieve separation with a charged resin (Heinzle, Biwer and Cooney, 2007). However, such methods of chromatography are not relevant for the separation of CBD.

More relevant to the proposed process are chromatographic methods like reversed-phase chromatography (a popular form of HPLC), which is commonly used for the separation of CBD (GALAK Chromatography Technology Company Ltd, 2023). In reversed phase chromatography, uneven distribution of solutes between immiscible liquid phases provides a basis for separation, with the less polar solvent acting as the stationary phase. Ultimately, elution occurs based on hydrophobicity (Heinzle, Biwer and Cooney, 2007).

Methanol and acetonitrile are the most commonly used solvents for this chromatographic technique, with common packing materials including polymeric resin, silica, or hydroxyapatite media (BioRad Laboratories, 2023).

This is the most commonly applied method for CBD purification, with reverse-phase liquid chromatography proving itself as reliably capable of producing CBD powder of 99 % or greater, especially when paired with additional purification mechanisms, and is known for having a very high recovery rate (90 % is higher than the average recovery rate for HPLC) (Rutz, 2016; Audo, 2018; Manufacturing Chemist, 2019; GALAK Chromatography Technology Company Ltd, 2023).

A viable alternative for reverse-phase liquid chromatography, however, is centrifugal partition chromatography (CPC). CPC is a method of particular interest for cannabis oil purifications, as it requires less solvent to achieve the same levels of separation, making it incredibly useful in the cases of producing pure cannabinoid products and larger-scale production plants (Abundant Labs, 2023).

HPLC also generally requires expensive resins and boasts a lower recovery rate compared to CPC - this, in addition to the larger volumes of required solvents, makes it a less efficient method for purifying cannabinoids overall. In fact, CPC uses up to five times less solvent to

purify cannabinoids than solid/liquid chromatography methods due to its capacity to continuously reuse solvents instead of requiring regular replacement of a solid stationary phase (Rutz, 2016; Manufacturing Chemist, 2019). It is for these reasons that CPC has been selected for use in the proposed process.

CPC columns contain spinning disks containing thousands of cells linked together in chain-like structures. The stationary phase is held in place by centripetal force while the mobile phase moves along the cells, allowing solutes to diffuse from the mobile phase to the stationary phase according to their respective affinities. For example, in a water-butanol system where water acts as the mobile phase, affinity will hinge upon polarity, with more polar solutes remaining in the water whilst non-polar solutes diffuse more readily into the butanol. This results in the solutes separating out into different cells (Audo, 2018; Manufacturing Chemist, 2019). A diagram of this process can be found in Figure 12. Alternatively, one can also reduce the flow direction of the process, so the desired phase remains in the rotor of the CPC rather than being accelerated along the column (Rutz, 2016).

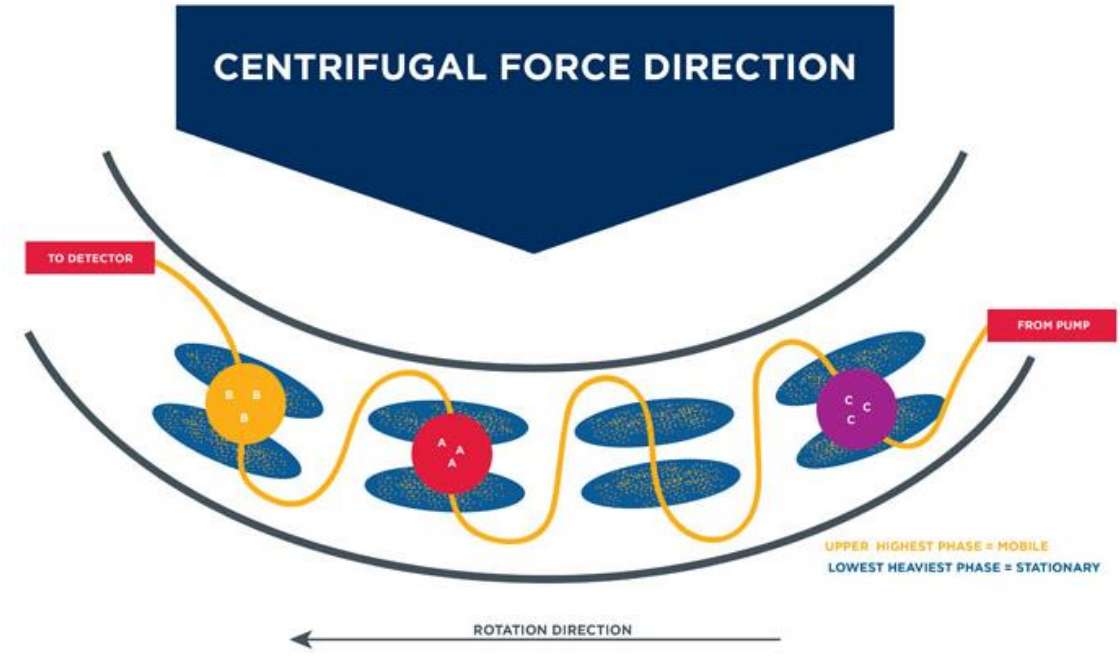


Figure 12: A diagrammatic representation of a CPC process, depicting the stationary phase (blue) and the mobile phase (yellow) as it separates out various solutes (A, B, and C) (Manufacturing Chemist, 2019).

An additional consideration regarding CPC - as with any chromatographic process - is the distribution coefficient K between the desired solute and the respective phases, with low K values resulting in elution so rapid that separation cannot take place, and too high K values extending the retention time too long. The ideal K value ranges between 0.5 and 5 - sometimes cited even more narrowly, as between 0.7 and 4.5 - to ensure separation (Rutz, 2016; Audo, 2018).

In an ideal CPC operation, the separation can be carried out entirely continuously, with the phases travelling counter-currently through a looped purification system - though whether or not this is possible depends on the degree of interaction the solutes have with the stationary phase, with discontinuous processes with the time-shifted departure of solutes from the column sometimes proving a necessity (Rutz, 2016).

The specific precise solvents used, along with other relevant parameters, will be broken down in more detail in Sections 3.8.4. Irrespective of the exact mechanism applied to the CPC operation and the precise solvents, though, the output will then need to be passed through a rotary evaporator or distillation column to separate the solvent from the desired solute, after which the solvents can be purified as required and reused (Manufacturing Chemist, 2019).

In addition to the previously listed advantages of CPC, it is also worth noting that aside from CBD, CPC can be used to purify many other cannabinoids such as CBG and CBDA (Audo, 2018; Manufacturing Chemist, 2019). This could potentially result in an additional source of income, providing an opportunity to purify multiple other cannabinoids alongside CBD without the necessity of developing alternative purification processes to achieve this. This potential income stream will be examined in greater detail in Section 5.2, as part of the economic analysis.

3.8.3. Distillation

Distillation is a pivotal part of many downstream processes. In terms of CBD oil purification, distillation will play a role in most variations of the process, either as the primary means of separation or as a final separation mechanism post-chromatography. As it has already been established that CPC will function as the primary downstream mechanism in the isolate process, distillation will function as a method to separate the cannabis compounds from the

respective solvents used during chromatography. In the production of full spectrum CBD blends, CPC will be bypassed entirely, and distillation alone will be relied upon to reduce THC concentrations to acceptable levels.

Using variation in the volatilities of the compounds to achieve separation, distillation involves feeding a pre-heated mixture into a column consisting of several theoretical stages, causing more volatile compounds to rise and exit the column as a gaseous distillate, which is then liquefied in a condenser. It is also common for this to be recycled into the column once again to improve the separation. Compounds with boiling points higher than the temperature of the mixture will remain in the liquid phase and leave the column from the bottom.

There are several different operation modes for distillation columns; some main types include vacuum distillation, destructive distillation, fractionating distillation, and batch distillation. An outline of the differences between these types can be found in Table 14.

Table 14: Explanation of the main distillation column operating modes (Téllez-Anguiano et al., 2017).

Vacuum distillation	Using a vacuum pump, a low-pressure system is induced to boil the mixture at low temperatures, resulting in separation.
Destructive distillation	The mixture is heated at elevated temperatures, resulting in decomposition into other products that can be separated by fractionating.
Extractive distillation	Different separating agents are added into azeotropic mixtures, raising the relative volatility of the components to facilitate separation.
Fractionating distillation	➢ Liquid mixtures are separated by heating the mixture to a temperature at which one or more fractions of the mixture will vaporize, ultimately using distillation to 'fractionate' the mixture. ➢ This distillation is often applied to separate composite mixtures where the components have similar boiling temperatures. ➢ This operation mode is generally continuous in nature, with a constant feeding flow through a feeding tray. The section above the feeding tray is called the rectifying section, whilst the section beneath the feeding tray is called the stripping section.
Batch distillation	➢ Often applied to small liquid quantities or when various products need to be obtained from a single set volume of the mixture. ➢ Mixture composition varies with time, making steady-state impossible due to its batch nature. ➢ Only the distillate can be rectified or enriched.

Distillation requires heat stability of the desired product. In terms of CBD oil purification, distillation is often operated below the boiling point of the compound (180 °C) to ensure degradation is minimal (Delta T Systems, 2023). Aside from substance boiling points, it is common practice to take note of the linear velocity of the vapour when designing a distillation process (Heinzle, Biwer and Cooney, 2007). In this case, fractionating distillation will be applied.

The required number of theoretical plates and tray design (or column packing) must also be selected with care. Column packing provides the surface area needed to facilitate mass transfer between vapour and liquid phases, and the choice of packing should be influenced by the mixture's properties and required operating conditions.

Packing is generally used in columns with small diameters, under low pressures, and with low pressure drops. It generally handles corrosive fluids and foaming liquids well, though this is not significant to our process, and is lighter in weight and cheaper to construct than tray columns (Towler and Sinnott, 2009). However, the packing itself is expensive, columns cannot cope well with high liquid flow rates, and distillate quality is not easily controlled; as the purpose of the distillation is to produce a quality product that is as pure as possible, this is not ideal (Jenkins, 2020).

As an alternative to packed columns, tray columns are also an option. Tray columns allow the feed to enter the column on a tray, also referred to as a plate, with the column separated into a top and bottom section. Vapour rises through the column, encountering liquid captured on the trays; during this period of contact, heat is exchanged, and some vapour condenses whilst some liquid evaporates.

Tray columns operate well under high liquid residence times, are easier to clean, and resist fouling. They are more robust, withstand thermal stress better, and offer smoother operation overall. Additionally, product quality and plate efficiency are more easily controlled, and a wider range of flow rates can be accommodated. However, these columns are not suited to low capacities, are thermally sensitive, and are often heavy enough to necessitate expensive support structures. Tray columns can handle a wide range of liquid flow rates without flooding, however, and are generally considered more reliable (Jenkins, 2020).

Ultimately, for the purpose of this column, tray internals were deemed the superior design as they can deal with a wider range of liquid flow rates, operate more smoothly, and most importantly, the quality of the distillate can be better controlled to produce a purer product.

Additionally: two primary categories of plates can be said to exist: counter-flow and crossflow plates. For this design, crossflow trays have been selected because they facilitate greater mixing between phases (Towler and Sinnott, 2009).

Tray design is also critical, providing the surface area required for efficient mass transfer. The most common designs include sieve trays (which consist of a perforated tray beneath a layer of packing material), bubble cap trays (a perforated tray with a series of inverted cups that capture the vapour), and valve trays (which contain valves that open and close to regulate the flow of liquid and vapour) (Henley and Seader, 1988; McCabe, Smith and Harriott, 1993).

Because the sieve trays are the most versatile and inexpensive type of trays, and because this system is simple in nature and does not require any kind of specialised plates for fluctuating flows and exceptionally high turndown ratios, sieve trays are selected for the distillation column (Kolmetz and Jaya, 2011).

The distillation column must also be sealed at its top and bottom end; these seals are referred to as the distillation column 'heads,' of which there are four primary types: flat plate heads, hemispherical heads, torispherical heads, and ellipsoidal heads. The shape selected is primarily dependent on the pressure in the column.

While flat plates are cheap to manufacture, they are only applicable to systems of low pressures. Hemispherical heads, on the other hand, are strong and able to withstand high pressures; however, this also makes them considerably more expensive. Generally, torispherical heads are considered the industry standard and are used for vessels operating at pressures up to 15 bar. As the pressure of this column will be kept relatively low, flat plates will be selected to construct a column that is both effective and economical (Towler and Sinnott, 2009).

In terms of operating conditions, temperature and pressure are usually controlled to maximize the separation efficiency whilst flow rate is regulated to maintain a steady-state operation. Rmin (reflux ratio (R)/minimum reflux ratio) in a distillation column is the minimum amount of liquid reflux required to achieve the desired separation, where the 'reflux ratio' is the ratio of liquid that is returned to the column as reflux compared to the amount of liquid removed as distillate. The Rmin value of a particular distillation procedure depends on the properties of the feed, the design of the column, and the desired product purity (Richardson, Harker and Backhurst, 2002).

The range of acceptable R/Rmin values is generally 1.2 - 1.5. A higher R/Rmin can result in better separation but can also increase energy consumption and operating costs whilst a lower R/Rmin achieves the opposite. Thus, this can be optimized depending on the process in question (Mavalal and Moodley, 2021).

The range of acceptable vapour linear velocity (u) in a distillation column also depends on several factors, such as column diameter, packing type, and the physical properties of the liquid and vapour phases. In general, the vapour linear velocity should be high enough to ensure proper mixing and mass transfer, but not high enough to cause flooding or entrainment (Treybal, 1980; Bennett, Kao and Wong, 1995).

The range of acceptable vapour linear velocity (u) in distillation columns can vary from around 0.05 m/s to 3 m/s. For example, Treybal (1980) reports that the acceptable range of vapour linear velocity is 0.1 – 2 m/s for packed columns whilst Holland (1981) reports 0.05 - 0.5 m/s; both sources reach a consensus of 0.3 – 3 m/s for tray columns. Thus, this is variable.

It is also worth noting the relationship between pressure and temperature, which often depend upon each other. For example, if the extraction is being conducted at a temperature greater than the solvent boiling point, pressure should be higher than atmospheric pressure to prevent the solvent from boiling off. On the other hand, if extraction is being conducted below the solvent boiling point, pressure should be lower than atmospheric pressure to prevent the solvent from condensing.

The pressure in a liquid extraction unit can also affect the solubility of the materials being extracted. Higher pressures can often increase the solubility of some materials; however, operating at high pressures can increase the risk of equipment failure and can cause damage to the materials being extracted. Therefore, the pressure should be maintained within the design limits of the relevant equipment and materials used.

It’s worth noting that traditionally, complex vapour-liquid equilibrium (VLE) calculations would be required in the design of a distillation column. However, in the case of the proposed process, much of this will be conducted by the simulation itself - thus the process of performing these calculations will not be outlined in detail. VLE calculations and other relevant design decisions that are made regarding the separation of cannabis oil from the CPC solvents will be discussed further in Section 4.5, Section 4.6, and Appendix B.

3.8.4. Details of the Proposed Process

In terms of CPC geared specifically towards CBD extraction, as mentioned, Rutz (2016) recommends a range of 0.7 - 4.5 for the partition coefficient, K. The specific K value of the operation performed by Rutz involving CBD is not specified - however, for the sake of the proposed process, we will assume its K value falls within this range.

According to Rutz, the first solvent phase used for CPC of CBD can be selected from heptane, cyclohexane, n-heptane, iso-heptane, octane, n-octane, or iso-octane - however, n-heptane is preferred. The second solvent is recommended to be acetonitrile, to which 1 – 15 % t-butyl methyl ether (TBME) could optionally be added (more ideally 9 – 15 %).

The solvents are to be fed continuously into the process at speeds of 50 – 600 mL/minute, more ideally 200 – 300 mL/min, and the rotation speed is recommended to be 50 – 1500 rpm, more ideally between 900 – 1100 rpm. A comprehensive collection of boiling points and densities can be found in Table 52, in Appendix A. In this process, acetonitrile has the heaviest density of the three solvents, forming the heavy, stationary phase while heptane forms the lighter, mobile phase.

With this method, the CBD extracts of purities greater than 95 % can be easily produced - this can increase up to 99.3 % if run optimally. When using a 12 500 mL rotor, Rutz suggests purifying up to 100 g of extract per run, with each separation running for between 120 - 160 minutes.

Taking 150 minutes as a basis (2.5 hours), this would mean 40 g of extract would be processed per hour, and between 30 L (200 mL/min) and 45 L (300 mL/min) of each solvent phase would be required per run, or a stream of between 12 L – 18 L. This can be varied as required through the relevant scale-up processes. The process used for the proposed process simulation will operate similarly but scaled down to a flow rate of 0.1252 kg/h. The calculations involved in this can be found in Table 15 and Table 16.

The relevant masses are found for the maximum (45 L) and minimum (30 L) solvent streams used for the 100 g process proposed by Rutz (2016), and then the ratio of these masses (assuming 11 % of the heavy phase stream will be TBME and 89 % will be acetonitrile) is used to find the relevant solvent masses for a feed stream of 0.1252 kg/h. The mass ratio for extract-to-acetonitrile-to-heptane-to-TBME is found to be 1: 20.99: 20.52: 2.44.

Table 15: Densities of CPC solvents and extract feed.

Acetonitrile density:	786	kg/m^3	0.786	*kg/L*	(Labchem, 2013)
Heptane density:	684	kg/m^3	0.684	*kg/L*	(ThermoFisher Scientific, 2021)
TBME density:	740	kg/m^3	0.740	*kg/L*	(The OSHA Standard 29, 2012)

Table 16: Calculations of maximum and minimum masses for CPC solvent streams for a 600 g run.

		(30L)			(45 L)		
	Extract	Acetonitrile	Heptane	TBME (11%)	Acetonitrile	Heptane	TBME (11%)
	0,1	2,986	2,052	0,244	3,148	3,078	0,366
per hour	0,125	2,627	2,569	0,306	3,941	3,853	0,459
		Total solvent:		5,623	Total solvent:		8,434

It is also worth noting that according to Ingkaninan and associates (2000), it is possible to separate out multiple cannabinoids using the same CPC system. Since CBD is a neutral cannabinoid, the same CPC used to separate out CBD can also be altered to separate out other neutral cannabinoids like THC, CBN, and CBG as well. All of these can function as additional sources of income, exiting the CPC in independent fractions which can then be separated from heptane in much the same manner as CBD.

In the study, two independent CPC systems were used. The system that separated out CBD and the other neutral cannabinoids used a hexane/acetone/acetonitrile system, while the system geared towards acidic cannabinoids like THCA, CBGA, and CBDA used hexane/methanol/water/formic acid and required that the extract not be decarboxylated before processing. This resulted in purities of above 90 % purity for all extracts, with CBD in particular ultimately achieving 92.7 % purity (Ingkaninan *et al.*, 2000). Some of the results yielded by this study can be found in Table 17.

Table 17: Cannabinoid yields from a hexane/acetone/acetonitrile CPC system (Ingkaninan et al., 2000).

Cannabinoid	Isolated mass (mg)	Relative yield (g/ 100 g plant mass)	Purity (%)
CBD	232.0	0.46	92.7
CBG	40.3	0.54	92.2
CBN	99.4	1.38	95.0
THC	90.0	0.83	93.1

CBG is the precursor of both CBD and THC and has been found to exhibit anti-inflammatory, antifungal, antibacterial, and neuromodulatory effects, showing significant therapeutic potential and gaining popularity as a cannabis product (Jastrząb, Jarocka-Karpowicz and Skrzydlewska, 2022). Thus, this is a viable secondary source of income.

CBN has received far less attention than either CBD or CBG, and studies have been limited on its effects. However, it has been found to possess anti-inflammatory, antibacterial, and orexigenic (appetite stimulation) effects. It is also possible that it possesses sleep induction capabilities, but this has not yet been fully scientifically substantiated (Maioli *et al.*, 2022). As a secondary product, CBD might not have the same market scope as CBD and CBG but could potentially hold potential for scientific studies or similar academic use.

As outlined in Section 2.3, THC has some positive attributes and has been used for medicinal purposes and research trials, despite its psychoactive properties. While its removal from the CBD products produced by the proposed process is critical for them to meet legal requirements, THC isolates do have niche appeal in the medical industry and could be sold as an ancillary product as well.

Because the majority of THC in the proposed process has been transformed into CBD, the isolated masses of CBD and THC measured by Ingkaninan (2000) have been combined into a single CBD value to estimate a collective mass to estimate a feasible ratio of CBG and CBN masses isolated by the CPC. This can be seen in Table 18 and will be further investigated in Section 5.2 as part of the economic analysis.

Table 18: Estimated ratio of CBG: CBG: CBN separated by CPC in the proposed process (Ingkaninan et al., 2000).

	CBD:	**CBG:**	**CBN**
Reported masses (mg)	322.0	40.30	99.40
Calculated ratio	100.0	12.52	30.87

Once the light phase stream, which will consist primarily of heptane and CBD (or CBG, or CBN, depending on the fraction, but it will be assumed to contain CBD going forward) exits the CPC process, it will need to be separated out by distillation. The bottom phase, or heavy phase, containing primarily acetonitrile, TBME, and the remaining unwanted cannabis compounds will also be distilled, independently. Referring to Table 52, Appendix A, boiling points can be found for the process, with CBD boiling at 160 °C – 180 °C, acetonitrile at 82.00 °C, TBME at 55.20 °C, and heptane at 98.42 °C. More details of the distillation processes can be found in Section 4.5, Section 4.6, and Section 5.3.

CBD oil can then be collected from the distillation column, in both processes. Further processing of the oil products, in the form of combination with a carrier oil, will be discussed as part of the economic analysis in Section 5.2.

3.9. Heat Exchanger System

Heat transfer operations are necessary to change and control the temperature of process equipment such as reactors, distillation columns, evaporators, and condensers, amongst others.

Steam is usually used for heating, with high-pressure steam providing more heat. Commonly, used cooling agents include cooling water (20 °C), chilled water (5 °C), or for lower temperatures, agents like glycol, Freon, sodium chloride brine, or calcium chloride brine. To ensure efficient heat transfer, the cooling agent's final temperature should be between 5 °C and 40 °C below the final temperature of the cooled liquid (Heinzle, Biwer and Cooney, 2007).

The rate of heat transfer will be proportional to the temperature difference between the heat exchange fluid and the process materials and the total heat exchange area. The rate of heat transfer will, in turn, determine the size of the exchanger required. Several types of heat exchangers exist, including:

1. Double-pipe exchangers (the simplest heat exchanger; used for both heating and cooling)
2. Shell and tube exchangers (commonly used for all kinds of applications)
3. Plate and frame exchangers (used for both heating and cooling)
4. Spiral heat exchangers
5. Plate-fin exchangers
6. Air-cooled (condensers and coolers)
7. Fired heaters

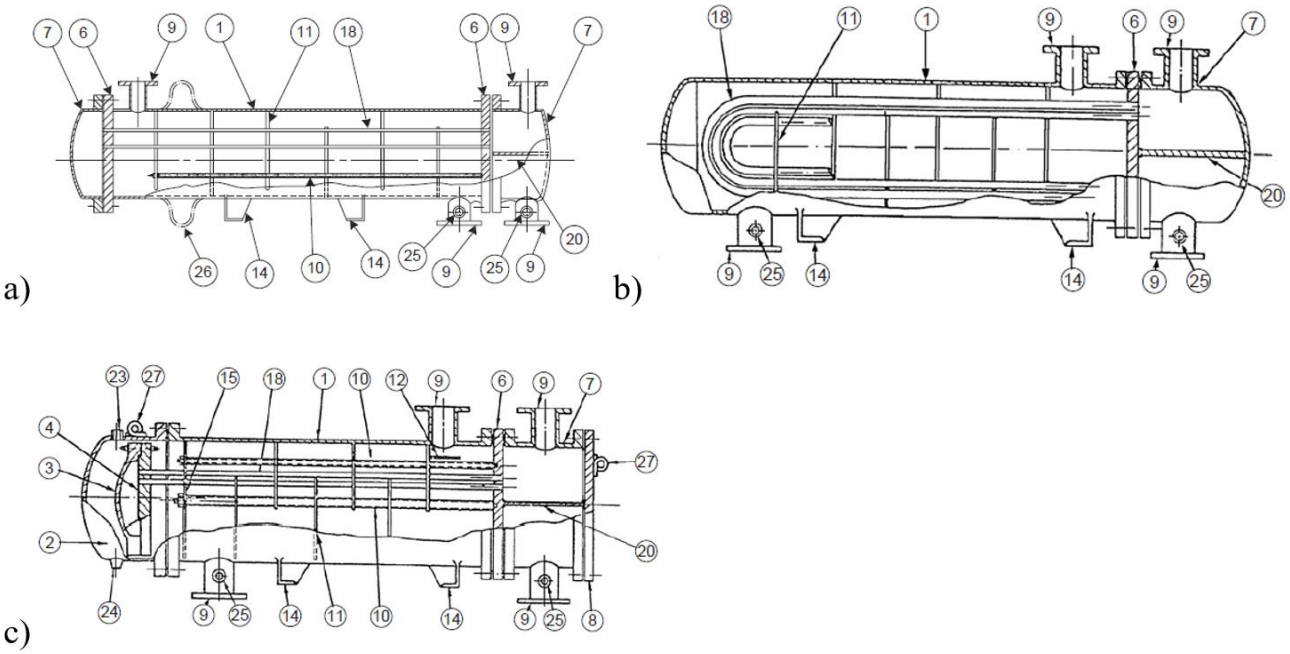
The most used heat exchanger in the chemical industry is the shell and tube exchanger, in which one section of the process flows through a bundle of tubes, and the other around the cylindrical shell that encloses the tubes. The tubes' ends fit into sheets which separate the fluids flowing through the tubes and the fluid flowing through the shell. Baffles in the shell, held together by spacers and rods, direct the flow and support the tubes (Towler and Sinnott, 2009).

Shell and tube construction provides several benefits, including easy cleaning, a wide range of feasible construction material options, as well as well-established design and fabrication techniques. Additionally, shell and tube exchangers also possess an effective layout, providing a large surface area to volume ratio, encouraging efficient heat exchange, and a shape ensuring reasonable pressure operation (Cengel, 2004; Towler and Sinnott, 2009).

Shell and tube exchangers come in three primary designs: fixed tube exchangers, U-tube exchangers (consisting of a single tube sheet), and floating-head designs (an internal floating-head and external floating-head variant exist; the internal variant houses the floating-head joint inside the shell whilst the external variant has it located outside of the shell) (Cengel, 2004; Towler and Sinnott, 2009). A comparison between these shell and tube exchanger designs can be seen in Table 19, and diagrammatic representations can be found in Figure 13.

Table 19: Comparison of the three major shell and tube heat exchanger designs. (Towler and Sinnott, 2009; Geurts Heat Exchangers, 2023)

Shell and tube design	Advantages	Disadvantages
Fixed tube exchanger	➢ The simplest and cheapest of the shell and tube exchanger designs. ➢ Design variations can be made to better accommodate differential expansion: ➢ Including an expansion loop in the shell, though this is limited to shell pressures below 8 bar. ➢ Fixing only one end of the tubes, thus allowing the tube bundle to expand.	➢ The tube bundle cannot be removed for cleaning, making effective cleaning difficult. ➢ There is no provision for differential expansion in the base fixed tube design. Due to variations in temperature and possibly materials between the shell and tube, differential expansion will be considerable. • For this reason, fixed tube exchangers are limited to temperature differences below 80 °C.
U-tube exchanger	➢ This design requires only one tube sheet and is therefore cheaper than the floating-head types. ➢ Requires less space than other types.	➢ Limited in application to clean fluids, due to difficulties in cleaning the tubes and bundle. ➢ It is also more difficult to replace tubes when necessary.
Internal floating head exchanger	➢ More versatile than the other designs. ➢ More effective for large temperature differentials. ➢ Due to the tubes' easy removal of the bundle, tubes are easier to clean and thus can be used for liquids more likely to induce fouling. ➢ Using a clamp ring (i.e.: a split flange design) reduces necessary clearance.	➢ Pull-through designs increase the clearance required between the outer tubes and the shell compared to fixed-tube and U-tube designs to allow fluid around the floating-head flange. ➢ Internal flanges induce leakage risks. • The risk of leakage limits shell-side pressures to below 20 bar. • It also means toxic or flammable materials should never be used on the shell side.



Part numbers:

1. Shell
2. Shell cover
3. Floating-head cover
4. Floating-tube plate
5. Clamp ring
6. Fixed-tube sheet (tube plate)
7. Channel
8. Channel Cover
9. Branch
10. Tie rod and spacer
11. Cross baffle or tube-support plate
12. Impingement barrier
13. Longitudinal baffle
14. Support bracket
15. Floating-head support
16. Weir
17. Split ring
18. Tube
19. Tube bundle
20. Pass partition
21. Floating-head gland (packed gland)
22. Floating-head gland ring
23. Vent connection
24. Drain connection
25. Test connection
26. Expansion bellows
27. Lifting ring

Figure 13: Diagrams of shell and tube heat exchanger designs: (a) fixed tube exchanger (b) U-tube exchanger (c) internal floating head exchanger (without clamp ring) (Towler and Sinnott, 2009).

Other heat exchangers, aside from the shell and tube variants, include kettle reboilers, which have vapour rising from the lower rows of tubes in the bundle and passing over the upper rows, thus facilitating heat transfer. Gasketed plate heat exchangers involve a stack of thin plates clamped together within a frame, with a gasket sealing the edges of the plates. Welded plates operate similarly but with the plate edges sealed using welding. Plate-fin exchangers separate the plates using corrugated sheets, and spiral heat exchangers have the plates formed into a spiral (Towler and Sinnott, 2009).

For this design, however, a shell and tube exchanger will be used for heat transfer processes unless specified otherwise. A U-tube shell and tube design has been selected based on the assumption that heat transfer fluids such as cooling water, chilled water, or steam will be predominantly used. This will not be an issue for a U-tube design, which is difficult to clean in the case of some process fluids and is more affordable than the floating head designs and more versatile than the fixed tube design.

Shell and tube heat exchangers can be configured either horizontally or vertically. For this design, a horizontal configuration was implemented due to its decreased pressure drop, lower pumping costs, and greater effective heat transfer coefficient, thus facilitating designs with higher heat exchange surface area requirements. Furthermore, due to being the standard within the industry, a one-pass shell will be used (Towler and Sinnott, 2009; The Engineering Concepts, 2020).

Some of the primary heat transfer equipment required by the process can be found in Table 19, where the heat exchange equipment required for the production of an isolate versus that required for the full spectrum CBD blend has been specified, as well as the relevant temperature differential and basic design requirements.

Some heat exchange units that require extra consideration are the condensers and reboilers associated with the distillation columns. The condenser will follow a U-tube design, as discussed, condensing the hot vapour, and returning it back to the top of the column. A total or partial condenser can be utilised, where a total condenser will condense the entire stream, whereas in a partial condenser only part of the vapour is condensed - in this case, even though the liquid is returned to the top of the column, the distillate will leave the column in a gaseous state (Towler and Sinnott, 2009). A total condenser will be implemented in this design.

Reboilers vaporise the liquid phase bottom stream as it exits the distillation column. A total reboiler like the ones applied in this process will completely vaporise the stream, operating at a temperature close to the stream’s boiling point, whilst a partial reboiler, as the name suggests, will vaporise only a fraction of the stream. There are primary types of this heat exchanger: a kettle reboiler, a thermosyphon, and a forced circulation reboiler (Towler and Sinnott, 2009).

A kettle reboiler contains tubes submerged in liquid, where the hot stream (or the fluid that will be used to heat the process stream) is contained within the tubes. It is easy to maintain, easy to control, and has no limit on the vapour stream, making it very versatile; however, they are somewhat more expensive than some of the alternatives and can face difficulties with fouling.

These reboilers are used within processes that require a large heat transfer area (Kilkovsky *et al.*, 2009).

A thermosyphon reboiler operates under natural circulation and can be arranged either vertically or horizontally. This unit can be arranged vertically or horizontally. In a vertical thermosyphon, the hot stream moves through the tubes, and in a horizontal thermosyphon, it will flow instead through the shell. This type of reboiler is less expensive than the kettle reboiler and allows for longer tubes to be utilised, but faces difficulties with fouling and often results in high boiling point components accumulating in the feedline, resulting in uneven column temperatures, with the column often becoming hotter towards the bottom (Kilkovsky *et al.*, 2009).

Forced circulation reboilers use an external pump to propel liquid through the exchanger. These reboilers are expensive, and only generally implemented in systems that contain very viscous or high fouling fluids. This is not especially relevant to this design – thus forced circulation reboilers will not be used (Kilkovsky *et al.*, 2009). Ultimately, a kettle-type reboiler will be implemented in this design due to its reliability, versatility, and simplicity.

Table 20: Overview of process heat exchange units as simulated using SuperPro Designer.

Process Unit	Isolate/ Blend	Temperature Differential	Design
Cooling of cannabis feed before ethanol extraction	Both	25 °C → -35 °C	Due to the required temperatures being lower than most commonly available cooling agents, an electric cooling unit will be used.
Cooling of ethanol feed before ethanol extraction	Both	78.3 °C → -35 °C	Due to the required temperatures being lower than most commonly available cooling agents, an electric cooling unit will be used.
Ethanol extraction unit/s	Both	Adiabatic	N/A
Decarboxylation oven	Both	-35 °C → 130 °C	Decarboxylation ovens use electrical power rather than traditional heat exchange units.
Pre-reactor cooling	Both	130 °C → 25 °C	U-tube exchanger using chilled water (5 °C).
Reactor	Both	Adiabatic	N/A
CPC	Isolate	Acetonitrile (boiling point: 82 °C) and heptane (boiling point: 98.42 °C) (Appendix A) will be recycled into the process post-distillation, alongside the crude oil at 25 °C; this will all collectively be cooled to 25 °C.	U-tube exchanger using chilled water (5 °C).
Distillation column/s	Both	Varies from exchanger to exchanger - see Section 4.5 and Section 4.6 for greater detail. One distillation column for each CPC solvent stream will be required for the isolate model and three consecutive columns will be used in the full spectrum blend model.	Reboiler: kettle using high-pressure steam (242 °C). Condenser: U-tube using cooling water (25 °C).
Condenser	Both	130 °C → 25 °C	U-tube exchanger using chilled water (5 °C).

3.10. Minimisation and Disposal of Waste

A final, pertinent consideration of the design will be the minimisation and proper disposal of waste produced by the process. More information on some of the methods available to monetize waste, such as CO_2 can be found in Section 2.8, but in the case of more hazardous waste, such as heptane, recycling and careful disposal are required. An overview of the waste products produced by the process can be seen in Table 21, where the level of toxicity and the acceptable concentration present in the product are specified.

Table 21: List of waste compounds produced by the process.

Waste Compound	LD50 (mg/kg)	Acceptable Product Concentration (ppm)	Source
Water	N/A	N/A - not hazardous	
CO_2	N/A	N/A - gaseous at room temperature, thus ingestion is unlikely	
Ethanol	9000	5000	(ThermoFisher Scientific, 2003; López-Olmos *et al.*, 2022)
Acetonitrile	170 - 520	N/A - not relevant to process; acetonitrile will not be present in the product.	(Hashimoto, 1991)
Heptane	3000	5000	(ThermoFisher Scientific, 2021; López-Olmos *et al.*, 2022)
TBME	2000	N/A - not relevant to process; acetonitrile will not be present in the product.	(The OSHA Standard 29, 2012)
Ethanol extraction cake	Refer to ethanol	The cake is composed of the solid remnants of the cannabis buds, as well as traces of ethanol and crude cannabis oil. It will not be present within the product.	

As mentioned in Section 3.10, the best middle-ground between minimizing waste and ensuring product purity is solvent recovery. To achieve this, purification processes and recycle streams for ethanol, heptane, and the acetonitrile/TBME mixture, respectively, will be incorporated into the design.

To ensure product purity, distillation columns will be applied to retrieve the CBD oil and the remaining cannabis oil from heptane and acetonitrile/TBME, respectively. Ethanol will be

purified and recycled using a simple evaporation unit. All the solvent recycling processes will include a purge stream to ensure sensible solvent purity is maintained. Thus, even with the recycles and the associated mitigation of solvent losses, some solvent waste is unavoidable.

Referring to the waste compounds listed in Table 21, disposal will be required for waste solvents (i.e.: hazardous liquid waste), CO_2 (gaseous waste), and the ethanol extraction cake (solid waste). The disposal mechanisms to be used for each type of waste will be discussed.

In terms of solvent waste, it is important to note that all solvents used - ethanol, heptane, acetonitrile, and TBME - are hazardous, flammable, organic compounds and require extreme care upon disposal. The cake - while primarily plant material - will also be removed from the filters whilst saturated with ethanol and should be treated accordingly. Wastewater, once purified, can be recycled and used either as a solvent, once again, or as part of the heat transfer system. Finally, the CO_2 waste gas will need to be captured and disposed of as well.

The potential processes for the disposal of these waste products are explored in greater detail in Table 22 and Table 23.

Table 22: Disposal methods of process waste streams.

Waste Compound	Disposal Method
Organic solvents (ethanol, heptane, acetonitrile, and TBME)	Direct landfilling of these solvents is not permitted - all will require treatment before disposal. A breakdown of the flashpoints, flammability, toxicity, treatment options, available disposal methods, and the potential hazards associated with these chemicals can be found in Table 23.
Filter cake	The cake can be washed using water to remove ethanol; following this, the remaining cake consists only of plant matter and water and can be landfilled or composted, whilst the ethanol can be purified and recycled to the process.
CO_2	The CO_2 waste gas must be captured - beyond this, it can be disposed of underground via pipelines and injected into storage sites like depleted oil or gas reservoirs (Luo *et al.*, 2023).

Table 23: Summary of waste solvent flammability, toxicity, associated hazards, treatment options, and disposal methods.

	Ethanol	**Heptane**	**Acetonitrile**	**TBME**
Flashpoint	13 °C	-4.4 °C	2 °C	-28 °C
Flammability	Flammable - introduces fire and explosion risk.	Highly flammable - introduces fire and explosion risk.	Flammable - introduces fire and explosion risk.	Flammable - introduces fire and explosion risk.
Toxicity	Moderate toxicity when ingested, inhaled, or absorbed in larger quantities, causing dizziness, nausea, and skin irritation.	Low acute toxicity - inhalation of high concentrations can cause dizziness, headaches, and central nervous system effects. Can also cause skin and lung irritation. Risks environmental contamination.	Potential to cause dizziness; headaches; eye, skin, and respiratory irritation; and potential permanent health effects with long-term exposure. Risks environmental contamination.	Low acute toxicity - exposure to high concentrations can cause dizziness, headaches, and long-term effects on the nervous system. Can also cause skin and respiratory irritation. Risks environmental contamination.
Treatment	• Chemical treatment • Biological treatment	• Adsorption • Chemical treatment • Solidification	• Chemical treatment • Biological treatment Solidification	• Adsorption • Solidification
Disposal	• Incineration • Conversion to biofuel	• Incineration • Landfill disposal (if treated and solidified)	• Incineration • Landfill disposal (if treated and solidified)	• Incineration • Landfill disposal (if treated and solidified)

(The OSHA Standard 29, 2012; Labchem, 2013; Vallero, McLeod and Cherrett, 2019; Mehmood et al., 2021; ThermoFisher Scientific, 2021; Louisiana State University, 2023)

Depending on the solvent, methods of chemical and biological treatments, as well as solidification, will vary significantly, with some posing more of a challenge in their treatment and safe disposal than others. However, the proposed process - which will be a commercial set-up, rather than a large-scale industrial process - should produce relatively modest amounts of waste. The capital and running costs required to perform the relevant treatments on each waste product, and then dispose of each one safely and carefully, would be excessive for smaller waste volumes.

Thus, the economic analysis in Section 5 will operate on the assumption that waste treatment and disposal will be outsourced and the detailed exploration of treatment and disposal methods,

beyond the brief summaries provided in Table 22 and Table 23 and the disposal prices estimated in Section 5.2, will fall outside the scope of this review.

3.11. Process Summary

In Table 24, a summary of the key design choices explored in the technical review can be found. Details regarding the cultivation and enzyme procurement will not covered here, as they will not be incorporated into the simulation, but a background discussion of these processes can be found in Sections 3.2 and 3.3, respectively.

It is also worth noting the inherent limitations of the research. One major limitation is the scarcity of chemical and experimental data involving cannabinoids. The limited availability of empirical data necessitated the use of estimations for several different parameters, such as enzyme conversion rates. To ensure that these estimations were as conservative and sensible as possible, the dissertation relied on theoretical frameworks and existing literature where applicable. Nonetheless, the inherently theoretical nature of the research introduces some level of error, as many values had to be assumed for the purpose of calculations. To address this, sensitivity analyses (Section 5.5) were incorporated to evaluate the impact of uncertainties in these assumptions and to provide a measure of the robustness of the results.

Additionally, simulations used in the research are inherently subject to limitations, as they may not always translate effectively to practical applications due to unaccounted-for factors. For example, simulations may not fully capture the complexities of enzyme-substrate interactions in a real-world setting, such as potential enzyme denaturation or substrate inhibition, which can affect the accuracy of the predicted conversion rates.

The research's progression also heavily depends on finding, modifying, and assessing a specific enzyme for THC-to-CBD conversion, which represents only a small fraction of the larger research expedition required to bring this process to fruition. The development of such an enzyme is reliant upon ongoing and future research efforts, including enzyme engineering and optimization. This reliance on future research advancements highlights the preliminary nature of this study and its role in paving the way for more tangible research.

In summary, while the dissertation makes every effort to address these limitations through conservative estimations and sensitivity analyses, it is important to recognize the constraints

imposed by the theoretical nature of the research and the current limitations in empirical data. This discussion aims to provide a clearer understanding of these limitations and the steps taken to ensure the validity of research findings.

A discussion on the limitations of the research regarding generalisability has been included to ensure an honest and realistic representation of the study. It is important to note that the research is based on theoretical models and assumptions tailored to the theoretical cannabinoid conversion process. These specific parameters may not fully reflect the variability encountered in practical applications, potentially limiting the applicability of the results to different contexts.

For example, assumptions regarding enzyme performance and reaction conditions may not hold up in all scenarios. These assumptions are necessary for theoretical calculations but may restrict the extent to which the findings can be generalised. The theoretical nature of the research, without empirical validation of enzyme performance, also means that findings are preliminary and may not directly translate to practical applications without further validation.

There are, however, some strategies for enhancing generalisability. For one, future research should include empirical testing of the enzyme under various conditions to validate the theoretical predictions and improve generalisability. This, of course, will only be possible once the enzyme has been found or created.

Once this has been done, more accurate data regarding the enzyme can be included in a more rigorous future simulation. Expanding the range of parameters and conditions in this future simulation also provides insights into how results might vary and identify the boundaries of applicability. In this way, theoretical findings with data from experimental studies or related enzyme systems can enhance the robustness and applicability of the results.

Specific recommendations for future research could include exploring multiple enzyme candidates and developing experimental methods to evaluate and refine any new theoretical models and simulations.

Table 24: Summary of design decisions covered in the technical review.

Purpose	Equipment	Details
Pre-extraction	Milling	➢ Resultant bud fragment sizes <0.5 mm or slightly smaller.
Extraction	Mixer-settler	➢ Cold ethanol extraction. ➢ Operated between 5℃ and – 80℃ (a temperature of -35 ℃ has been selected for the simulation). ➢ Between 5 L ethanol/kg cannabis and 44 L ethanol/kg cannabis. ➢ Total extraction time of 55 minutes.
Upstream processing	Filters	➢ Series of 3 plate and frame filters of decreasing pore size, operated under pressure.
	Decarboxylation unit	➢ Operated at 130˚C. ➢ No buffers were added. ➢ Oil moves slowly and continuously through the cylindrical heating unit. ➢ 22.3 % of total mass lost (20 % water and 2.3 % carbon dioxide)
Reactor	PFR	➢ CLEAs immobilised onto the walls of a PFR, using a silica base. ➢ Operated at room temperature. ➢ Operated at a neutral pH. ➢ Assume no required cofactors, buffers, or inhibition. ➢ The enzyme is assumed to function as an isomerase.
Downstream processing	CPC	➢ Heptane (light phase) and acetonitrile mixed with TBME (heavy phase) will be used as solvents. ➢ 11 % TBME will be added to the acetonitrile stream. ➢ 150-minute runtime. ➢ For every 0.24 kg of feed entering the process per hour, the following amounts of solvent will be used: o 5.04–7.56 kg acetonitrile o 4.92–7.39 kg heptane o 0.59–0.88 kg TBME ➢ Can be altered to separate out other neutral cannabinoids like THC, CBN, and CBG as well. ➢ Operated at ambient pressure and temperature.
	Distillation	➢ Operated below the boiling point of CBD (180 °C). ➢ Crossflow, sieve trays with flat plate heads will be used. ➢ R/Rmin should be between 1.2 and 1.5. ➢ Vapour linear velocity (u) should be between 0.05 m/s and 3 m/s (0.3 – 3 m/s for tray columns, specifically). ➢ Separate distillation columns will be used to separate CBD from heptane, and the remaining cannabis compounds from acetonitrile, respectively, for the isolate model. ➢ Three consecutive distillation columns will be applied in the full spectrum model to reduce THC concentrations to acceptable levels.
Heat exchangers	➢ U-tube shell and tube heat exchangers ➢ Kettle reboilers (distillation columns)	➢ U-tubes will be horizontally configured, with a one-pass shell. ➢ Total condensers and -reboilers will be applied in the distillation columns. ➢ See Table 19 for greater detail on individual units.
Waste minimisation	➢ Solvent and wastewater purification and recovery ➢ Waste disposal	➢ Ethanol, heptane, and the acetonitrile/TBME mixture are recovered through distillation and recycling. ➢ Disposal will be required for purged solvents (hazardous liquid waste), CO_2 (gaseous waste), and ethanol extraction cake (solid waste). ➢ Wastewater, once purified, is recycled and used as a solvent part of the heat transfer system.

4. Process Modelling

4.1. Design Outline

An overview of each process stage can be found in Figure 5 and Figure 6, Section 3, in the form of block flow diagrams, where the former represents the process to produce a CBD isolate, and the latter produces a full spectrum blend containing other cannabinoids beyond CBD, as well as other compounds like flavonoids and terpenes.

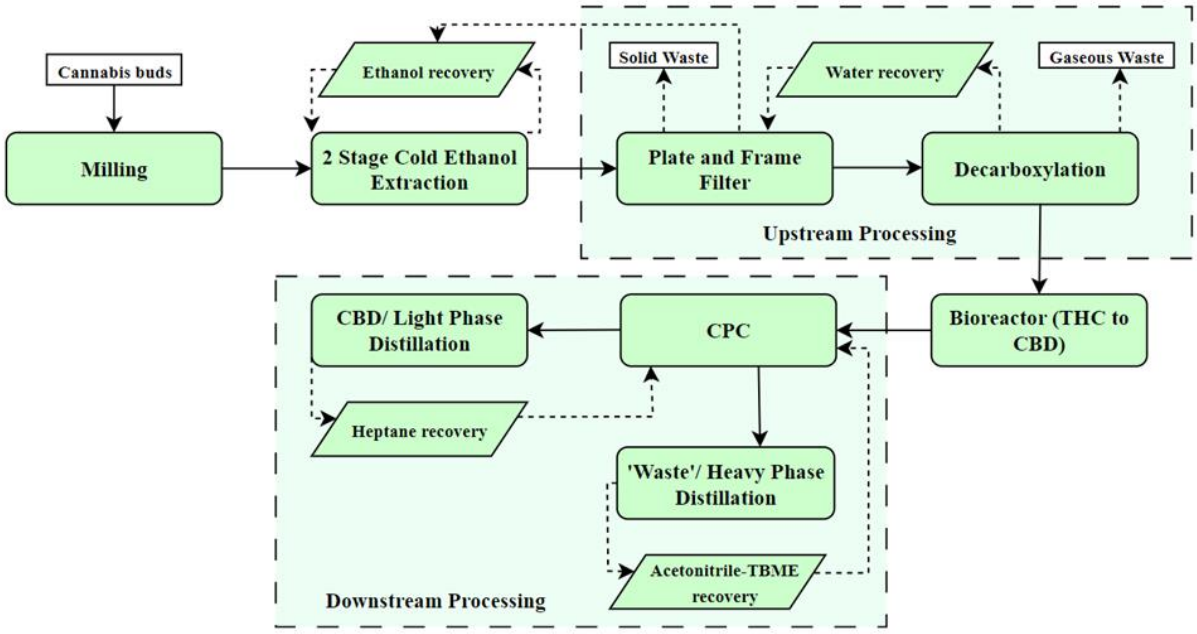


Figure 14: Block flow diagram overview of the process producing CBD isolates.

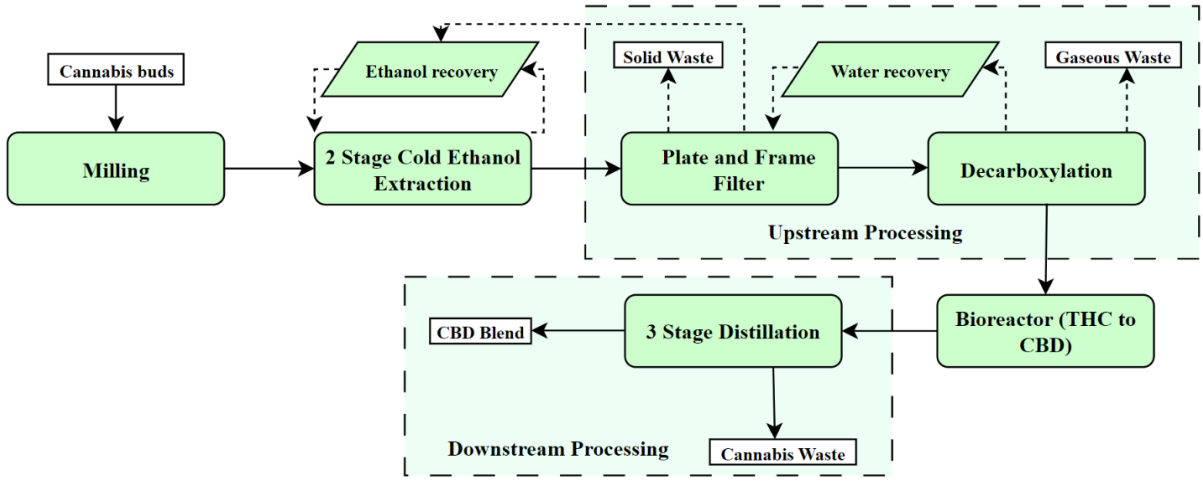


Figure 15: Block flow diagram overview of the process producing CBD blends.

A more detailed overview of the respective processes can be found in Figure 16 for the isolate process, and Figure 17 for the full spectrum blend process, in the form of process flow diagrams. An overview can be found in Section 3.11, which details each stage, including the processes occurring in each design and an overview of the equipment and parameters relevant to each. For a step-by-step description of each process unit, see Section 4.1.

What will henceforth be referred to as the isolate model – i.e., the process producing CBD isolate – is composed of four primary parts: the extraction of the crude oil (including the pre-extraction process) (1), the upstream processing of the oil (2), the reaction of THC into CBD (3), and the downstream processing of the resultant oil (4). The alternate process, henceforth referred to as the blend model, is similarly structured but includes a different variation of the fourth process stage (the downstream processing) which excludes CPC. Beyond this, solvent recovery systems, heating and cooling apparatus, and purification processes exist in both cases.

After milling (SR-101), the cannabis buds will pass through cooler EC-101, followed by two consecutive mixer-settler extraction units MSX-101 and MSX-102, inside which the buds will undergo cold ethanol extraction. The ethanol used in the process is cooled before entering the extractors using electric cooling units EC-103 and EC-104. This is the extraction segment (1).

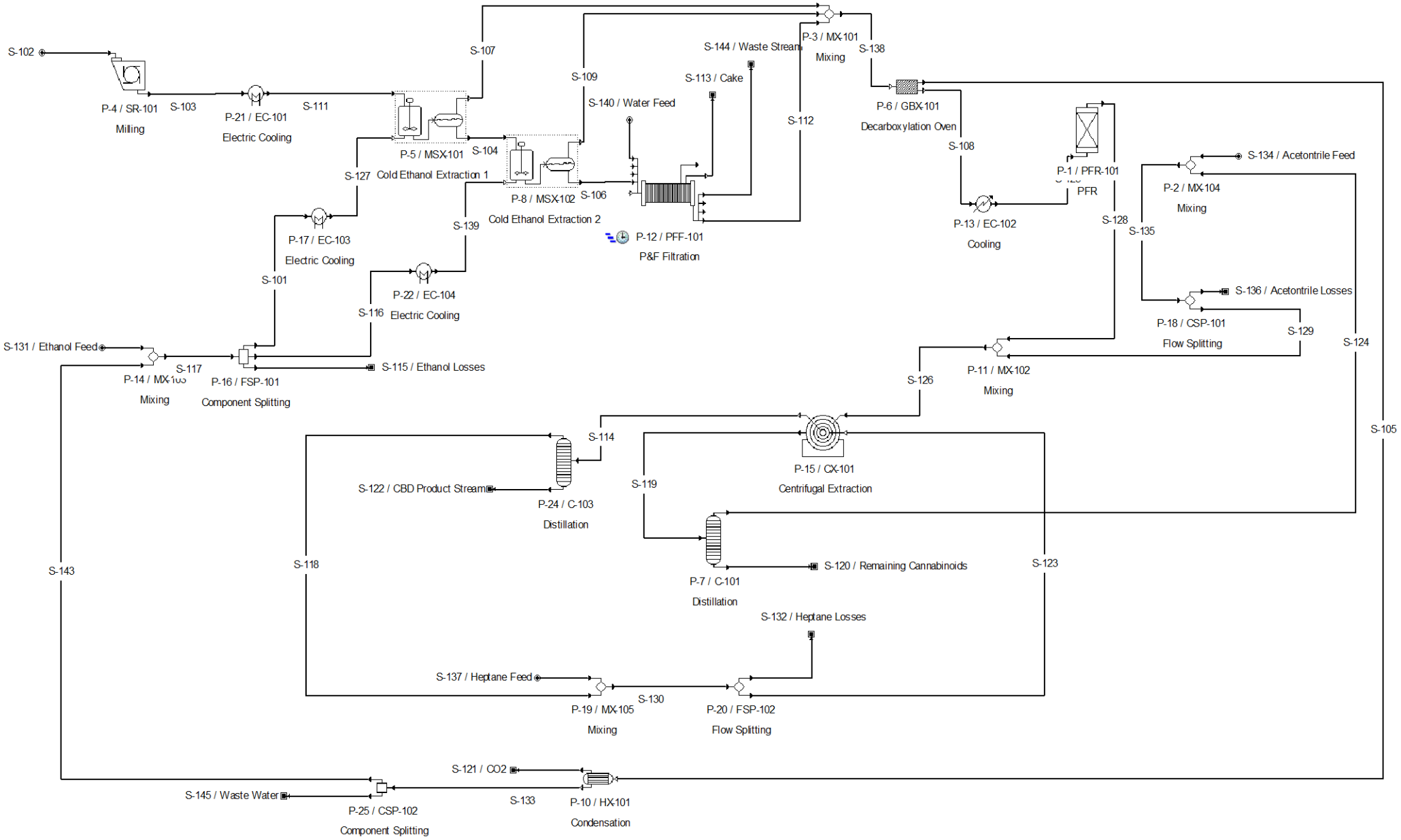


Figure 16: Process flow diagram for the proposed production of CBD isolates designed using SuperPro Designer.

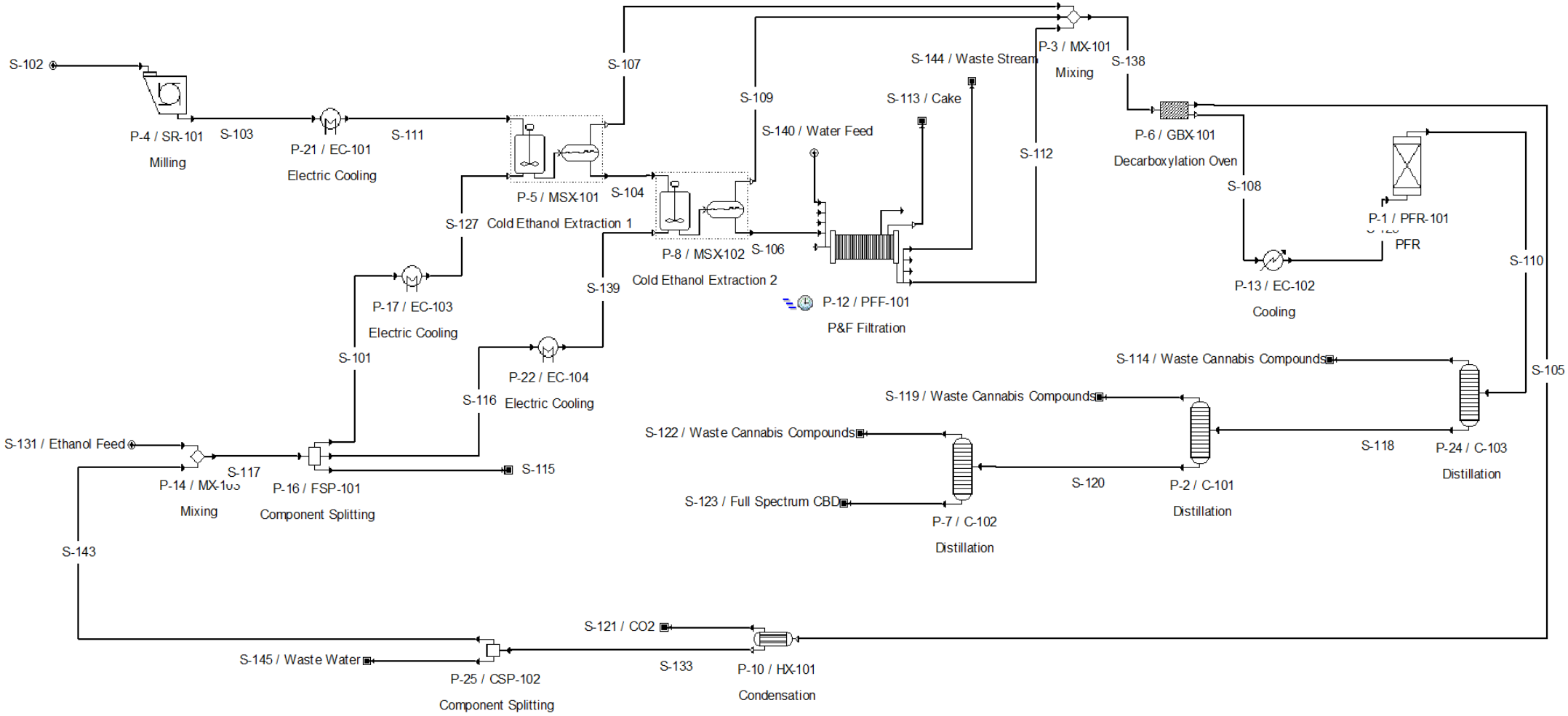


Figure 17: Process flow diagram for the proposed production of full spectrum CBD tinctures designed using SuperPro Designer.

Next, the oil-plant matter mix will pass through the plate-and-frame filtering system PFR-101 alongside a water feed stream and will be separated into a cake consisting primarily of solid matter, a waste stream consisting primarily of water and ethanol, and the cannabis crude oil. The filtrate will be reunited with the light phases of the extraction units using mixer MX-101.

The oil will then move the decarboxylation oven GBX-101, which will convert the cannabinoids into their neutral forms, producing CBD and THC from CBDA and THCA respectively, and releasing CO_2. The heat of the oven results in the vaporisation of any residual ethanol and water, which will then pass through two separating units (condenser HX-101, which separates out the CO_2, and splitter CSP-102, which separates out the water). The remaining ethanol enters the recycle-purge loop consisting of mixing unit MX-103 and splitting unit FSP-101, with the recycled ethanol being fed back into the extraction units. The cannabis oil is then cooled EC-102. This is the upstream processing (2).

The oil then passes into a PFR, PFR-101, where the CLEAs will catalyse the reaction of THC into CBD. This is the reaction segment (3) and going forward into segment (4) is where the designs diverge.

In the case of the isolate process, the oil moves into the downstream processing section (4), and the oil is mixed MX-102 with an acetonitrile-TBME stream before entering the CPC, CX-101, alongside an accompanying heptane stream. During the chromatographic process, most of the CBD will move into the heptane stream, which upon exiting the CPC process, will then move to a distillation column C-103, with the CBD isolate product stream emerging from the final distillation column.

The top streams will consist primarily of heptane and will be recycled via mixer MX-105 and splitter FSP-102, before being fed back into the CPC. It is important to reiterate that the chromatographic process separates cannabinoids into distinct fractions rather than all components moving together through the system. Referring specifically to CBD moving into the heptane stream is done for simplicity. In practice, other cannabinoids will also move into the hexane phase, but they will exit in separate fractions as part of the CPC's purpose of isolating each compound.

The acetonitrile-TBME stream exiting the CPC will contain the cannabinoids and remaining cannabis compounds apart from CBD and will enter the distillation column C-101 to separate them, after which the solvent stream will be recycled via mixer MX-104 and splitter FSP-101, before being mixed with more crude oil and fed back into the CPC. The bottom stream of the

distillation column C-101 can then be separated further into other valuable cannabinoids using the same CPC, but this will be discussed further in Section 5.

The full spectrum model involves a different downstream process (4); instead of CPC, the oil is passed into a series of distillation columns to reduce THC to the required concentrations (> 0.001 %). It requires three consecutive distillation columns (C-101, C-102, and C-103) to reduce THC to acceptable limits; the full spectrum CBD oil product can be collected from the bottom stream of the final column (C-102). The top streams of each column consist primarily of mixed cannabis compounds.

In this section, the final details of each piece of equipment used in the process will be given, section by section. For greater detail regarding the process units, see Appendix E. An overview of the process mass balance can be found in Appendix D. In particular, the relevant vapour-liquid equations for CBD and THC were attempted (see Appendix B) but due to a scarcity of relevant data and conflicting results, the relevant properties where instead predicted by SuperPro through comparison to compounds with similar boiling points – this should be noted as a potential source of predictive errors in the simulation.

A breakdown of equipment cost and capacity for both processes can be found in Section 5.4 and Section 6, and energy consumption tables, as well as mass and energy balances, can be viewed in Appendix F.

4.2. Extraction

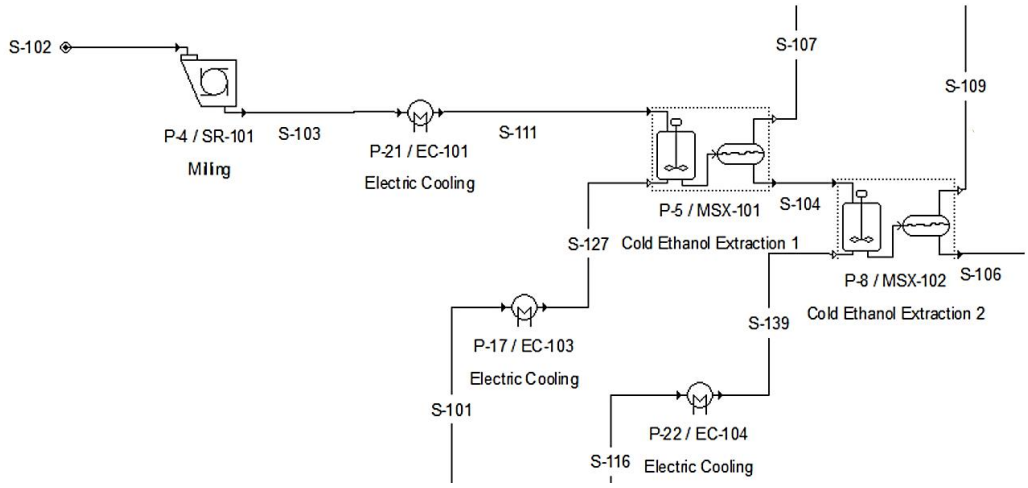


Figure 18: The extraction section for both the production of CBD isolates and CBD blends.

Cannabis Mill (SR-101)

The cannabis buds enter the mill operating at atmospheric pressure and room temperature. For the sake of the simulation, the 'reaction' occurring within the process will be as follows, as discussed in Section 3.4, in terms of mass percentages:

$$100 * Cannabis\ Buds \rightarrow 5 * CBD + 60 * Solids + 5 * THC + 10 * Undesirable\ Compounds + 20 * Water$$

Equation 1

In reality, no reaction occurs, and the surface area of the buds is simply increased through milling, allowing the extraction solvent (cold ethanol) a greater mass transfer area, and thus greater access to the compounds. This is simply a method of inputting the way the cannabis buds 'break down' into constituents of interest in the simulation. An overview of how a unit mass of cannabis bud breaks down into these constituents, in terms of mass percentages, can be found in Table 2 – this was used to formulate Equation 1.

The milling unit is modelled as a continuous process operating at a rate of 0.65 kg/h (the basis value for the mass of bud fed into the process) and constructed from carbon steel. While carbon steel is more susceptible to rust than a material like stainless steel, it is mechanically strong and comparatively inexpensive; due to the buds being milled dry, without the use of solvent or water, the rusting issue is not particularly pertinent, thus making carbon steel an economical choice.

Mixer-Settler Extraction Units (MSX-101 and MSX-102)

Both extraction units are operated adiabatically, at atmospheric pressure. Due to the lack of available VLE data (see Appendix B), component splits were specified according to the discussion in Section 3.5.4, where the percentages of CBD and THC recovered using ethanol extraction were calculated in Table 5. It is assumed the undesirable compounds are split evenly between streams. The component splits applied in both extraction units can be found in Table 25, where each percentage listed indicates the mass percentage of that specific component

exiting each individual extraction unit from the top phase; the remainder exits via the bottom phase.

Table 25: Extraction unit component splits.

Component	Percentage to Top Phase
Ethanol	90
CBD	58.32
THC	54.91
Undesirable Compounds	50
Water	90
Solids	0

The mixer residence time was assigned as 30 minutes and the settler residence time as 25 minutes (55 minutes total) with both extraction units being constructed of stainless steel. The corrosion-resistant nature of stainless steel (as opposed to carbon steel, for example) will increase the lifespan of the extraction units, reducing long-term maintenance costs.

SuperPro Designer calculates extraction unit 1 (MSX-101) to have a throughput of 6.01 L/h, an ethanol flow of 5.557 L/h (giving an ethanol-cannabis ratio of 8.55 L/kg, which falls within the acceptable range given in Section 3.5.4), a mixer volume of 3.005 L and a settler volume of 2.504 L.

For extraction unit 2 (MSX-102), throughput is calculated to be 4.07 L/h, the mixer volume is calculated as 2.036 L, and the settler volume as 1.696 L, with an ethanol flowrate of 3.242 L/h (for a ratio of 7.84 L/kg cannabis, which is also within the acceptable range). The heavy phase stream, containing the solid impurities, is then passed through the filtration system.

Pre-Extraction Cooler 1, 2 and 3 (EC-101, EC-103 and EC-104)

The three heat exchangers are required to cool the extraction input streams (cannabis buds for EC-101 and ethanol for EC-103 and EC-104) to the temperature required by the process (-35 °C). The cannabis must be cooled from room temperature, whilst the streams from the ethanol recycle must be cooled from 78.3 °C; because of the extremely cold temperature required, the traditional heat exchangers modelled by SuperPro were incapable of achieving this change due to the heat transfer medium limitations.

Thus, to simplify heat transfer for the process, electric coolers are used in the simulation. All coolers are composed of stainless steel with performance coefficients of 4.5. EC-101 has an operating power of 0.0118 kW, EC-103 has an operating power of 0.08 kW and EC-104 has an operating power of 0.0638 kW.

4.3. Upstream Processing

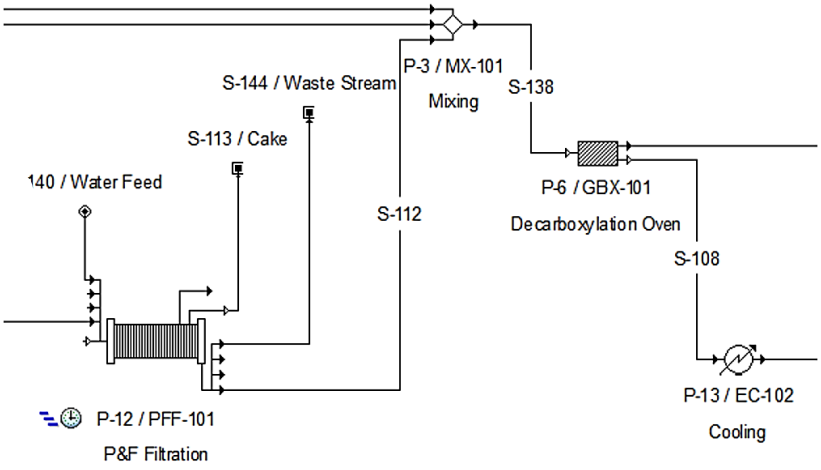


Figure 19: The upstream processing section for both the production of CBD isolates and CBD blends.

Plate-and-Frame Filtering System (PFF-101)

The plate-and-frame filter (PFF) has two input streams, both entering at atmospheric pressure and -35 °C to ensure the removal of the undesirable waxes (as discussed in Section 3.5.4) – consisting of the cannabis stream exiting the extraction units and one composed of wash water. The flow rate of water required is calculated as 0.6839 kg/h.

The filter area is reported as 0.0077 m^2, with a vessel volume of 3.871 L, where the vessel is constructed of stainless steel. The three exiting streams are the cake, a liquid waste stream, and the cannabis crude oil. A crude oil stream absent of solid plant residue is produced, consisting primarily of ethanol (91.42 %), with a collective flow rate of 0.3308 kg/h. The cake is reported as 0.4107 kg/h, composed of solid plant residues and water, and consisting of negligible CBD and THC, post-washing. Finally, the waste stream has a flowrate of 0.6805 kg/h and consists primarily of water (96.45 %) – though 0.00028 kg/h of CBD and 0.00033 kg/h of THC are also lost to this stream.

Ethanol Extraction Mixer (MX-101)

The mixer is operated at atmospheric pressure and constructed using stainless steel, combining the light phase output streams from both extraction units and the extraction unit heavy phases, post-filtration, before entering the decarboxylation oven.

Decarboxylation Oven (GBX-101)

The crude oil stream exiting the filter enters the decarboxylation oven at -35 °C and contains both ethanol and water alongside the cannabis crude oil. The decarboxylation oven was modelled from a stainless steel 'generic box,' and is heated to 130 °C with a continuous throughput of 7.619 kg/h and an input stream composed primarily of ethanol (96.60 %).

The oven uses steam (152 °C) as a heat transfer agent at a rate of 4.43 kg/h and splits the feed into two streams – one gaseous and one liquid. The gaseous exit stream consists of water, ethanol, trace amounts of undesirable compounds, and the CO_2 liberated due to the decarboxylation process (a waste stream to be purified and partially recycled). The liquid exit stream consists of decarboxylated crude oil.

Due to a lack of specific literature regarding the specific reaction coefficients, a reaction formula was compiled using the baseline equation shown in Equation 2, with each mole of THCA and CBDA (combined into the broader category of 'undesirable compounds') releasing one carboxyl group, released in the form of CO_2, as they are decarboxylated into THC and CBD respectively. Thus, for every one mole of 'undesirable compounds,' two CO_2 molecules are released, alongside one mole of THC and one mole of CBD. These molar values were then converted into mass ratios using molar mass values and, using the estimated mass percentage of CO_2 produced from decarboxylation (2.3 %), as discussed in Section 3.6, Equation 3 was developed for the decarboxylation reaction in terms of mass fractions. Iterative methods were used alongside the simulation to find a conversion rate capable of achieving a 2.3 % mass ratio of CO_2 upon exiting the decarboxylation oven, ultimately yielding a 40 % conversion rate for the decarboxylation reaction. A full breakdown of the calculations performed can be found in Appendix C.

$$Undesirable\ Compounds \rightarrow 2 * CO2 + CBD + THC$$

Equation 2

$$Undesirable\ Compounds \rightarrow 0.1228 * CO2 + 0.4386 * CBD + 0.4386 * THC$$

Equation 3

Upon exiting the oven, the oil will be composed of 30.54 % undesirable chemicals, 34.71 % THC, and 34.75 % CBD, with a flow rate of 0.1252 kg/h.

Post-Decarboxylation Cooler (EC-102)

The stainless steel cooler uses chilled water (5 °C) at a flow rate of 1.07 kg/h to change the temperature of the crude oil stream from 130 °C to 25 °C.

CO_2 Separating Unit (HX-101)

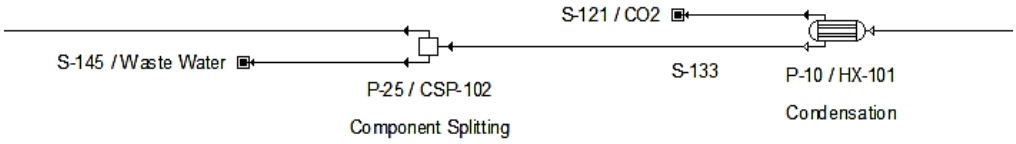


Figure 20: The separation of CO2 and water in both the production of CBD isolates and CBD blends.

The stream entering HX-101 is composed primarily of ethanol (98.89 %), as well as water, CO_2, and undesirable cannabis compounds in smaller amounts, with a total continuous flow rate of 7.50 kg/h.

The temperature is cooled to 25 °C at atmospheric pressure using chilled water (366.90 kg/h, entering at 5 °C). All CO_2 remains in its gaseous phase, exiting the process in an output stream also containing trace amounts of water and undesirable compounds. The gas stream also contains a percentage mass of 11.76 % ethanol and a collective flowrate of 0.0036 kg/h; it can then be collected and disposed of while the remaining components exit the unit in a liquid stream.

The separating unit is constructed using stainless steel, with a heat transfer area of 0.019 m^2.

Water Separating Unit (CSP-102)

The exit stream from the CO_2 separating unit now enters a stainless steel water separating unit (CSP-102). The stream – containing predominantly ethanol (98.92 %) – is heated to 90 °C, at a pressure of 2.5 bar, with an operating throughput of 7.495 kg/h. Two streams are produced – a wastewater stream of 0.1301 kg/h, containing 99.85 % water with the remainder consisting of undesirable compounds, and an ethanol stream of 7.365 kg/h, composed of over 99.99 % ethanol with trace amounts of undesirable compounds. The ethanol stream then enters the ethanol recycle.

Ethanol Recycle (Mixer MX-103 and Splitting Unit FSP-101)

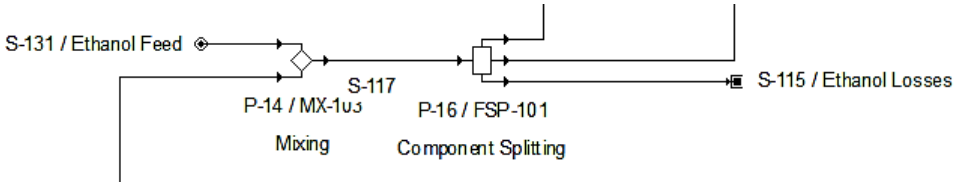


Figure 21: The ethanol recycle section for both the production of CBD isolates and CBD blends.

The ethanol stream from the water-separating unit enters the mixer alongside a fresh ethanol feed of 0.4 kg/h. The combined stream comes to 7.765 kg/h at a temperature of 78.3 °C. The splitting unit then produces three streams – a purge (5 % of the input), a stream leading into extraction unit 1 (MSX-101) (60 % of the input), and a stream leading into extraction unit 2 (MSX-102) (35 % of the input).

Both units are constructed from stainless steel, operating at atmospheric pressure, producing ethanol streams of above 99.99 % purity, containing only trace amounts of undesirable compounds.

4.4. Reaction

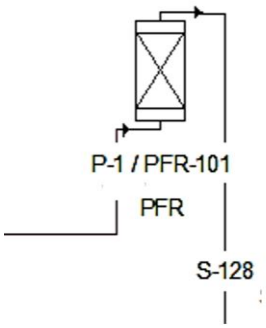


Figure 22: The reactor used in the production of CBD isolates and CBD blends.

PFR (PFR-101)

The PFR, constructed from stainless steel, operates adiabatically at 25 °C and atmospheric pressure. The PFR uses CLEAs immobilised to the reactor walls using a silica base to catalyse the remediation reaction shown in Equation 4.

$$THC \rightarrow CBD$$

Equation 4

The reactor has a 1-hour residence time with a 0.15 L working volume. The flow rate through the PFR is 0.1252 kg/h, and a conversion of 85 % is assumed. It is worth noting that enzyme conversion rates can be highly variable, even among isomerases, due to differences in substrate specificity, reaction conditions, and enzyme modifications. This broad range of possible conversion rates presents a significant challenge in providing a detailed analysis of what constitutes a 'sensible' conversion rate for the theoretical enzyme.

Considering this, a more detailed analysis is constrained by the current stage of the research. The chosen conversion rate reflects a balance between realism and practicality, aiming to ensure the process design is both feasible and conservative.

CBD has a mass percentage of 34.75 % in the input stream, increasing to 64.26 % in the exit stream, whereas THC has a mass percentage of 34.71 %, decreasing to 5.21 % in the exit stream.

To achieve this conversion, the PFR is calculated to be 0.15 L, with a length-to-diameter ratio of 3 and a design pressure of 1.520 bar. From this data, the mass of the enzyme required to catalyse the reaction was calculated, assuming a negligible change in volume and constant pressure. The calculation can be found in Appendix C, yielding an amount of 37.46 kg of the enzyme per year to achieve conversion, assuming replacement is required every seven days of operation.

4.5. Downstream Processing: Isolate Model

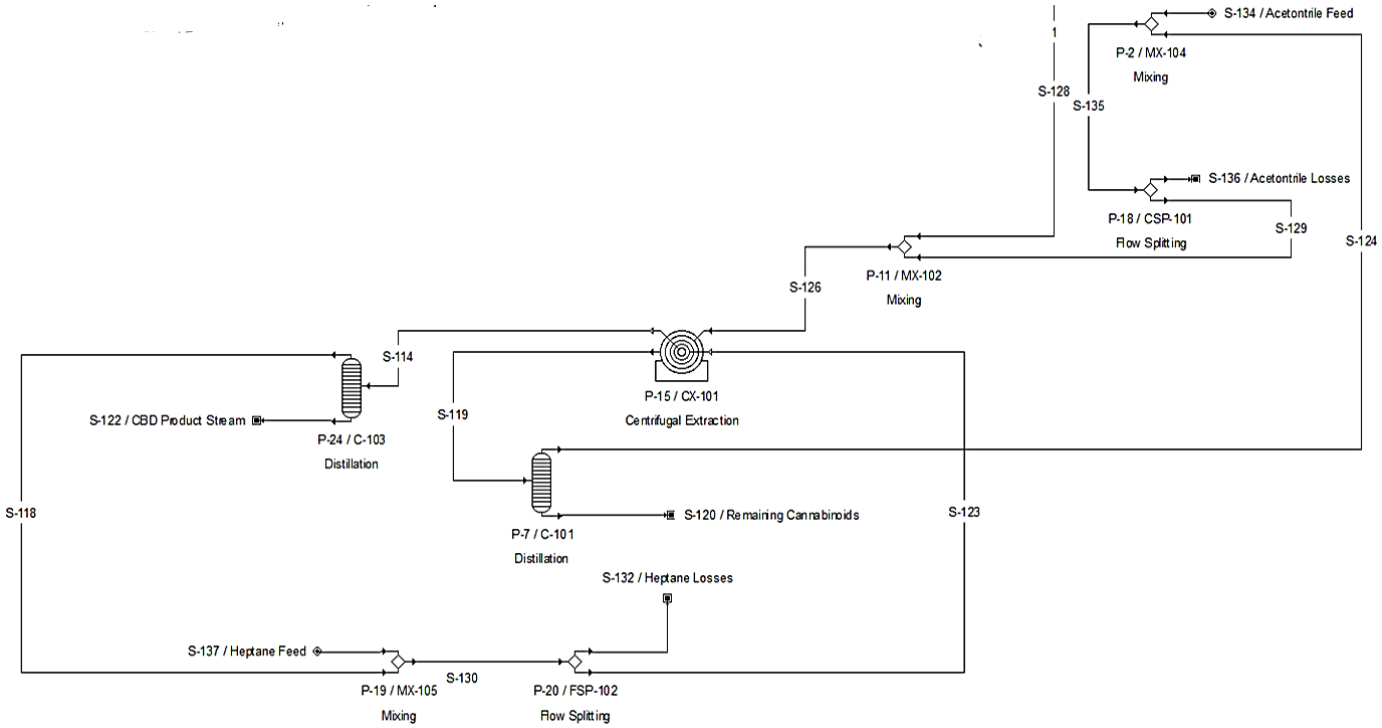


Figure 23: The downstream processing section in the production of CBD isolates.

Oil-Acetonitrile-TBME Mixer (MX-102)

The mixer is operated at atmospheric pressure and was constructed using stainless steel, combining the acetonitrile-TBME solvent stream as it exits the solvent recycle, and the oil stream. The collective flow rate through the mixer is 3.596 kg/h and the mixture temperature is 61.6 °C.

CPC (CX-101)

The acetonitrile-TBME-oil stream enters the CPC as the heavy phase, and the heptane stream (94.8 °C) from the solvent recycle enters as the light phase. The CPC is operated continuously, adiabatically, and at atmospheric pressure. It has 5 equivalent theoretical extraction stages.

Because of the notable lack of partition coefficient information, the process is simulated according to component splits (estimated using the data in Section 3.8.4), and 99 % of the CBD is separated by the light phase. The CPC has a throughput of 8.91 L/h and is composed of stainless steel, with a crude oil feed of 0.1252 kg/h.

Upon entering the CPC, the light phase (2.846 kg/h, which is within the acceptable range calculated in Table 16) is composed primarily of heptane (99.97 % mass percentage), with trace amounts of CBD and undesirable chemicals due to the heptane recycling process.

Upon entering the CPC, the heavy phase (3.596 kg/h) is composed of 86.43 % acetonitrile, 10.08 % TBME, and the remainder consisting of the cannabis crude stream mixed into the heavy phase (THC, CBD, and the remaining cannabis undesirable compounds). The acetonitrile segment of the stream makes up 3.108 kg/h of the aforementioned flow rate, while TBME has a flow rate of 0.3626 kg/h (both within the range calculated in Table 16).

Upon exiting the CPC, the light phase has a flow rate of 2.926 kg/h and is composed of 97.23 % heptane, and 2.76 % CBD, with the remainder consisting of undesirable compounds. The output heavy phase has a flow of 3.516 kg/h and is composed of 88.40 % acetonitrile, and 10.31 % TBME, with the remainder of the stream consisting of cannabis compounds, including 0.01 % of the CBD originally entering the CPC.

Heptane Distillation Column (C-103)

The light phase enters distillation column C-103. According to the guidelines put forth in Section 3.8.3, the R/Rmin is input as 1.250 and the vapour linear velocity is input as 3.00 m/s for the column. The simulation calculated the remaining variables required to achieve the desired CBD product purity (>99 % CBD) with heptane concentrations below the limits discussed in Section 3.10 (5000 ppm, or 4.994 g/L).

The column, constructed using stainless steel, is calculated to have 19 actual stages, a diameter of 0.011 metres, a height of 7.6 metres, a stage height of 0.4 metres, a reflux ratio of 0.013, and a design pressure of 1.013 bar. Column pressure is atmospheric. The heat transfer area of both the condenser and reboiler is calculated as 0.004 m^2. The cooler has a temperature of 98.5 °C, a duty of 0.3 kW, and uses cooling water (25 °C) at a rate of 44.0 kg/h as a heat transfer agent. The reboiler has a temperature of 159.7 °C, a duty of 0.3 kW, and uses high-pressure steam (242 °C) at a rate of 0.6 kg/h as a heat transfer agent.

The distillate stream has a flowrate of 2.846 kg/h, and is composed of 99.97 % heptane, with trace amounts of CBD and undesirable chemicals; after distillation, it enters the heptane recycle. The product stream has a flowrate of 0.0804 kg/h and is composed of 99.47 % CBD (above the 99 % purity mark, and thus acceptable for an isolate), and 0.35 % heptane, at a concentration of 3.378 g/L (i.e., below the 4.9943 g/L limit).

Heptane Recycle (Mixer MX-105 and Splitter FSP-102)

The distillate stream from the heptane distillation column enters the mixer alongside a fresh heptane feed of 0.15 kg/h. The combined stream comes to 2.996 kg/h at a temperature of 94.8 °C. The splitting unit then produces two streams – a purge (5 % of the input) and the solvent stream leading into the CPC).

Both units are constructed from stainless steel, operating at atmospheric pressure, and producing a heptane solvent stream of 99.97 % purity with only trace amounts of undesirable compounds and CBD.

Acetonitrile-TBME Distillation Column (C-101)

The heavy phase enters distillation column C-101 after exiting the CPC. According to the guidelines put forth in Section 3.8.4, the R/Rmin is input as 1.25 and the vapour linear velocity is input as 3.00 m/s for the column.

The column, constructed using stainless steel, is calculated to have 14 actual distillation stages, a diameter of 0.018 metres, a height of 5.6 metres, a stage height of 0.4 metres, a reflux ratio of 0.043, and a design pressure of 1.013 bar. Column pressure is assumed to be atmospheric.

The cooler has a temperature of 80.3 °C, a heat transfer area of 0.013 m^2, a duty of 0.7 kW, and uses cooling water (25 °C) at a rate of 119.4 kg/h as a heat transfer agent. The reboiler has a temperature of 159.2 °C, a heat transfer area of 0.008 m^2, a duty of 0.7 kW, and uses high-pressure steam (242 °C) at a rate of 1.4 kg/h as a heat transfer agent.

The distillate stream has a flowrate of 3.454 kg/h, and is composed of 89.54 % acetonitrile, and 10.45 % TBME, with trace amounts of undesirable chemicals; after distillation, it enters the acetonitrile-TBME recycle. The bottom stream has a flow rate of 0.0623 kg/h and is composed primarily of undesirable compounds (61.06 %), acetonitrile (24.93 %), TBME (2.91 %), with lesser amounts of THC and CBD as well. For a more detailed breakdown of the bottom stream, see the discussion on alternative cannabinoid products in Section 3.8.4, and the economic breakdown of this in Section 5.

Acetonitrile-TBME Recycle (Mixer MX-104 and Splitter FSP-101)

The distillate stream from the acetonitrile-TBME distillation column enters the mixer alongside a fresh acetonitrile-TBME feed of 0.20 kg/h (89.55 % acetonitrile and 10.45 % TBME). The combined stream comes to 3.654 kg/h at a temperature of 62.6 °C. The splitting unit then produces two streams – a purge (5 % of the input) and a stream to be passed to mixer MX-102, which will then be combined with the cannabis oil before entering the CPC).

Both units are constructed from stainless steel and operated at atmospheric pressure, producing a solvent stream containing 89.54 % acetonitrile and 10.45 % TBME, with trace amounts of undesirable compounds.

4.6. Downstream Processing: Full Spectrum Blend Model

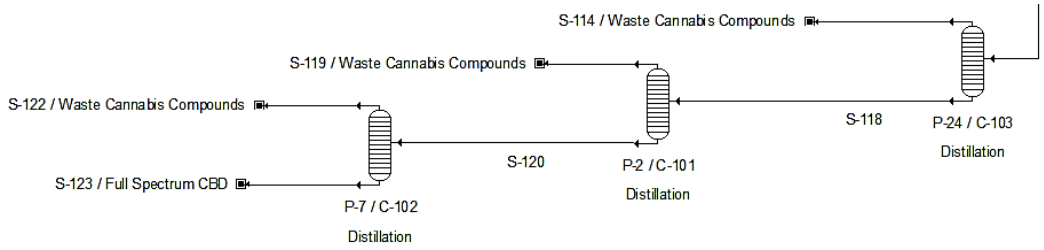


Figure 24: The downstream processing section in the production of CBD blends.

Consecutive Distillation Columns (C-101, C-102 and C-103)

After exiting the PFR, the oil moves through 3 consecutive distillation columns to reduce the THC concentrations to below acceptable limits (< 0.001 %). The oil first moves through column C-103, whereafter the bottom stream passes into column C-101, after which the bottom stream enters column C-102. The bottom stream from C-102 is the final full spectrum CBD blend produced by the process.

According to the guidelines put forth in Section 3.8.4, the R/Rmin is input as 1.30 and the vapour linear velocity is input as 3.00 m/s for all three columns. All three columns are also constructed using stainless steel, operated at 0.5 bar, with a reflux ratio of 0.013, and are calculated to have 34 actual distillation stages each.

Column C-103 has a diameter of 0.003 metres, a height of 13.60 metres, and a stage height of 0.4 metres. The cooler has a temperature of 153.0 °C, a heat transfer area of 1.706 cm^2, a duty of 21.4 W, and uses cooling water (25 °C) at a rate of 3.7 kg/h as a heat transfer agent. The reboiler has a temperature of 149.4 °C, a heat transfer area of 3.126 cm^2, a duty of 28.9 W, and uses high-pressure steam (242 °C) at a rate of 0.1 kg/h as a heat transfer agent.

Column C-101 has a diameter of 0.002 metres, a height of 13.60 metres, and stage height of 0.4 metres. The cooler has a temperature of 153.2 °C, a heat transfer area of 0.850 cm^2, a duty of 10.7 W, and uses cooling water (25 °C) at a rate of 1.8 kg/h as a heat transfer agent. The reboiler has a temperature of 146.8 °C, a heat transfer area of 1.121 cm^2, a duty of 10.7 W, and uses high-pressure steam (242 °C) at near-negligible rates as a heat transfer agent.

Column C-102 has a diameter of 0.001 metres, a height of 13.60 metres, and stage height of 0.4 metres. The cooler has a temperature of 153.1 °C, a heat transfer area of 0.427 cm^2, a duty

of 5.4 W, and uses cooling water (25 °C) at a rate of 0.9 kg/h as a heat transfer agent. The reboiler has a temperature of 143.6 °C, a heat transfer area of 0.542 cm^2, a duty of 5.3 W, and uses high-pressure steam (242 °C) at near-negligible flow as a heat transfer agent.

The bottom stream of column C-102, the final column of the sequence, has a flowrate of 0.0828 kg/h and is composed of 94.23 % CBD, negligible THC, with the remainder consisting of other cannabis compounds. Collectively, the three distillate streams have a flow of 0.0424 kg/hour, resulting in the loss of 0.0024 kg/h CBD (a loss of 0.0289 kg per every kg of the CBD blend produced).

4.7. Product Preparation

After the CBD oil (whether full spectrum or isolate) has been collected from the bottom stream of the distillation column, it should be mixed with a carrier oil, as discussed in Section 3.8.1 and Section 5.2.

Once processing of the oil is completed, it should be stored and, when relevant, transported in dark, cool conditions, in sealed containers; this is because light, heat, and contact with air tend to accelerate the rate of cannabinoid decomposition, and thus precautions must be taken to maintain product integrity (Golombek *et al.*, 2020; Mazzetti *et al.*, 2020).

5. Optimisation & Economic Analysis

5.1. Economic Analysis Overview

The bulk of the economic analysis is performed internally by SuperPro in terms of US dollars and will be presented as such with the methods of calculation and base values used presented alongside it where relevant. Any economic values taken from data have been converted into dollars and thus may vary slightly based on conversion rate fluctuations. It is also worth noting that variables such as utility costs, construction costs and material costs have been taken from the SuperPro databanks unless stated otherwise. As this is not a South African programme, some of these costs may not be entirely accurate if one wishes to view the design in a strictly South African context.

Several additional calculations outside of the scope of SuperPro are required to properly understand the economic implications of the process as described, however. These calculations include:

- The cost of the required enzyme.
- Comparison between producing one's own enzyme and purchasing the enzyme.
- Net revenue received from producing CBD isolates and CBD full spectrum blends after incorporating them into a carrier oil.
- Cost comparison between growing one's own cannabis and purchasing it.
- Potential income from separating additional cannabinoids using the CPC and selling them as ancillary products.

Therefore, this economic analysis will first involve a sensitivity analysis and optimisation based on SuperPro's simulated values; this can be found in Sections 5.3 and 5.5. The ancillary calculations listed above will then be incorporated into the SuperPro-generated economic analysis in Section 5.2. Finally, the profitability of the CBD isolate will then be compared to that of the full spectrum blend in Section 5.4.

5.2. Ancillary Calculations

Enzyme Cost (Produced versus Purchased)

The costs and immobilisation prices of enzymes are highly variable and further complicated by the theoretical nature of the enzyme. From Ferreira and associates' research (2018) on free cellulase enzymes, overall production costs of $ 40 – 70 /kg where reported. This was amongst the cheaper examples of enzyme production. Han and associates (2012), on the other hand, produced enzymes to convert starch into inositol via complex enzymatic biosystems consisting of *α-glucan phosphorylase* aGP, *phosphoglucose mutase* PGM, *inositol 1-phosphate* synthase IPS, and *inositol monophosphatase* IMP. A free enzyme mixture, in this case, costs $ 150 /kg, with co-immobilised multi-enzymes costing $ 750 /kg. However, this was for a complex enzyme system, rather than for a singular enzyme, as is the case in the proposed process.

From these values, loose costs for the theoretical enzymes can be assumed. For the sake of gauging the process's financial viability, intermediate values of $ 100 /kg to produce free enzymes and $ 500 /kg for immobilisation will be assumed, yielding an overall value of $ 600 /kg for the production and preparation of the theoretical enzyme. Theoretically, this would be assumed to cover capital and running costs as well. Due to the lack of specific information available about the specific nature and production requirements of this enzyme, this was deemed an acceptable estimate.

From the producer DC DiagnoCine, glucose isomerase would cost $ 6480.60 /g, and immobilisation would still be required (coming to approximately $ 6981.10 /g). This is a marked price difference. From calculations made in Section 4.4 and Appendix C, it is known that 37.46 kg of the enzyme would be required per year. Thus, the cost of purchasing the enzyme would come to $ 261.51 million/year and the cost of producing the enzyme would come to $ 22 476 /year, with producing one's own enzyme being the more economical option.

The potential issues involved with on-site enzyme production were discussed in Section 3.3. An additional consideration would be that the niche nature of the enzyme would likely make purchasing it impossible anyway, necessitating the on-site production of the enzyme irrespective of cost. Furthermore, while the amount of enzyme required for the process is notably small, the potential profitability of a scaled-up process will also be analysed further in Section 7; considering the hefty price of such enzymes, the on-site production of the enzyme will become ever-more crucial in the scenario of the process being upscaled.

The precise process required to produce the enzyme falls outside the scope of this work but can be assumed to be a feasible sister-process for the proposed process based on its small scope. Thus, the cost of producing and preparing the enzyme has been incorporated into the simulation at a yearly expense of $ 22 476.

It is worth noting, however, that this estimate does not account for the additional expenses inherent to GMP processes such as cleanrooms, QA/QC measures, and the Certificate of Analysis requirements. These factors have the potential to dramatically increase the production cost of enzymes due to the stringent regulatory standards. Thus, enzyme production costs were also investigated based on the assumption that GMP-grade enzymes would be required for the process, to investigate how these additional protocols might impact the profitability of the process. Based on estimates from Tufvesson (2011), enzyme production costs could be in the range of € 250 /kg and € 1000 /kg for unpurified enzymes, and € 2000 /kg for the finalised enzymes, including immobilisation. This is equivalent to a maximum of approximately $ 2625 /kg to produce immobilised enzymes ($ 98 330 /year). It is worth noting that this is still exponentially less expensive than purchasing enzymes at $ 6981.10 /g. This GMP-grade enzyme value will be used as a comparative maximum enzyme price in Section 5.4. and Section 7, to gauge to what extent the incorporation of GMP procedures would impact profits.

Carrier Oil Use for Isolates and Full Spectrum Blends

The CBD isolate product, once mixed with an MCT oil carrier, has been priced at $ 39.00 per 30 ml, where every 30 ml contains 600 mg CBD, as advertised by Goodleaf (Goodleaf, 2023). Thus, in the production of the CBD isolate final product, 50 L MCT oil will be required per every 1 kg of CBD isolate; this can be sold in 30 ml units for a collective $ 65 000.00 per every kilogram of isolate produced.

The full spectrum oil, once combined with the MCT oil carrier, has been priced at $ 40.00 per 30 ml, where every 30 ml contains 1500 mg of cannabis oil, as advertised by Lazarus Naturals (Lazarus Naturals, 2023). Thus, in the production of the CBD isolate final product, 20 L MCT oil will be required per every 1 kg of full spectrum CBD; this can be sold in 30 ml units for a collective $ 26 666.67 per every kilogram of full spectrum oil produced.

MCT oil can be bought for $ 18.20 per litre, as per Amazon (Amazon, 2023). Thus, the MCT oil expense for the 50 L required per 1 kg CBD isolates comes to $ 910.07, and for the 20 L

required per 1 kg full spectrum CBD, the cost is $ 364.03. If the MCT costs required per kilogram of cannabis oil are deducted from the selling price per kilogram of cannabis oil produced, this yields a net revenue of $ 64 089.93 per kilogram of CBD isolate and $ 26 302.64 per kilogram of full spectrum CBD.

For the sake of simplicity, these will be the revenue values used for the simulation; the value for the full spectrum CBD product will be used directly in the simulations, while the value for the CBD isolates will be incorporated into further calculations of the profitability of selling the additional cannabinoids as ancillary products, found later in Section 5.2, to evaluate the collective profits yielded for all cannabis isolates produced by the process.

A full comparative breakdown of the difference in profitability between the production of CBD isolates and CBD full spectrum blends can be found in Section 5.4.

Purchasing/ Cultivating Cannabis

Cannabis cultivation can be performed alongside the proposed process, essentially producing the raw material required for the process on-site, or it can be outsourced, with the cannabis being purchased. The price of cannabis, if purchased, is recorded as $ 3 /g (Bloomberg, 2021). If cannabis enters the process at 0.65 kg/h (5148 kg/year), this amounts to staggering raw material expenses of $ 15 444 000 per year.

Cultivating one's own cannabis is considerably more complex. To grow the plant, licensing from the South African Health Products Regulatory Authority (SAHPRA) costs upwards of $ 1330 per year (Funding Hub, 2022). To estimate the cost of growing the cannabis, the following data was used:

- A drip-based irrigation system for 4 to 5 hectares of cannabis costs between $ 79 500 and $ 106 000 (The Best Grow, 2020).
- The average cannabis yield per square foot is 39.5 g (Cannabis Business Times, 2016), where 1 hectare is equivalent to 107 639 ft^2.
- Once the cultivation process is past its initial set-up stage, cannabis can be grown for $ 1.48 /kg (approximately $ 7619 for the yearly feed of 5148 kg).

As calculated in Table 70, Appendix D, a capital expense of approximately $ 23 311.90 will be required to prepare a drip-based irrigation system capable of meeting processing

requirements – i.e. producing 5148 kg of cannabis per year. This assumes a relatively simple set-up of 1.2108 hectares, without the use of advanced greenhouse technology, which would require significantly higher capital and running expenses but would also produce a higher yield; the technicalities involved in cannabis cultivation, including greenhouse use, were detailed in Section 3.2. Combined with licensing costs, this yields a capital cost of $ 24 641.90.

Following this, a yearly cultivation cost of $ 7629.76 ($ 1.48 /kg cannabis) would be required to meet raw material demands. Thus, in the first year of operation alone, if the combined capital and cultivation costs ($ 32 271.66) are compared to the cost of purchasing the cannabis required for a single year of processing ($ 15 444 000), the cost of cultivating one's own cannabis in the first year is 0.21 % the cost of purchasing the cannabis. In the proceeding years, this percentage lowers further to 0.05 %, with cultivating one's own cannabis emerging as the more economical option.

Non-CBD Cannabinoids as Ancillary Products

As discussed in Section 3.8.4, CPC can also be used to separate out other cannabinoids aside from CBD, which can be sold as ancillary products, thus providing an additional source of revenue in the case of the process producing CBD isolates. This will not be applicable in the process producing full spectrum blends, as these additional cannabinoids will remain as part of the CBD oil product, and thus a single output will be produced. The additional cannabinoid products would include THC (which can be sold for medicinal purposes exclusively, due to the illegality of its recreational use), CBG, and CBN.

Separating the additional cannabinoids would be relatively simple but would require careful collection of the different cannabinoids during their relative elution. In an industrial or large commercial scale CPC extractor, such is the case, this would be automated to achieve effective separation; thereby, the same distillation columns would be used to separate the various neutral cannabinoids, but ultimately each one would emerge in stepwise periods for collection. Care must be taken in ensuring these elutions are kept separate, and pure.

Neglecting to make use of these cannabinoids as an additional source of revenue would be senseless and, in fact, wasteful, because by the very nature of CPC, they would invariably be separated into isolated fragments during the separation of CBD. The only additional cost involved would be that of the MCT carrier oil, which has also been used to stabilise CBD.

From Table 18, it was assumed CBD, CBG, and CBN isolated would be retrieved from the process in a mass ratio of 100: 12.52: 30.97, where a 633.65 kg yearly mass of CBD isolate is produced, as per the simulation. The mass of THC in the cannabis mixture can also be taken from the simulation, yielding 51.63 kg/yr. As in the case of the CBD isolate, 50 L MCT oil bought for $ 18.20 per litre will be used as a carrier for every 1 kg of cannabinoid isolate produced (Amazon, 2023). Once again, it will be assumed that the isolates will be sold in unites of 30 ml, with each containing 600 mg of the relevant cannabinoid.

The neutral cannabinoids can be sold for the following selling prices:

- CBN isolates: $ 4180 /kg (Open Book Extracts, 2023b)
- CBG isolates: $ 1540 /kg (Open Book Extracts, 2023a)
- THC isolates: $ 100 000 /kg (Brown, 2023)

The relevant calculations for the potential profitability of each cannabinoid isolate can be found in Table 26, following the same logic as that used in the CBD-MCT oil calculations. The sale of all four isolates, including CBD at a net profit of $ 64 089.93 /kg (or $ 40 610 584.14 /year at an output of 633.65 kg/year, as per the simulation), are deemed viable sources of income.

Per every kilogram of CBD isolate separated by the CPC, it can thereby be calculated (see Table 71, Appendix D) that a net profit of $ 73 255.41 ($ 64 089.93 for the CBD and $ 9165.48 for the ancillary cannabinoids, collectively) can be made post deduction of the MCT oil costs, after the collective sale of all isolates produced per kilogram of CBD (1 kg CBD, 125.20 g CBG, 309.70 g CBN and 81.48 g THC, as per the cannabinoid ratio and the simulated data).

As 0.5164 kg of the ancillary cannabinoid products yields $ 9165.48, 1 kg will give $ 17 749.48. This value will be incorporated into the simulation as the revenue earned from the ancillary product stream.

Table 26: Cannabinoid isolates, the MCT oil required for each, and the potential revenue of each isolate.

Cannabinoid	Extracted Mass (kg/year)	Base Income ($/year)	Required MCT Oil (L/year)	Cost of MCT Oil ($/year)	Net Profits ($/year)	Net Profits ($/kg)
CBN	196.24	820 289.07	9812.07	178 579.68	641 709.39	3270.00
CBG	79.33	122 172.79	3966.65	72 193.01	49 979.78	630.00
THC	51.63	5 163 000.00	2581.50	46 983.30	5 116 016.70	99 090.00

Waste Disposal

From literature from the Department of Environment Forestry & Fisheries, the disposal of hazardous waste was recorded as $ 0.17031 /kg; this value was assigned to disposing of the solvent waste streams. CO_2 disposal, as a recoverable waste stream, was assigned a disposal cost of $ 0.07665 /kg; the distillate streams from the three consecutive distillation columns in the full spectrum model are composed primarily of cannabis compounds, and thus categorised as organic waste and assigned a disposal cost of $ 0.04482 /kg (South African Department of Environment Forestry & Fisheries; 2012).

The solid waste from the filter is composed of water and plant matter and thus can be used as fertiliser for the cultivation sector, thus incurring no disposal costs. The wastewater stream can be repurposed for either cultivation purposed, fed back into the process as a water feed stream, or used for heat transfer purposes, thus negating disposal costs for this waste stream as well.

Further discussion into the process waste streams can be found in Section 3.10.

Summary

In short, the following values (see Table 27) will be incorporated into the economic analysis, based on the ancillary calculations detailed above.

Table 27: Result summary of ancillary calculations.

Calculation	Relevance to Process	Output Values
Enzyme costs	Calculation of the yearly expenses associated with the use of the enzyme catalyst in the PFR, to be associated with the running costs of the PFR. The possibility of either producing or purchasing the enzyme was considered.	Producing the enzyme worked out more economically viable, at a yearly cost of $ 22 476 (or $ 98 330, for GMP-grade enzymes).
Comparative profitability of cultivating or purchasing cannabis	A comparison between the capital and running costs associated with both cultivating one's own cannabis and purchasing it from a third-party supplier is to be recorded as part of the raw material expenses required for the process.	Cultivating one's own cannabis worked out to be more economically viable. A drip-based irrigation system of 1.2108 hectares will be incorporated into the process expenses, requiring capital costs of $ 24 641.90 and a yearly cultivation cost of $ 7629.76 ($ 1.48 /kg cannabis grown).
Use of MCT oil as a carrier in isolates and full spectrum blend	Deduction of the relevant MCT oil expenses from the selling prices of the CBD oils, yielding a more accurate idea of revenue earned from yearly sales. This value was used in the simulation as the final product values for the isolate and full spectrum blend, rather than simply the baseline selling price.	CBD isolates will be sold for $ 39.00 per 30 ml, with each unit containing 600 mg of CBD, with the remainder being MCT oil. 50 L of MCT oil will be required per every 1 kg of CBD isolate to produce the final product. Full spectrum CBD oil will be sold for $ 40.00 per 30 ml, where every 30 ml contains 1500 mg of cannabis oil. 20 L MCT oil will be required per every 1 kg of full spectrum CBD oil. Once the costs of the MCT oil were deducted from the theoretical revenue values, the net revenue values came to $ 64 089.93 per kilogram for CBD isolate and $ 26 302.64 per kilogram for the full spectrum CBD oil.
Non-CBD cannabinoids as ancillary profits	An analysis of the profitability of selling THC, CBG, and CBN as ancillary products, once the cost of the MCT has been deducted from the prices, in the same manner as the calculations performed for CBD. Note that these additional products are applicable only for the process of producing isolates; in the case of the full spectrum blends, these cannabinoids will be part of the same final product as CBD, producing a single output.	Collectively, the ancillary cannabinoid products (CBG, CBN, and THC) yielded a net revenue (less the cost of the required MCT oil) of $ 17 749.48 /kg. In the same vein as the CBD isolate products, 30 ml units will be sold, each containing 600 mg of the relevant cannabinoid.
Waste disposal costs	Disposal costs for the solvent waste streams and the CO_2 waste streams were determined; the solid waste from the filter and the wastewater stream were repurposed to avoid disposal costs.	The cost of hazardous waste was recorded as $ 0.17031 /kg; the solvent waste streams fall into this category. CO_2, as a recoverable waste stream, has disposal costs of $ 0.07665 /kg. Distillate streams from the full spectrum model distillation columns are categorised as organic waste and assigned a disposal cost of $ 0.04482 /kg.

5.3. Equipment Optimisation

In terms of optimisation, many of the process components are limited in terms of being designed around component splits derived from literature data (e.g.: the extraction units, decarboxylation oven, and the CPC) while the PBR itself is designed around an entirely theoretical enzyme. Thus, optimisation will focus primarily on the effects of process variables on the costs of the distillation columns C-101 and C-103, where pressure and reflux ratios will be varied.

Firstly, changes in the price of the heptane distillation column, C-103, were explored; a detailed tabulated analysis as seen in Table 62, Appendix D. The effects of R/Rmin and column pressure on the required distillation stages, capital expense and cost of collective heating agents required by the process was compared as these values were varied. This was then used to minimise the costs of the columns while maximising performance.

Reflux ratio values (R/Rmin) of 1.2, 1.25 (the basis value), 1.3, 1.4, and 1.5 and pressures of 0.5 bar, 1.013 bar (the basis value), 1.5 bar, 2.0 bar, 2.5 bar, and 3.0 bar were used for the analysis. Of the three R/Rmin values yielding a capital cost of $ 94 000 (namely R/Rmin 1.30, 1.40, and 1.50), a R/Rmin of 1.30 gave the lowest combined cost of high-pressure steam and cooling water at $ 662.34 per year. The R/Rmin value yielding the lowest combined heating agent cost overall was R/Rmin 1.20, at $ 662.20 /year, but required a capital investment of $ 5000 more and an additional distillation column stage, yielding a capital requirement of $ 99 000.

For context, if one uses an R/Rmin of 1.20 as opposed to 1.30, it will require a theoretical 35 715 years of operation until the savings made from the reduced heating agent requirements account for the additional $ 5000 spent on capital expenses. This is unrealistic and thus R/Rmin 1.30 emerges as the optimal value.

Applying the same logical to a column pressure of 0.5 bar (yielding the lowest capital expenses of $ 83 000 and the lowest cooling water cost of $ 94.60, but the highest cost of steam at $ 569.41) and a pressure of 3.0 bar (yielding the highest capital expenses of $ 125 000 and highest cooling water cost of $ 64.70, but the lowest cost of steam of $ 567.38), it would require 21 762 years of operating at 3.0 bar to make up for the additional $ 42 000 in capital expenses required for the additional 8 stages required to facilitate the higher pressure. The relevant values

used in these calculations can be found in Table 62, Appendix D. Thus, 0.5 bar emerges as the superior operating pressure.

Next, the acetonitrile-TBME distillation column, C-101, was considered and the same logic was followed. Of the three R/Rmin values yielding a capital cost of $ 26 000 (namely R/Rmin 1.30, 1.40, and 1.50), R/Rmin 1.30 gave the lowest combined cost of high-pressure steam and cooling water at $ 663.08 per year; a full tabulated analysis can be found in Table 63, Appendix D. The R/Rmin value yielding the lowest combined heating agent cost overall of $ 661.44 per year, however, was R/Rmin 1.20, which required a capital investment of $ 2000 more for the additional distillation column stage required ($ 28 000, in total). If one used a R/Rmin of 1.20 as opposed to 1.30, it would require a theoretical 1220 years until the savings made from the heating agents account for the additional $ 2000 spent on capital expenses. Thus, 1.30 is once again the optimal R/Rmin value.

A column pressure of 0.5 bar yielded the lowest capital expenses of $ 26 000, and a combined heating agent cost of $ 663.77; 1.013 bar required capital expenses of $ 28 000 and cooling water cost of $ 94.60 but had the lowest cost of steam of $ 567.66; 1.5 bar required a capital expense of $ 28 000, steam cost of $ 568.70, but had the lowest cooling water cost of $ 94.53. It would require 3704 years of operating at 1.5 bar as opposed to 0.5 bar to make up for the additional $ 2000 in capital expenses required for the additional stage. At 1.013 bar as opposed to 0.5 bar, 1325 additional years would be required. The relevant values used in these calculations can be found in Table 63, Appendix D. Thus, 0.5 bar is the superior column pressure once again.

Graphical representations of the sensitivity analysis can be seen in Figure 25. If the optimal values for both columns are applied simultaneously, the relevant changes to the operating units from the baseline simulation can be found below, in Table 28.

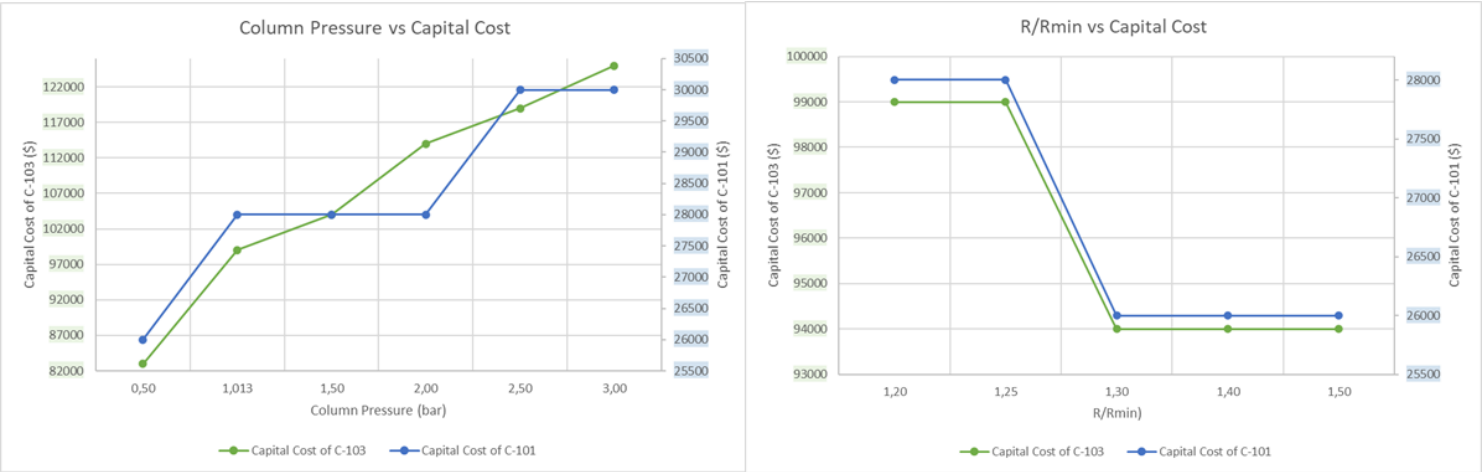


Figure 25: Sensitivity analysis graphs for distillation columns C-103 and C-101.

Table 28: Results of distillation column optimisation.

	Initial		Optimised	
	C-103	**C-101**	**C-103**	**C-101**
R/Rmin	1.25		1.30	
Column Pressure (bar)	1.013		0.5	
Stages	19	14	16	13
Process Heating Agent Cost ($/yr)	662,28		666,07	
Capital Cost	99000	28000	83000	26000
Column Diameter	0,011	0,018	0,015	0,024
Column Height (m)	7,6	5,6	6,4	5,2
Reflux Ratio	0,013	0,043	0,013	0,025
Cooler Heat Transfer Area (m2)	0,004	0,013	0,005	0,022
Cooler Temperature (°C)	98,5	80,3	76,1	59
Cooling Water (kg/hr)	44	119,4	44,9	120,4
Reboiler Heat Transfer Area (m2)	0,004	0,008	0,003	0,007
Reboiler Temperature (°C)	159,7	159,2	133,1	138,4
High Pressure Steam (kg/hr)	0,6	1,4	0,6	1,4

Overall, with an increase in overall heat transfer agent costs of $ 3.79 per year and a decrease in distillation column capital expenses of $ 18 000, it would take 4750 years of operation for the savings made on the capital expenses to be rendered moot by the heat transfer agent costs. This is too long to hold any practical relevance – thus, the distillation columns can be said to be optimised.

Pivotal values which have not been mentioned can be assumed to be unchanged, such as the flowrate of the CBD output stream or purity. The optimised values of R/Rmin 1.30 and column pressure of 0.5 bar will be applied in place of the base values used in Section 4.5.

5.4. Economic Analysis

The cost of producing CBD isolates and CBD full spectrum blends via the biocatalytic remediation of THC was analysed using SuperPro Designer. This software allowed for the estimation of capital costs, operating costs, revenues, and economic viability indicators based on the inputs of costs such as raw materials, utilities, disposal/treatment costs of waste streams, and selling prices of product streams. Additionally, the software can estimate equipment costs using built-in or user-defined models, as well as estimate the required labour costs and other such expenses. Capital expenses like startup, construction, and piping, and operating expenses like research and development, maintenance, and depreciation were estimated in this manner using SuperPro's cost factors and relevant parameters. For the economic analysis, the following assumptions have been made, in addition to the ancillary values calculated in Section 5.2:

- The depreciation period for both processes is 10 years.
- The annual operating time (AOT) is 7920 hours (330 days).
- The uninstalled unit purchase costs for basic equipment are pulled from the SuperPro database and calculated using the standard SuperPro equipment purchase cost models for each unit; a detailed breakdown of these values can be found later in this section. The exception to this is reactor PFR-101, which was designed to facilitate an 85% conversion rate, as seen in Section 4.4 and Appendix C.
- The direct capital costs to produce CBD isolates and blends are approximately six times the total equipment purchase cost. Indirect costs are 60% of the direct costs, with the contractor's fee being 5 % of the combined value of the direct and indirect costs. The contingency cost is set at 10 % of the combined direct and indirect costs. These values were estimated based on SuperPro standards.
- The processes use electricity purchased at a unit cost of $ 0.10 /kWh, as per the SuperPro database.
- The purchasing price of tap water is $1.0 /MT. It is important to note that the values for both water and electricity, having been taken from the SuperPro database, are not

specific to the prices of these utilities in a strictly South African context, and should be viewed in the context of a more generalised analysis.

- Disposal of hazardous waste was recorded as approximately $ 0.17 /kg; CO_2 disposal, as a recoverable waste stream, was assigned a disposal cost of $ 0.08 /kg; organic waste was assigned a disposal cost of $ 0.04 /kg (Section 5.2).
- CBD isolates will be sold for $ 39.00 per 30 ml, with each unit containing 600 mg of CBD, with the remainder being MCT oil. Full spectrum CBD oil will be sold for $ 40.00 per 30 ml, where every 30 ml contains 1500 mg of cannabis oil. (Section 5.2)
- Additional cannabinoid products, produced in the isolate process and sold as an ancillary product, will be sold in unites of 30 ml, with each containing 600 mg of the relevant cannabinoid, with the volume made up using MCT oil. CBG isolates will be sold for approximately $ 2.51 per 30 ml, CBG isolates for $ 0.92 per 30 ml, and THC isolates for $ 60.00 per 30 ml.
- MCT oil can be bought for $ 18.20 per litre. (Section 5.2)
- The enzyme will be produced at a yearly cost of $ 22 476 in both processes, where 37.46 kg of the enzyme would be required per year for use in the reactor. (Section 5.2)
- A drip-based irrigation system of 1.2108 hectares will be used in the cultivation of cannabis for the processes, requiring capital costs of approximately $ 24 600 and a yearly cultivation cost of $ 7630 ($ 1.48 /kg cannabis grown). (Section 5.2)

SuperPro produced several reports related to the process's economic viability, including the Economic Evaluation Report (EER), the Cash Flow Analysis Report (CFR), and the Itemized Cost Report (ICR). The Executive Summary from the EER for both processes can be seen in Table 29. Within it, the total capital investment (TCI), total operating cost, and total unit operating cost for the isolate process producing 637 kg/year of CBD isolate (31 850 L after the addition of MCT oil) and 494 kg/year of the ancillary cannabinoids (24 700 L after the addition of MCT oil), and the full spectrum process producing 656 kg/year of the CBD blend (13 120 L after the addition of MCT oil).

According to Table 29, the TCI for the production of CBD isolates is approximately $ 31.2 million, with a total operating cost of approximately $ 8.0 million and a total unit production cost of $ 12 500/kg for the isolate product prior to MCT dilution. An NPV of $ 190.4 million at a 7% discount rate and a gross margin of 84 % is achieved, with an ROI of 102 % and a payback time of approximately 1 year. In contrast, the TCI for the production of

CBD blends is approximately $ 33.9 million, with a total operating cost of around $ 8.5 million and a total unit production cost of $ 12 900/kg. A Net Present Value (NPV) of $ 14.2 million at a 7% discount rate and a gross margin of 51 % is achieved, with an ROI of 22 % and a payback time of approximately 5 years.

Table 29: Executive summaries of the economic analyses for the CBD isolate and CBD blend processes.

CBD Isolate	
Total Capital Investment	31236000 $
Capital Investment Charged to This Project	31236000 $
Operating Cost	8005000 $/yr
Main Revenue	40828000 $/yr
Other Revenues	8764198 $/yr
Total Revenues	49592000 $/yr
Cost Basis Annual Rate	637,05 kg MP/yr
Unit Production Cost	12565,48 $/kg MP
Unit Production Revenue	77847,50 $/kg MP
Gross Margin	83,86 %
Return On Investment	101,58 %
Payback Time	0,98 years
IRR (After Taxes)	49,38 %
NPV (at 7,0% Interest)	190458000 $
MP = Total Flow of Stream 'S-122'	
CBD Blend	
Total Capital Investment	33857000 $
Capital Investment Charged to This Project	33857000 $
Operating Cost	8475000 $/yr
Revenues	17257000 $/yr
Cost Basis Annual Rate	656,11 kg MP/yr
Unit Production Cost	12917,35 $/kg MP
Unit Production Revenue	26302,64 $/kg MP
Gross Margin	50,89 %
Return On Investment	21,75 %
Payback Time	4,60 years
IRR (After Taxes)	12,06 %
NPV (at 7,0% Interest)	14199000 $
MP = Total Flow of Stream 'S-123'	

Table 30 shows a section of the Profitability Analysis table of the EER that further breaks down the TCI of both processes. The Direct Fixed Capital (DFC) for the CBD isolate process is approximately $ 5.68 million, the startup cost is $ 284 thousand, and working capital is $ 631 thousand; for the CBD blend process, the values are $ 8.18 million for the DFC, $ 409 thousand for the startup cost, and $ 630 thousand working capital.

Table 30: Total capital investment for the CBD isolate and CBD blend processes.

CBD Isolate Production		
A.	Direct Fixed Capital	5680000 $
B.	Working Capital	631000 $
C.	Startup Cost	284000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	31236000 $
G.	Investment Charged to This Project	31236000 $

CBD Blend Production		
A.	Direct Fixed Capital	8176000 $
B.	Working Capital	630000 $
C.	Startup Cost	409000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	33857000 $
G.	Investment Charged to This Project	33857000 $

Table 31, which is also extracted from the EER, provides a breakdown of the DFC. The DFC consists of direct and indirect costs. Direct costs include tangible items such as equipment and installation costs, electrical equipment, piping, buildings, insulation, and auxiliary facilities. Indirect costs include intangible items such as construction, engineering, contractor's fees, supervision, and contingency. SuperPro estimates direct costs based on multipliers of the installed equipment costs. SuperPro also splits indirect costs into two segments; the first of which pertains to indirect costs such as construction and engineering, calculated based on multipliers of the sum of the direct costs, while the second segment pertains to indirect costs such as contractor's fee and contingency, and is based on multipliers of the sum of direct costs and the previously described group of indirect costs.

According to Table 31, the total plant direct cost (TPDC) for the production of CBD isolates is approximately $ 3.09 million, the total plant indirect cost (TPIC) is $ 1.85 million, and the contractor's fee and contingency (CFC) comes to $ 741 thousand. The TPDC for the CBD blend process is approximately $ 4.44 million, the TPIC is $ 2.67 million, and the CFC is $ 1.07 million. The total plant cost can then be calculated through the addition of the TPDC and the TPIC, and the DFC can be calculated through the addition of the TPC and the CFC.

Table 31: Fixed capital expenses for the CBD isolate and CBD blend processes.

CBD Isolate Production	
3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	929000
2. Installation	410000
3. Process Piping	325000
4. Instrumentation	372000
5. Insulation	28000
6. Electrical	93000
7. Buildings	418000
8. Yard Improvement	139000
9. Auxiliary Facilities	372000
TPDC	3087000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	772000
11. Construction	1080000
TPIC	1852000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	4939000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	247000
13. Contingency	494000
CFC = 12+13	741000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	5680000

CBD Blend Production	
3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	1392000
2. Installation	435000
3. Process Piping	487000
4. Instrumentation	557000
5. Insulation	42000
6. Electrical	139000
7. Buildings	626000
8. Yard Improvement	209000
9. Auxiliary Facilities	557000
TPDC	4444000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1111000
11. Construction	1555000
TPIC	2666000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	7110000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	355000
13. Contingency	711000
CFC = 12+13	1066000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	8176000

Table 32: Equipment breakdown summary for the CBD isolate and CBD blend processes.

CBD Isolate Production				
Name	*Type*	*Capacity*		*Price ($)*
CX-101	Centrifugal Extractor	9,22	L/h	43000
HX-101	Condenser	0,02	m2	108000
C-101	Distillation Column	2,43	L	26000
C-103	Distillation Column	1,07	L	83000
GBX-101	Generic Box	7,54	kg/h	3000
EC-102	Heat Exchanger	-	m2	4000
MSX-102	Mixer-Settler Extractor	4,02	L/h	9000
MSX-101	Mixer-Settler Extractor	5,94	L/h	9000
PFF-101	Plate and Frame Filter	0,01	m2	73000
PFR-101	Plug Flow Reactor	0,15	L	81000
SR-101	Shredder	0,65	kg/h	86000

CBD Blend Production				
Name	*Type*	*Capacity*		*Price ($)*
HX-101	Condenser	0,02	m2	108000
C-102	Distillation Column	0,02	L	174000
C-103	Distillation Column	0,1	L	174000
C-101	Distillation Column	0,05	L	174000
GBX-101	Generic Box	7,54	kg/h	3000
EC-102	Heat Exchanger	-	m2	4000
MSX-102	Mixer-Settler Extractor	4,02	L/h	9000
MSX-101	Mixer-Settler Extractor	5,94	L/h	9000
PFF-101	Plate and Frame Filter	0,01	m2	73000
PFR-101	Plug Flow Reactor	0,15	L	81000
SR-101	Shredder	0,65	kg/h	86000

According to Table 32, which is a breakdown of the primary equipment involved in the processes, the collective costs of the isolate process equipment come to $ 525 000, while the collective costs of the blend process equipment come to $ 895 000 - 170 % higher than the isolate process. This is due primarily to the costs of the distillation required to separate the cannabinoids.

In the isolate process, the cost of distillation is significantly reduced by the incorporation of the CPC, modelled as a centrifugal extractor (CX-101). In the isolate process, distillation costs R 109 000 (about 21 % of the total costs), while the CPC costs $ 43 000 (1% of the total costs). In the blend process, distillation costs a total of $ 522 000 for the 3 columns, constituting the majority of the equipment costs for the process (58 %). The cost of distillation for the blend process is 343% more than the distillation costs for the isolate process, emerging as the primary

cost variation between the processes. The total operating costs can be found in Table 33, as taken from the ICR.

In addition to the aforementioned costs, operating costs will also be incurred. Variable operating costs include raw material costs (in this case, the cost of the cannabis, solvents, and enzymes) and waste treatment costs. Fixed operating costs would include regular, repeating costs such as labour. A breakdown of the operating expenses can be found in Table 33, while material costs (and the amounts of material used) can be found in Table 34.

Table 33: Annual operating costs for the CBD isolate and CBD blend processes.

CBD Isolate Production		
Cost Item	**$**	**%**
Raw Materials	12000	0,15
Labor-Dependent	6918000	86,42
Facility-Dependent	1068000	13,35
Waste Treatment/Disposal	1000	0,01
Utilities	5000	0,07
TOTAL	8005000	100,00

CBD Blend Production		
Cost Item	**$**	**%**
Raw Materials	10000	0,12
Labor-Dependent	6918000	81,62
Facility-Dependent	1542000	18,20
Waste Treatment/Disposal	1000	0,01
Utilities	5000	0,06
TOTAL	8475000	100,00

Table 34: Material costs for the CBD isolate and CBD blend processes.

CBD Isolate Production					
Bulk Material	**Unit Cost ($)**	**Annual Amount**		**Annual Cost ($)**	**%**
Acetonitrile / TBME	1,24	1584	kg	1964,16	15,86
Ethanol	0,75	3168	kg	2376	19,18
Heptane	0,36	1188	kg	427,68	3,45
Plant Material	1,48	5148	kg	7619,04	61,51
Total		11088	kg	12386,88	
CBD Blend Production					
Bulk Material	**Unit Cost ($)**	**Annual Amount**		**Annual Cost ($)**	**%**
Ethanol	0,75	3168	kg	2376	23,77
Plant Material	1,48	5148	kg	7619	76,23
Total		8316	kg	9995	

In Table 33 and Table 34, it can be seen that the collective material costs for the isolate process are approximately $ 12 000, 1.2 times greater than the costs of material for the blend process, at $ 10 000. The unit costs of each component can also be seen in Table 34. Returning to Table 33, the utility and facility-dependent costs make up approximately 0.07 % and 13.35 % of the total operating costs, respectively, for the isolate process, with facility-dependent costs including maintenance, depreciation, and other overhead costs. In the blend process, these values come to 0.06 % and 18.20 %, respectively. In both cases, these values are dwarfed by the labour-dependent costs, which contribute the most sizeable portion of the process operating costs – 86.42 % in the case of the isolate process and 81.62 % for the blend process. To further explore the contribution of utility costs to the total operating cost, Table 35 was also taken from the ICR.

Table 35: Utility costs for the CBD isolate and CBD blend processes.

CBD Isolate Production					
Utility	**Unit Cost ($/kW-h)**	**Amount (/year)**		**Cost ($/year)**	**%**
Std Power	0,10	1724	kW-h	172,41	100,00
TOTAL		1724	kW-h	172,41	100,00
Utility	**Unit Cost ($)**	**Amount (/year)**		**Cost ($/year)**	**%**
Steam	32,00	60	MT	1929,16	37,02
Steam (High P)	36,00	16	MT	570,71	10,95
Cooling Water	0,10	954	MT	95,36	1,83
Chilled Water	0,50	5232	MT	2615,98	50,20
TOTAL				5211,21	100,00
CBD Blend Production					
Utility	**Unit Cost ($/kW-h)**	**Amount (/year)**		**Cost ($/year)**	**%**
Std Power	0,10	1719	kW-h	171,95	100,00
TOTAL		1719	kW-h	171,95	100,00
Utility	**Unit Cost ($)**	**Amount (/year)**		**Cost ($/year)**	**%**
Steam	32,00	60	MT	1929,04	42,20
Steam (High P)	36,00	1	MT	26,27	0,57
Chilled Water	0,50	5232	MT	2615,80	57,22
TOTAL				4571,11	100,00

Table 35 indicates that the cost of electricity consumed by the production of CBD oils comes to $ 172.41 for isolate production (3.20 % of the total utility costs for the process) and $ 171.95 for blend production (3.63 % of the total utility costs for the process). For both processes, cooling and heating agents contributed the vast majority of the utility costs.

In Appendix E, Table 72 displays cash flow analyses for the first 15 years of the processes, where the first year of operation (in which revenue begins to come in) is year 3; this can also be seen represented graphically in Figure 26, for the first 10 years of the process.

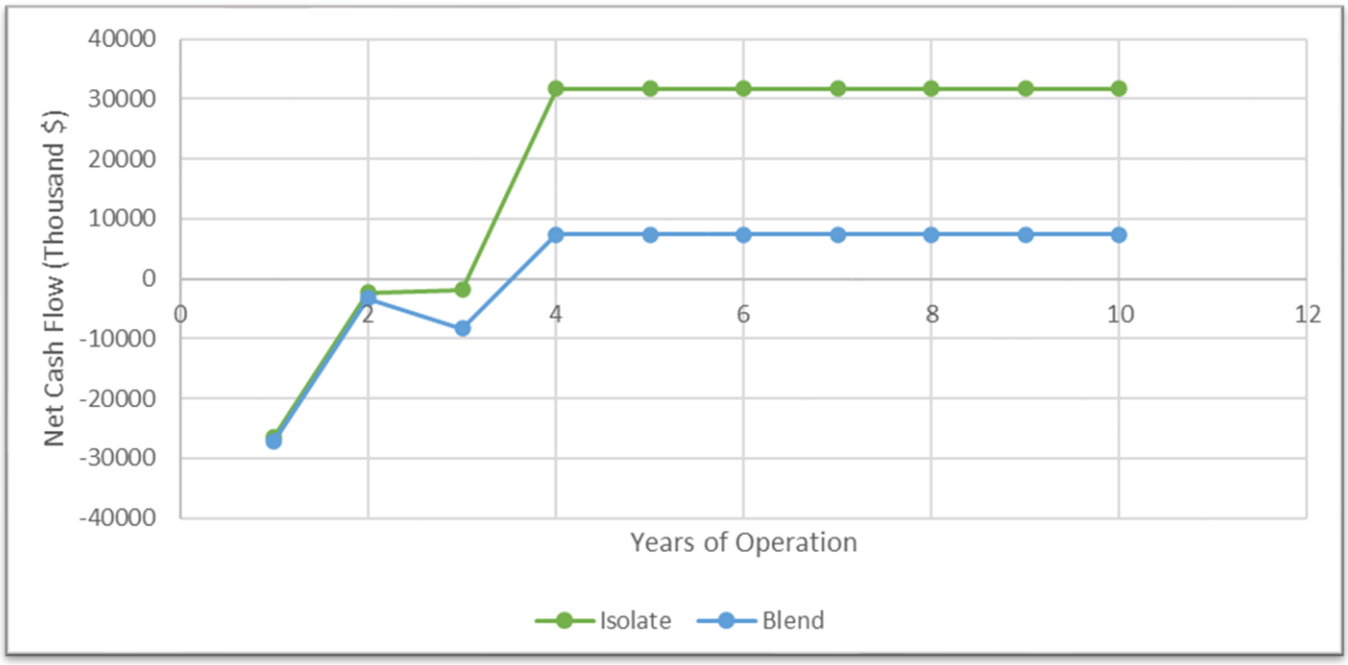


Figure 26: Net cash flow comparison of the production of CBD isolates and CBD full spectrum blends.

In summary, as the economic analysis shows, the production of isolates is more profitable overall than the production of CBD blends. The isolate product boasts lower capital costs ($ 13 236 000 versus $ 33 857 000) and lower operating costs ($ 8 005 000 /year versus $ 8 475 000 /year), as well as greater revenue ($ 49 592 000 /year versus $ 17 257 000 /year) than the full spectrum blend process. In the case of applying the GMP-grade enzyme costs,

Both processes require the same raw labour costs, utility costs, and waste disposal costs ($ 6 918 000 /year, $ 5000 /year, and $ 1000 /year, respectively). The only minor advantage the CBD blend process has over the isolate process is a slightly lower raw material cost ($ 10 000 /year versus $ 12 000 /year); despite this, the isolate process achieved superior project indices in all instances.

The isolate process has a gross margin of 83.86 %, an ROI of 101.58 %, a payback time of 0.98 years, an IRR of 49.38 %, and an NPV of $ 190 458 000. If GMP-grade enzymes are used in the process, as discussed in Section 5.2 and calculated in Appendix E, these parameters will instead manifest as a gross margin of 83.71 %, an ROI of 101.41 %, a payback time of 0.99 years, an IRR of 49.30 %, and an NPV of $ 190 153 965. The full spectrum blend has a

gross margin of 50.89 %, an ROI of 21.75 %, a payback time of 4.60 years, an IRR of 12.06 %, and an NPV of $ 14 199 000. Using GMP-grade enzymes, these values become instead a gross margin of 50.46 %, an ROI of 21.58 %, a payback time of 4.63 years, an IRR of 11.97 %, and an NPV of $ 14 128 764.

Thus, while both processes boast favourable project indices, the isolate process emerges as superior and has been selected as the preferred process design. It is also worth noting that the GMP-grade enzymes have minimal financial impact when compared to the use of non-GMP enzymes.

5.5. Sensitivity Analysis

The stability of the process in the face of fluctuations in process variables can be gauged using a sensitivity analysis. In this section, the sensitivity of the process to 'theoretical' parameters (enzyme conversion rate and input CBD percentage) as well as to more concrete variables such as inflation of raw material costs (in this case, the cannabis buds, specifically) and labour costs will be explored.

Raw Material Costs and Labour Costs

The cost of the plant material – i.e.: the buds, priced at $ 1.48/kg, or approximately $ 7619 for the yearly feed of 5148 kg – was first reduced by 50 %, then by 25 %, then inflated by 25 %, 50 % and 100 % in turn to determine how vulnerable the process is to changes in material costs. This was achieved through observation of the effect these changes had on the Return on Investment (ROI) of the processes. The cost of the plant was selected to evaluate the processes' stability in the face of raw material cost variations because it contributes the largest fraction of the raw material costs in both processes – 61.51 % of raw material costs in the case of the isolate process and 76.23 % of raw material costs in the case of the blend process. More information about the ROI and raw material costs of the processes can be found in Section 5.4.

When the cost of the plant material in the isolate process was incrementally increased from 50 % of the original price ($ 0.74/kg, or approximately $ 3810 for the yearly feed) to 200 % of the original price ($ 2.96/kg, or approximately $ 15 238 for the yearly feed), the ROI of the

process very marginally decreased, peaking at 101.59 % when costs are halved, with a minimum value of 101.56 % when costs are doubled.

If the same analysis is performed on the blend process, altering the prices for the cannabis buds within the range of 50 % of the original price ($ 0.74/kg) and 200 % of the original price ($ 2.96/kg), the ROI of the process once again very marginally declines, peaking at 21.76 % when costs are halved, with a minimum value of 21.73 % when costs are doubled. These changes are minuscule, varying by only 0.03 % in both processes – a negligible amount. Thus, it can be said that the processes exhibit extremely low sensitivity to changes in material costs.

A graphical representation of this analysis can be found in Figure 27, while the tabulated values of the plant material costs and their relevant ROIs can be found in Appendix D for a more detailed breakdown of the sensitivity of the processes.

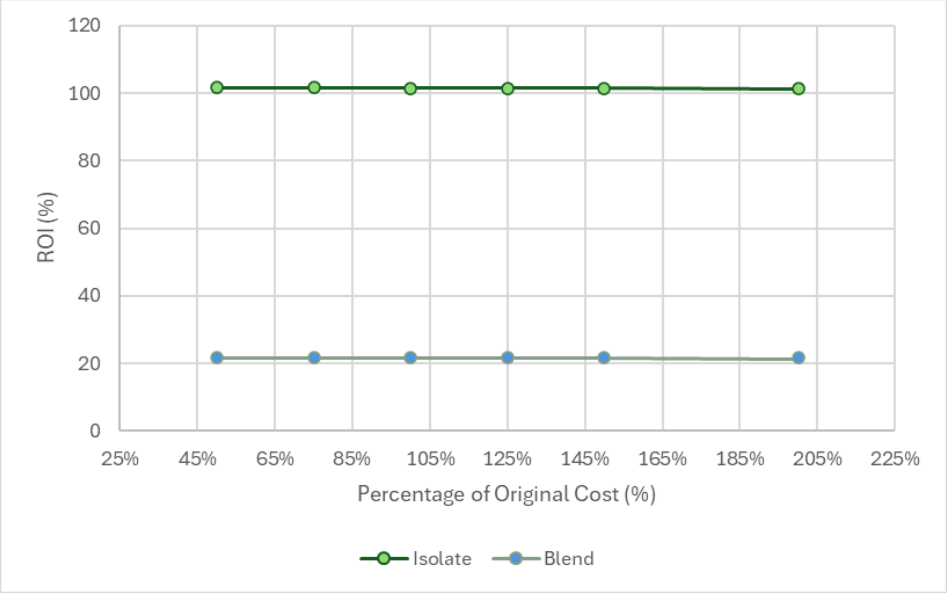


Figure 27: Sensitivity analysis graph for the effects of plant material costs on ROI for the CBD isolate and CBD blend processes.

The cost of the labour initially came to $ 6 918 000 for both processes, which is exponentially higher than the costs of the plant material and, indeed, than the raw material costs in their entirety. More precisely, the labour costs are approximately 577 times higher than the raw material costs in the isolate process, and 692 times higher than those in the blend process.

Thus, it follows that variation in labour costs has the potential to impact profitability of the process to a much larger degree than any fluctuation in raw material costs. This was assessed

by, once again, reducing the costs by 50 %, then by 25 %, then inflating them by 25 %, 50 % and 100 % in turn, and observing the resulting variation in ROI.

When the cost of labour in both processes was increased incrementally from 50 % of the original price ($ 3 459 000) to 200 % of the original price ($ 13 836 000), the ROI of the process decreased much more exponentially than in the case of the plant material costs. In the isolate process, the ROI peaked at 111.00 % when costs were halved, with a minimum value of 83.29 % when costs were doubled, whereas ROI for the blend process peaked at 29.69 % when costs were halved, with a minimum value of 6.31 %, when costs are doubled.

Both processes are significantly more sensitive to fluctuation in labour costs than they are to changes in raw material costs, with ROI varying by 27.71 % in the case of the isolate process and 23.38 %. In the case of the blend process, particularly, this would be cause for concern, as when labour costs are doubled, ROI is decreased to a meagre 6.31 %, which could have serious implications for the profitability of the process. Thus, care must be taken to control and minimise labour costs wherever possible, potentially opting for automation where possible to minimise the labour required, though this would elevate capital expenses.

Graphical representations of the sensitivity analysis can be seen in Figure 28, while the tabulated values of the plant material costs and their relevant ROIs can be found in Appendix D for a more detailed breakdown of the sensitivity of the processes.

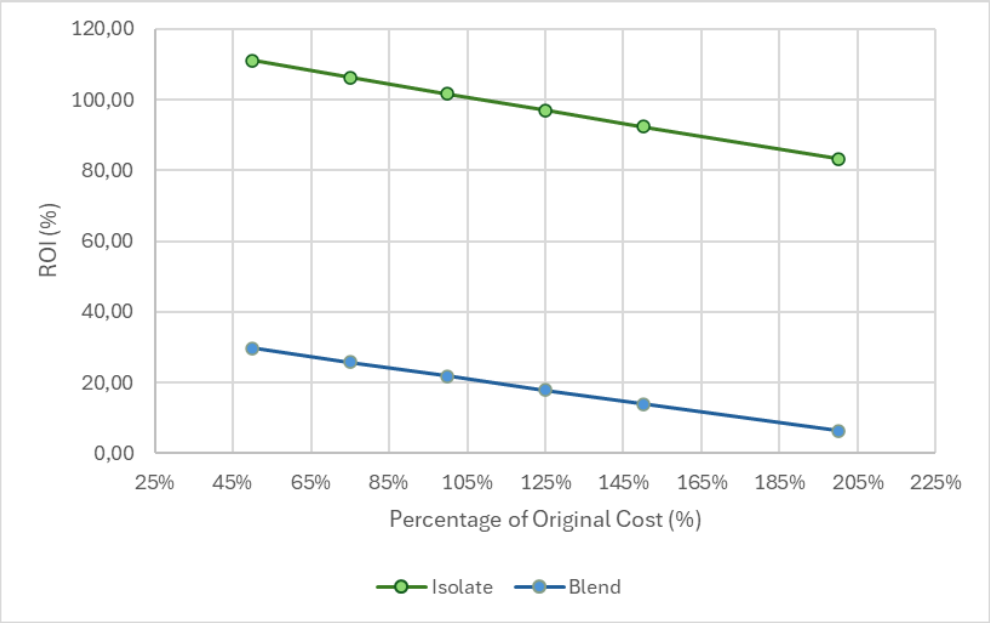


Figure 28: Sensitivity analysis graph for the effects of labour costs on ROI for the CBD isolate and CBD blend processes.

Enzyme Conversion and Input CBD Percentage

The isolate process has already been identified as the more profitable of the processes (Section 5.4), and thus the more in-depth sensitivity analysis and model validation were centred around this process.

For enzyme conversion, a series of numbers was generated using the Inverse of the Normal Cumulative Distribution function on Excel, assuming a mean of 70, a standard deviation (Ơ) of 10, and the Rand() function in place of probability to generate random values for each iteration. These generated values were used as conversion inputs for the simulation (for more information about the precise calculations and detailed numerical results, see Appendix D). The output mass flowrate of the product stream, as well as the percentages of both CBD and THC, were recorded for each conversion value input into the simulation. The standard deviations of the output flow and the CBD output percentage concentrations were then recorded; THC concentrations were noted to ensure that THC was never present in the product stream, irrespective of how low the enzyme conversion dropped or how low input CBD percentage became – this was confirmed, and THC remained absent throughout.

To ensure the consistency of the results generated by the process model, the CBD percentage in the product stream and output flow values were displayed in scatterplots, and trendlines were added for both variables. Using the trendline equation, 'graphical values' were generated for each conversion value input into the simulation, alongside their 'simulation value' counterparts. The standard deviation between the two datasets was then calculated to gauge the extent to which the simulation values aligned with their graphically predicted counterparts, thus providing insight into the reliability and consistency of the modelled process.

The same procedure was undertaken using percentage CBD in the input feed, using the Inverse of the Normal Cumulative Distribution function once again, this time with a mean of 5 and a standard deviation of 2.5 to generate values for input percentage CBD. It is also worth noting that the combined composition of THC and CBD in the feed, as specified in Section 3.2 was maintained at 10% of the total feed, so varying CBD percentages altered THC percentages in turn, so that their sum always equated to 10 %. Once again, simulation- and graphical values were obtained for percentage output CBD and output flow, so that standard deviations could

be calculated for both datasets, as well as the standard deviation between them. The results of these calculations as well as the relevant graphs produced can be found below in Table 36 and Figure 29 respectively.

Table 36: Mean and standard deviation for the effect of enzyme conversion and input CBD percentage on output flow and output CBD percentage in the CBD isolate process.

Enzyme Conversion		**Output Flow**		**Ouput CBD %**	
Mean	*σ*	*Simulation Mean*	*Simulation σ*	*Simulation Mean*	*Simulation σ*
70	10	581,8514	42,3377	99,4125	0,0447
		Graphical Mean	*Graphical σ*	*Graphical Mean*	*Graphical σ*
		581,8508	42,3377	99,4124	0,0446
		Comparative σ	5,8478E-04	*Comparative σ*	1,3838E-03

Input CBD %		**Output Flow**		**Ouput CBD %**	
Mean	*σ*	*Simulation Mean*	*Simulation σ*	*Simulation Mean*	*Simulation σ*
5	2,5	640,3322	18,8606	99,468475	1,5677E-02
		Graphical Mean	*Graphical σ*	*Graphical Mean*	*Graphical σ*
		640,3320	18,8377	99,4683	0,0156
		Comparative σ	0,9027	*Comparative σ*	7,1575E-04

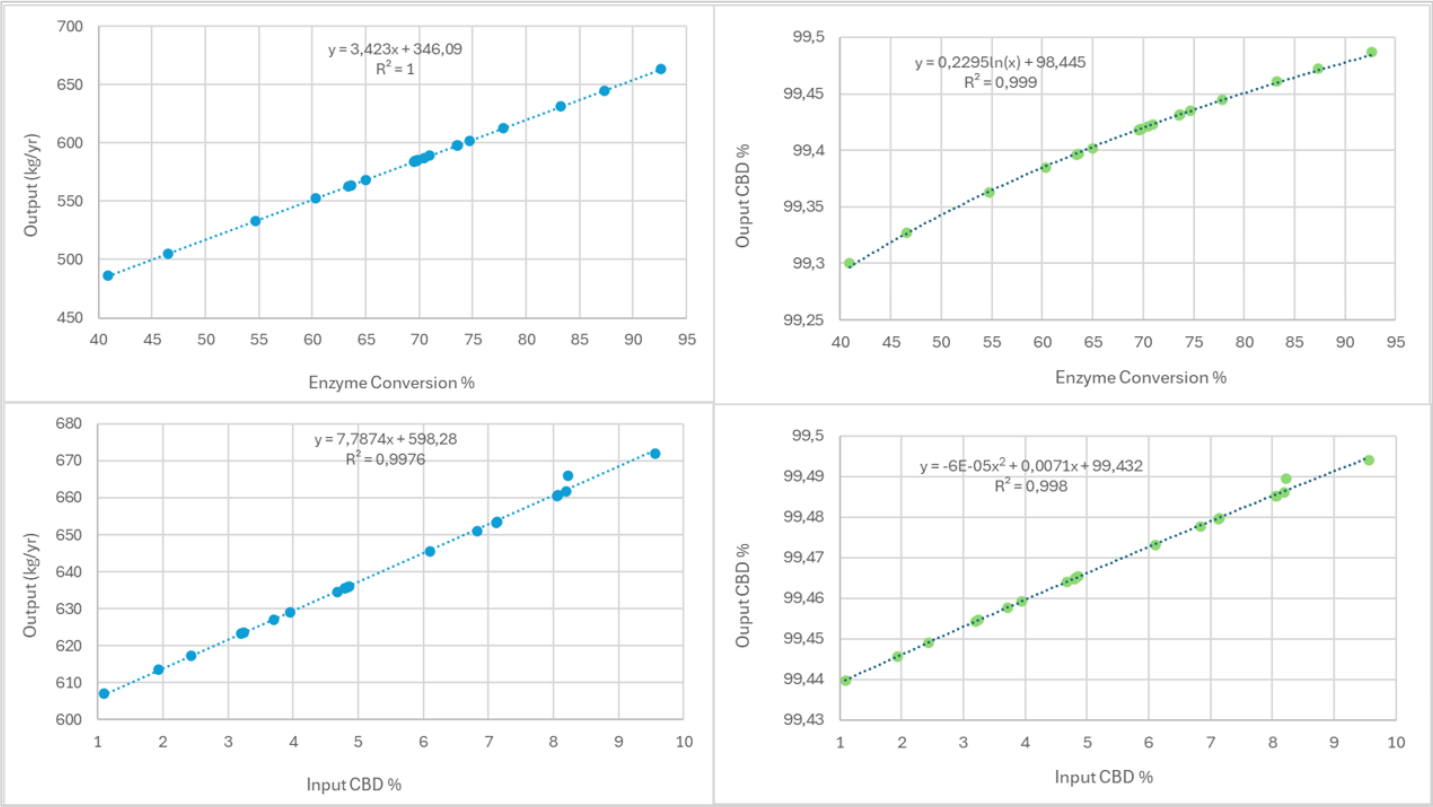


Figure 29: Sensitivity analysis graph for the effect of enzyme conversion and input CBD percentage on output flow and output CBD percentage in the CBD isolate process.

In terms of the reliability of the model, the fact that the comparative standard deviations (in this case, defined according to

Equation 5) were all less than 1 (ranging from 5.85E-04 to 0.9027) is indicative of the fact that the output values vary predictably in response to variation in the input values. The consistency between the simulation and graphical datasets supports the reliability of the simulation.

$$Comparative\ \sigma = \sqrt{\frac{\sum(simulation\ datapoint(n) - graphical\ datapoint(n))^2}{N}}$$

Equation 5

The 'simulation standard' deviations can also offer insight into how drastically the output flow and output CBD percentages fluctuate according to variations in conversion and input CBD percentage. Table 36 revealed that the standard deviation due to fluctuations in enzyme conversion rate was 42.337 for output flow rate and 0.0447 for output CBD percentage.

Standard deviation, unlike the comparative standard deviation defined above, was calculated according to Equation 6.

$$\sigma = \sqrt{\frac{\sum(datapoint(n) - mean)^2}{N}}$$

Equation 6

This suggests that the output flow rate is sensitive to variations in the enzyme conversion rate, while the output CBD percentage is relatively unaffected by these changes. The enzyme conversion rate selected for the initial simulation (85%) was based on a theoretical enzyme, which introduces a degree of uncertainty. Given the significant variability observed in the output flow rate during the sensitivity analysis, it is apparent that even slight deviations in enzyme conversion during practical application could result in substantial differences in the actual output flow.

This indicates that the process's practical applicability could be heavily influenced by the accuracy and reliability of the enzyme's conversion rate. If an enzyme with a different or less consistent conversion rate was used, the output flow could deviate significantly from the estimated values, which must be accounted for in real-world applications. Therefore, rigorous refinement and testing of the enzyme conversion rate would be critical in ensuring the scalability and reliability of the proposed process, once the theoretical enzyme has been found and/or created.

The resulting standard deviations for output flow rate and output CBD percentage were 18,8606 and 1,5677E-02, respectively, due to variations in input CBD percentage. These results demonstrate that both the output flow rate and the output CBD percentage are notably less sensitive to changes in the input CBD percentage compared to the enzyme conversion rate analysis. While variations in input CBD percentage do have some impact, the relatively small standard deviations indicate that changes in the input feed's CBD concentration lead to less significant deviations in both the process flow rate and the resulting CBD percentage in the output.

The limited sensitivity of the output variables to input CBD percentage suggests that fluctuations in feed composition are less likely to drastically affect the overall process performance, comparative to the conversion rate. Thus, while input CBD percentage plays a

role in the process, its impact on output flow and CBD percentage is more manageable and less of a concern in terms of variability. The primary focus, therefore, remains on the enzyme conversion rate as the more critical factor influencing output reliability.

6. Alternate Extraction Method

The profitability of the production of CBD isolates and CBD full spectrum blends has already been compared in Section 5.4, above, with the isolate production emerging as the more profitable of the two. However, both processes relied upon cold ethanol extraction. An alternative extraction method that will now be briefly explored is the use of supercritical CO_2 extraction to produce CBD isolates; this method was also discussed in Section 3.5.2. A PFD depicting this process can be seen in Figure 31.

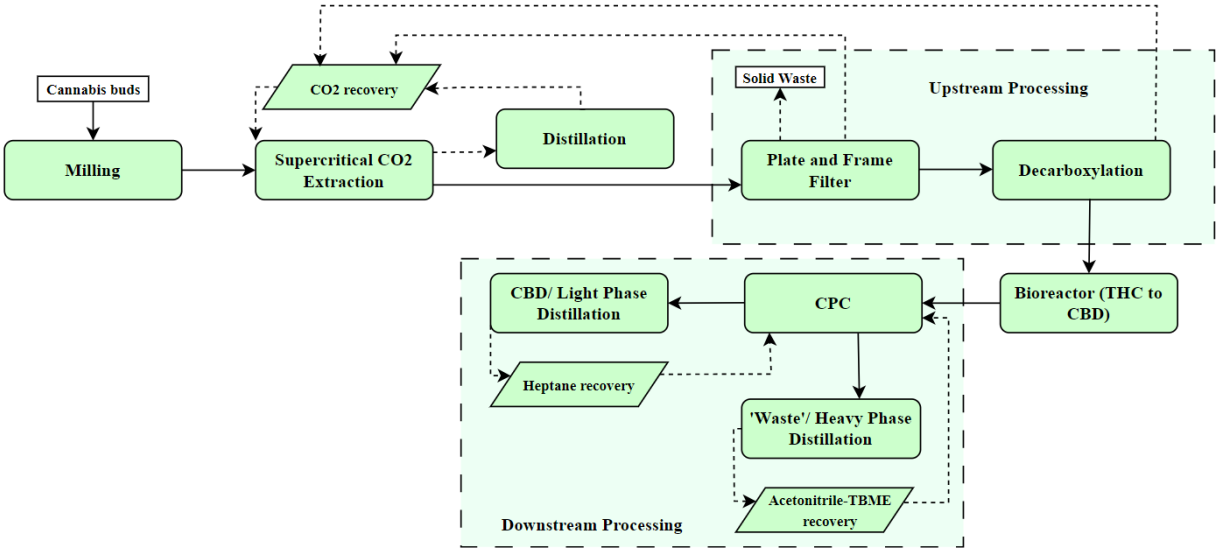


Figure 30: Block flow diagram overview of the process producing CBD isolates using supercritical CO_2.

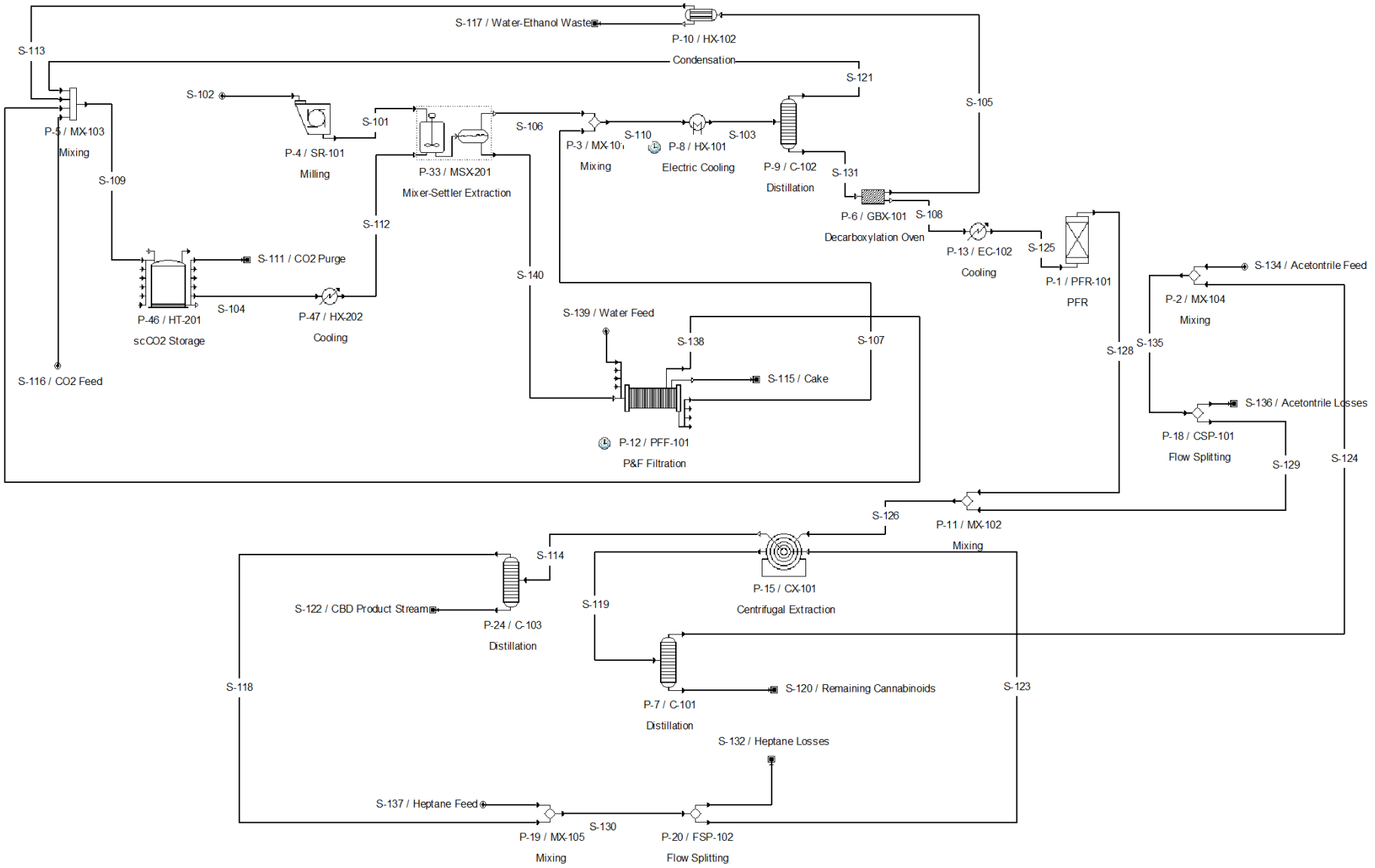


Figure 31: Process flow diagram for an alternative, supercritical CO_2 extraction-based production of CBD isolates designed using SuperPro Designer.

Regarding the supercritical CO_2 extraction-based process, a patent from Müller (2002) provides a basis for the various parameters required for the process. Ethanol has been added to the CO_2 to function as an entrainer; a concentration between 1 – 10 % in reference to the amount of CO_2 used is recommended. Thus, the new solvent stream (the CO_2 feed) for the alternative process will consist of 6 kg/h CO_2 and 0.5 kg/h ethanol (8.33 %).

CO_2 enters the supercritical range at a temperature between 31 ° C and 80 ° C, at pressures between 75 bar and 500 bar, with optimal process parameters of 60 ° C and 250 bar – thus, these are the parameters that have been used. The CO_2 extraction process, without the addition of the CPC, was reported to achieve 90 % CBD purity once it was separated from the solvent (Müller, 2002).

One issue with the CO_2 extraction process is that, unlike the primary process, it will require winterisation to remove waxes, triglycerides, and chlorophyll from the CBD product. This is inconvenient, especially in the case of an otherwise continuous process, as each winterisation can only occur in batches, requiring 24 hours per batch, thus holding up process flow. Winterisation will occur at -20 °C. Further discussion on winterisation can be found in Section 3.5.4.

Much like the original isolate model, the supercritical CO_2 model also produces CBD isolates as a primary product and THC, CBG, and CBN isolates as ancillary products. It is also composed of four primary parts: extraction of the crude oil (now using supercritical CO_2 extraction instead of cold ethanol extraction) (1), upstream processing of the oil (now including winterisation) (2), the reaction of THC into CBD (3), and the downstream processing of the resultant oil (4).

After milling (SR-101), the cannabis buds will now pass through the supercritical CO_2 extraction unit; due to a lack of specialised equipment within the SuperPro database, this has once again been modelled as a mixer-settler (MSX-201), though it must be noted that this is not entirely accurate. In the spirit of costing the process more realistically, the purchase price of the unit was altered to $ 85 000 to more accurately reflect the costs of a supercritical CO_2 extraction process (extraktLAB, 2023).

A CO_2-ethanol feed at room temperature and atmospheric pressure enters the process and is mixed with three CO_2 recycle streams (to be detailed later) in mixer MX-103. The output CO_2 stream then enters the CO_2 storage unit (HT-201), which is pressurised to 500 bar; two output streams exit HT-201, a purge stream, and the solvent feed stream. The solvent feed stream’s

temperature is set to 60 °C via heat exchanger HX-202; this ensures supercritical conditions as the CO_2 stream enters extraction unit MSX-201 alongside the cannabis stream.

The top stream consists of primarily CO_2, as well as containing ethanol and the extracted cannabis compounds; upon exiting the extraction unit, it enters mixer MX-101. The bottom stream from extraction unit MSX-201 consists largely of solid plant matter, as well as smaller concentrations of CO_2, cannabis compounds, and ethanol; upon exiting the extraction unit, it passes into a plate-and-frame filtration system, PFF-101, alongside a water feed stream. It is then separated into a cake consisting primarily of plant matter, a gaseous stream consisting primarily of CO_2, and a water-cannabis crude oil stream. The cake can then be disposed of.

The gaseous CO_2 output stream is recycled and fed back into mixer MX-103, alongside the CO_2 feed and two other recycle streams. The water-cannabis oil stream is fed into mixer MX-101, alongside the top stream from extraction unit MSX-201. This is the extraction segment (1).

The combined oil stream exiting the mixer then moves to cooling unit HX-101 – this is where winterisation takes place. The cooling unit does not operate continuously like most of the process, instead operating in day-long cycles. The oil then moves to distillation column C-102, consisting of 7 distillation stages and operating at 1.5 bar, with a condenser temperature of 173.4 °C and a reboiler temperature of 172.9 °C. The distillate is composed of 99.87 % CO_2 and is recycled back into mixer MX-103 alongside the CO_2 feed and the other two recycles. The bottom stream consists primarily of water and cannabis compounds, with small concentrations of CO_2 and ethanol – this stream then enters the decarboxylation oven (GBX-101), which will convert the cannabinoids into their neutral forms, producing CBD and THC releasing CO_2.

The oven vaporises any lingering ethanol and water, which will all then pass through condenser HX-102 alongside the CO_2 (both that which remains of the solvent and the CO_2 released in the decarboxylation reaction). The vapour stream is composed of 99.04 % CO_2, which is fed back into mixer MX-103. The liquid waste stream consists primarily of ethanol and water. The cannabis oil stream from decarboxylation oven GBX-101 is then cooled to 25 °C in heat exchanger EC-102 and continues onto the reactor. This is the upstream processing segment (2).

The remainder of the process, including the reactor and the CPC system, continues in the same manner as the original cold ethanol extraction isolate process (see Section 4). The relevant

ancillary calculations still apply, in much the same vein as the original cold ethanol extraction isolate design.

The product stream (590.48 kg/year) achieves CBD purity of 99.29 %, which is acceptable, and contains 0.37 % heptane, at a concentration of 3.749 g/L (i.e., below the 4.9943 g/L limit).

The cost of producing CBD oil by cold ethanol extraction versus by supercritical CO_2 extraction was analysed using SuperPro Designer. Unless specified otherwise, all the same baseline values are used as was applied in the economic analysis in Section 5.4.

Much like in Section 5.3, the Executive Summary from the EER for both processes can be seen in Table 37. Within it, the total capital investment (TCI), total operating cost, and total unit operating cost for the cold ethanol process (the same process referred to as the 'isolate process' in the economic analysis carried out in Section 5.3) producing 637 kg/year of CBD isolate (31 850 L after the addition of MCT oil) and 494 kg/year of the ancillary cannabinoids (24 700 L after the addition of MCT oil), and the supercritical CO_2 process producing 591 kg/year of the CBD isolate (29 550 L after the addition of MCT oil) and 432 kg/year of the ancillary cannabinoids (21 600 L after the addition of MCT oil).

According to Table 37, the TCI for the production of CBD isolates via cold ethanol is approximately $ 31.2 million, with a total operating cost of approximately $ 8.0 million and a total unit production cost of $ 12 500/kg for the isolate product prior to MCT dilution. An NPV of $ 190.4 million at a 7% discount rate and a gross margin of 84 % is achieved, with an ROI of 102 % and a payback time of approximately 1 year. In contrast, the TCI for the supercritical CO_2 is approximately $ 30.6 million, with a total operating cost of around $ 8.0 million and a total unit production cost of $ 12 600/kg for the isolate product prior to MCT dilution. An NPV of $ 173.4 million at a 7% discount rate and a gross margin of 84 % is achieved, with an ROI of 95 % and payback time of approximately 1 year.

Table 37: Executive summaries of the economic analyses for the cold ethanol and supercritical CO_2 processes.

Cold Ethanol Separation	
Total Capital Investment	31236000 $
Capital Investment Charged to This Project	31236000 $
Operating Cost	8005000 $/yr
Main Revenue	40828000 $/yr
Other Revenues	8764198 $/yr
Total Revenues	49592000 $/yr
Cost Basis Annual Rate	637,05 kg MP/yr
Unit Production Cost	12565,48 $/kg MP
Unit Production Revenue	77847,50 $/kg MP
Gross Margin	83,86 %
Return On Investment	101,58 %
Payback Time	0,98 years
IRR (After Taxes)	49,38 %
NPV (at 7,0% Interest)	190458000 $
MP = Total Flow of Stream 'S-122'	

Supercritical CO_2 Separation	
Total Capital Investment	30641000 $
Capital Investment Charged to This Project	30641000 $
Operating Cost	7251000 $/yr
Main Revenue	37855000 $/yr
Other Revenues	7668768 $/yr
Total Revenues	45524000 $/yr
Cost Basis Annual Rate	590,66 kg MP/yr
Unit Production Cost	12276,78 $/kg MP
Unit Production Revenue	77073,29 $/kg MP
Gross Margin	84,07 %
Return On Investment	95,28 %
Payback Time	1,05 years
IRR (After Taxes)	47,24 %
NPV (at 7,0% Interest)	173404000 $
MP = Total Flow of Stream 'S-122'	

Table 38 shows a section of the Profitability Analysis table of the EER that further breaks down the TCI of both processes. The DFC for the cold ethanol process is approximately $ 5.68 million, the startup cost is $ 284 thousand, and working capital is $ 631 thousand; for the supercritical CO_2 process, the values are $ 8.18 million for the DFC, $ 409 thousand for the startup cost, and $ 630 thousand working capital.

Table 38: Total capital investment for the cold ethanol and supercritical CO_2 processes.

Cold Ethanol Separation		
A.	Direct Fixed Capital	5680000 $
B.	Working Capital	631000 $
C.	Startup Cost	284000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	31236000 $
G.	Investment Charged to This Project	31236000 $

Supercritical CO_2 Separation		
A.	Direct Fixed Capital	5170000 $
B.	Working Capital	571000 $
C.	Startup Cost	259000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	30641000 $
G.	Investment Charged to This Project	30641000 $

Table 39, which is also extracted from the EER, provides a breakdown of the DFC. As explained in Section 5.3, the DFC consists of direct and indirect costs. SuperPro estimates direct costs based on multipliers of the installed equipment costs. SuperPro also splits indirect costs into two segments; the first of which pertains to indirect costs such as construction and engineering, calculated based on multipliers of the sum of the direct costs, while the second segment pertains to indirect costs such as contractor's fee and contingency, and is based on multipliers of the sum of direct costs and the previously described group of indirect costs.

According to Table 39, the total plant direct cost (TPDC) for the cold ethanol process is approximately $ 3.09 million, the total plant indirect cost (TPIC) is $ 1.85 million, and the contractor's fee and contingency (CFC) comes to $ 741 thousand. The TPDC for the supercritical CO_2 process is approximately $ 2.81 million, the TPIC is $ 1.69 million, and the CFC is $ 674 000. The total plant cost can then be calculated through the addition of the TPDC and the TPIC, and the DFC can be calculated through the addition of the TPC and the CFC.

Table 39: Fixed capital expenses for the cold ethanol and supercritical CO_2 processes.

Cold Ethanol Separation	
3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	929000
2. Installation	410000
3. Process Piping	325000
4. Instrumentation	372000
5. Insulation	28000
6. Electrical	93000
7. Buildings	418000
8. Yard Improvement	139000
9. Auxiliary Facilities	372000
TPDC	3087000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	772000
11. Construction	1080000
TPIC	1852000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	4939000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	247000
13. Contingency	494000
CFC = 12+13	741000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	5680000

Supercritical CO_2 Separation	
3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	849000
2. Installation	364000
3. Process Piping	297000
4. Instrumentation	340000
5. Insulation	25000
6. Electrical	85000
7. Buildings	382000
8. Yard Improvement	127000
9. Auxiliary Facilities	340000
TPDC	2810000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	702000
11. Construction	983000
TPIC	1686000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	4496000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	225000
13. Contingency	450000
CFC = 12+13	674000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	5170000

Table 40: Equipment breakdown summary for the cold ethanol and supercritical CO_2 processes.

CBD Isolate Production				
Name	*Type*	*Capacity*		*Price ($)*
CX-101	Centrifugal Extractor	9,22	L/h	43000
HX-101	Condenser	0,02	m2	108000
C-101	Distillation Column	2,43	L	26000
C-103	Distillation Column	1,07	L	83000
GBX-101	Generic Box	7,54	kg/h	3000
EC-102	Heat Exchanger	-	m2	4000
MSX-102	Mixer-Settler Extractor	4,02	L/h	9000
MSX-101	Mixer-Settler Extractor	5,94	L/h	9000
PFF-101	Plate and Frame Filter	0,01	m2	73000
PFR-101	Plug Flow Reactor	0,15	L	81000
SR-101	Shredder	0,65	kg/h	86000
Alternative CBD Isolate (Supercritical CO2) Production				
Name	*Type*	*Capacity*		*Price ($)*
CX-101	Centrifugal Extractor	9,21	L/h	43000
HX-102	Condenser	0,01	m2	108000
C-101	Distillation Column	2,42	L	26000
C-102	Distillation Column	5,79	L	15000
C-103	Distillation Column	1,07	L	83000
HT-201	Flat Bottom Tank	-	m3	66000
GBX-101	Generic Box	1,54	kg/h	3000
EC-102	Heat Exchanger	-	m2	4000
HX-202	Heat Exchanger	-	m2	9000
MSX-201	CO2 Extraction Unit	1,02	L/h	85000
PFF-101	Plate and Frame Filter	0,01	m2	73000
PFR-101	Plug Flow Reactor	0,15	L	81000
SR-101	Shredder	0,65	kg/h	86000

According to Table 40, which is a breakdown of the primary equipment involved in the processes, the collective costs of the cold ethanol process equipment come to $ 525 000, while the collective costs of the supercritical CO_2 process equipment come to $ 682 000 - 130 % higher than the cold ethanol process.

In the supercritical CO_2 process, distillation costs are relatively similar to those found in the cold ethanol extraction process ($ 15 000 greater). Far more significant are the additional costs of the CO_2 extraction unit MSX-201 and the supercritical CO_2 storage tank HT-201, which contribute 12 % and 10 % to the total equipment costs, respectively. The total operating costs

can be found in Table 41, as taken from the ICR, while material costs (and the amounts of material used) can be found in Table 42.

Table 41: Annual operating costs for the cold ethanol and supercritical CO_2 processes.

Cold Ethanol Separation		
Cost Item	**$**	**%**
Raw Materials	12000	0,15
Labor-Dependent	6918000	86,42
Facility-Dependent	1068000	13,35
Waste Treatment/Disposal	1000	0,01
Utilities	5000	0,07
TOTAL	8005000	100,00

Supercritical CO_2 Separation		
Cost Item	**$**	**%**
Raw Materials	13000	0,18
Labor-Dependent	6240000	86,05
Facility-Dependent	973000	13,41
Waste Treatment/Disposal	4000	0,06
Utilities	21000	0,29
TOTAL	7251000	100,00

Table 42: Material costs for the cold ethanol and supercritical CO_2 processes.

Cold Ethanol Separation					
Bulk Material	**Unit Cost ($)**	**Annual Amount**		**Annual Cost ($)**	**%**
Acetonitrile / TBME	1,24	1584	kg	1964,16	15,86
Ethanol	0,75	3168	kg	2376	19,18
Heptane	0,36	1188	kg	427,68	3,45
Plant Material	1,48	5148	kg	7619,04	61,51
Total		11088	kg	12386,88	
Supercritical CO_2 Separation					
Bulk Material	**Unit Cost ($)**	**Annual Amount**		**Annual Cost ($)**	**%**
Carbon Dioxide	0,06	47520	kg	2741,52	17,44
Acetonitrile / TBME	1,24	1584	kg	1964,16	12,49
Ethanol	0,75	3960	kg	2970	18,89
Heptane	0,36	1188	kg	427,68	2,72
Plant Material	1,48	5148	kg	7619,04	48,46
Total		11880	kg	15722,40	

In Table 41 and Table 42, it can be seen that the collective material costs for the cold ethanol process are approximately $ 12 000, 92.31 % of the costs of material for the supercritical CO_2 process, at $ 13 000. Returning to Table 41, the utility and facility-dependent costs make up approximately 0.07 % and 13.35 % of the total operating costs, respectively, for the cold ethanol process, with facility-dependent costs including maintenance, depreciation, and other

overhead costs. In the supercritical CO_2 process, these values come to 0.29 % and 13.41 %, respectively. In both cases, these values are dwarfed by the labour-dependent costs, which contribute the most sizeable portion of the process operating costs – 86.42 % in the case of the cold ethanol process and 86.05 % for the supercritical CO_2 process. To further explore the contribution of utility costs to the total operating cost, Table 43 was also taken from the ICR.

Table 43: Utility costs for the cold ethanol and supercritical CO_2 processes.

Cold Ethanol Separation					
Utility	**Unit Cost ($/kW-h)**	**Amount (/year)**		**Cost ($/year)**	**%**
Std Power	0,10	1724	kW-h	172,41	100,00
TOTAL		1724	kW-h	172,41	100,00
Utility	**Unit Cost ($)**	**Amount (/year)**		**Cost ($/year)**	**%**
Steam	32,00	60	MT	1929,16	37,02
Steam (High P)	36,00	16	MT	570,71	10,95
Cooling Water	0,10	954	MT	95,36	1,83
Chilled Water	0,50	5232	MT	2615,98	50,20
TOTAL				5211,21	100,00
Supercritical CO_2 Separation					
Utility	**Unit Cost ($/kW-h)**	**Amount (/year)**		**Cost ($/year)**	**%**
Std Power	0,10	531	kW-h	53,10	1,50
High Voltage	20,60	169	kW-h	3484,80	98,50
TOTAL		700	kW-h	3537,90	100,00
Utility	**Unit Cost ($)**	**Amount (/year)**		**Cost ($/year)**	**%**
Steam	32,00	2	MT	54,20	6,80
Steam (High P)	36,00	16	MT	566,98	71,12
Cooling Water	0,10	952	MT	95,18	11,94
Chilled Water	0,50	6	MT	3,01	0,38
Steam-2	30,00	0	MT	2,66	0,33
Glycol	0,35	215	MT	75,14	9,43
TOTAL				797,16	100,00

Table 43 indicates that the cost of electricity consumed by the production of CBD oils comes to $ 172.41 for the cold ethanol process (3.20 % of the total utility costs for the process) and $ 3537.90 for isolate production (81.61 % of the total utility costs for the process). For the cold ethanol process, cooling and heating agents contributed most of the utility costs, while for the supercritical CO2 process, the electricity prices were incredibly high, primarily due to the necessity of high-voltage power.

The profitability of the original isolate process versus that of the CO_2 extraction-based process is more evenly matched than that of the cold ethanol process (the original isolate process) versus the full spectrum blend process. The original process has lower capital costs ($ 13 236 000 versus $ 30 641 000) but higher operating costs ($ 8 005 000 /year versus

$ 7 251 000 /year), and greater revenue ($ 49 592 000 /year versus $ 45 524 000 /year) than the CO_2 extraction process.

The original process has much lower raw material ($ 12 000 /year versus $ 13 000 /year), waste disposal ($ 1000 /year versus $ 4400 /year), and solvent costs ($ 4775 /year versus $ 5369 /year), but higher labour ($ 6 918 000 /year versus $ 6 240 000 /year) and utility costs ($ 5000 /year versus $ 21 000 /year).

The isolate process also scores higher in four of the five project indices compared, emerging once again as the superior process. The CO_2 extraction process has a slightly higher gross margin (84.07 % versus 83.86 %), but the original isolate process has a higher ROI (101.58 % versus 95.28 %), shorter payback time (0.98 years versus 1.05 years), a higher IRR (49.38 % versus 47.24 %), and a higher NPV ($ 190 458 000 versus $ 173 404 000).

Thus, while the difference in profitability is not as vast as it is in the case of the CBD blend process, the original cold ethanol extraction remains the most profitable of the CBD oil processes.

A comparative graphical net cash flow analysis is displayed in Figure 32, comparing the net cash flow of the two processes. It can be seen that the net cash flows of the original isolate process and the supercritical CO2 process err considerably closer than those of the original isolate process and the blend process, as compared in Section 5.3. However, the original isolate process does still emerge as slightly higher.

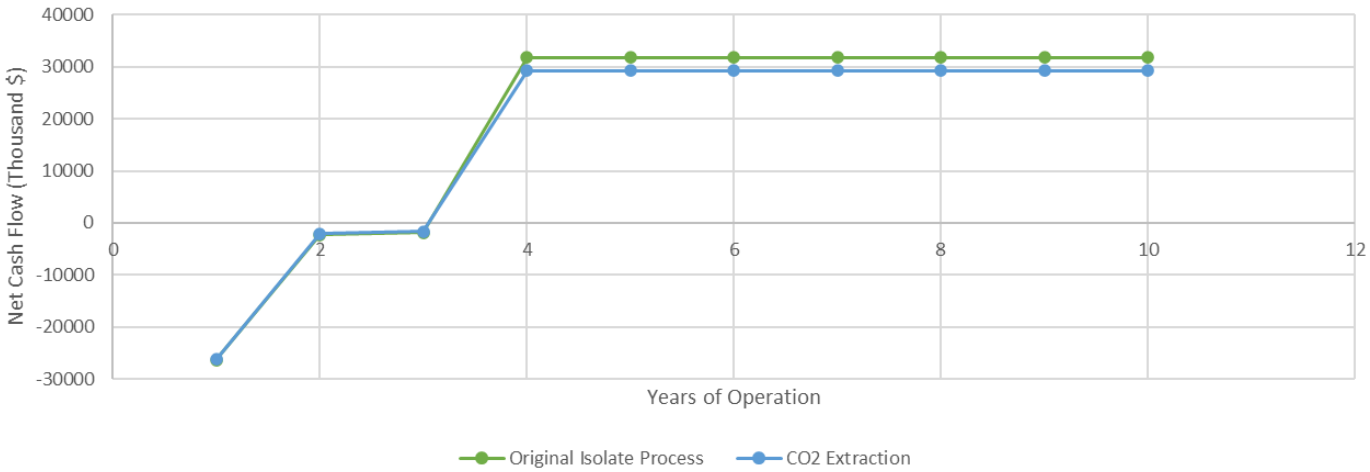


Figure 32: Net cash flow comparison of the production of CBD isolates using the original cold ethanol extraction process versus the supercritical CO_2 process.

7. Effects of Production Scale-Up

While the isolate process using cold ethanol has proved profitable in its current form, it is also important to assess the process for its potential profitability on an industrial scale. As the demand for CBD isolate continues to rise, there is a need to design and optimize industrial-scale plants capable of handling far more substantial throughputs while ensuring economic viability. This section aims to scale up the process from its initial conservative scale to a more robust industrial scale; this was accomplished by introducing a significantly increased feed rate of 10 kg/hr of cannabis buds, where the initial feed rate was 0.65 kg/hr, or 79 200 kg/year. The cost of cannabis is assumed to remain the same.

In scaling up the process, several parameters were recalculated. Notably, the solvent inputs (the acetonitrile/TBME blend, ethanol, and heptane), were proportionally scaled up to maintain the efficacy of the process, using the same ratio as that used for the scale-up of the plant material feed. Additionally, the quantity of the enzyme required for catalysing the reaction has been recalculated based on the increased feed rate, as well as the size of the reactor. According to the calculations made in Appendix C, 576.25 kg of enzymes will be required per year, coming to a yearly cost of $ 345 750 (or $ 1 512 656 per year if GMP-grade enzymes are utilised, as discussed in Section 5.2).

In terms of product streams, the CBD isolate (prior to MCT dilution) exhibits a purity of 99.47% and a production rate of 1.24 kg/hr, equivalent to 9800 kg per year. The rate of the ancillary cannabinoid product came to 0.96 kg/hr, translating to 7587 kg annually. For each product stream, MCT oil will still serve as the diluent prior to sale, in the form of 30 ml units containing 600 mg of the relevant cannabinoid. Thus, the new MCT oil requirement per year was also calculated, with 490 000 L being required for the dilution of the CBD isolates, and 370 350 L being required for the ancillary cannabinoid products. Collectively, this adds up to 869 350 L per year.

While the configuration of the process remains unchanged, the scale-up also necessitates changes to several aspects, including the multiplication of certain process units. The specific equipment and corresponding quantities are given in Table 44, where initially only singular units were installed.

Table 44: Multiples of equipment necessitated by scale-up.

Equipment		Required units
Mill	SR-101	10
Ethanol Extraction Unit 1	MSX-101	5
Ethanol Extraction Unit 2	MSX-102	4
Decarboxylation Oven	GBX-101	6
Acetonitrile/TBME Mixer	MX-104	6
Acetonitrile/TBME Splitter	CSP-101	6
CPC Feed Mixer	MX-102	6
CPC	CX-101	8
Heptane Mixer	MX-105	3
Heptane Splitter	FSP-102	3

An economic analysis akin to that in Section 5.4 was also conducted to evaluate the financial viability of the industrial-scale operation, where all variables that have remained unspecified here are assumed to have remained constant.

According to Table 45, the TCI for the scaled-up process is approximately $ 42.1 million, with a total operating cost of approximately $ 11.2 million and a total unit production cost of $ 1130/kg for the CBD isolate product, prior to MCT dilution. An NPV of $ 3.99 billion at a 7% discount rate and a gross margin of 99 % is achieved, with an ROI of an exponential 1340 % and a payback time of only 0.07 years.

Table 45: Executive summary of the economic analysis for the scaled-up CBD isolate process.

Total Capital Investment	42068000 $
Capital Investment Charged to This Project	42068000 $
Operating Cost	11158000 $/yr
Main Revenue	628101000 $/yr
Other Revenues	134680846 $/yr
Total Revenues	762782000 $/yr
Cost Basis Annual Rate	9800 kg MP/yr
Unit Production Cost	1138,52 $/kg MP
Unit Production Revenue	77832,45 $/kg MP
Gross Margin	98,54 %
Return On Investment	1343,63 %
Payback Time	0,07 years
IRR (After Taxes)	226,07 %
NPV (at 7,0% Interest)	3959192000 $

Table 46 shows a section of the Profitability Analysis table of the EER that further breaks down the TCI of the scaled-up process. The DFC is approximately $ 16.0 million, the startup cost is $ 799 thousand, and the working capital is $ 655 thousand.

Table 46: Total capital investment for the scaled-up CBD isolate process.

A.	Direct Fixed Capital	15972000 $
B.	Working Capital	655000 $
C.	Startup Cost	799000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	42068000 $
G.	Investment Charged to This Project	42068000 $

Table 47, which is also extracted from the EER, provides a breakdown of the DFC. According to the table, the TPDC for the scaled-up process is approximately $ 8.68 million, the TPIC is $ 5.21 million, and the CFC comes to $ 2.08 million. The total plant cost can then be calculated through the addition of the TPDC and the TPIC, and the DFC can be calculated through the addition of the TPC and the CFC.

Table 47: Fixed capital expenses for the scaled-up CBD isolate process.

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2586000
2. Installation	1232000
3. Process Piping	905000
4. Instrumentation	1035000
5. Insulation	78000
6. Electrical	259000
7. Buildings	1164000
8. Yard Improvement	388000
9. Auxiliary Facilities	1035000
TPDC	8681000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	2170000
11. Construction	3038000
TPIC	5208000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	13889000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	694000
13. Contingency	1389000
CFC = 12+13	2083000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	15972000

Table 48: Equipment breakdown summary for the scaled-up CBD isolate process.

Name	Type	Units	Size (Capacity)		Unit Price ($/Unit)	Total Price ($)
CX-101	Centrifugal Extractor	8	17,59	L/h	43000	344000
HX-101	Condenser	1	0,28	m2	108000	108000
C-103	Distillation Column	1	16,28	L	91000	91000
C-101	Distillation Column	1	37,16	L	31000	31000
GBX-101	Generic Box	6	19,08	kg/h	3000	18000
EC-102	Heat Exchanger	1		m2	4000	4000
MSX-102	Mixer-Settler Extractor	4	15,30	L/h	9000	36000
MSX-101	Mixer-Settler Extractor	5	18,07	L/h	9000	45000
PFF-101	Plate and Frame Filter	1	0,12	m2	73000	292000
PFR-101	Plug Flow Reactor	3	0,77	L	81000	243000
SR-101	Shredder	10	1,00	kg/h	86000	860000

According to Table 48, which is a breakdown of the primary equipment involved in the processes, the collective costs of the process equipment come to $ 2.07 million. In the scaled-up process, the mills (SR-101) make up the majority of the equipment costs, contributing 42 % of the combined costs. This is due to the elevated plant material feed now entering the process, necessitating 10 units in place of the 1 unit installed in the original process. Other major contributors to the equipment cost are the 8 CPCs (CX-101), comprising 17% of the costs, and the 3 PFRs (PFR-101), comprising 12 % of the costs. Collectively, these process units contribute approximately 71 % of the total equipment costs. The total operating costs can be found in Table 49, as taken from the ICR, while material costs can be found in Table 50.

Table 49: Annual operating costs for the scaled-up CBD isolate process.

Cost Item	$	%
Raw Materials	191000	1,71
Labor-Dependent	6918000	62,00
Facility-Dependent	3002000	26,91
Consumables	951000	8,52
Waste Treatment/Disposal	15000	0,13
Utilities	82000	0,73
TOTAL	11158000	100,00

Table 50: Material costs for the scaled-up CBD isolate process.

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Acetonitrile / TBME	1,24	24369	kg	30334	15,91
Ethanol	0,75	48738	kg	36554	19,17
Heptane	0,36	18277	kg	6580	3,45
Plant Material	1,48	79200	kg	117216	61,47
Total		11088	kg	190684	

Table 50 shows collective material costs for the process are approximately $ 191 thousand per year. As shown in Table 49, the utility and facility-dependent costs make up approximately 0.73 % and 26.91 % of the total operating costs, respectively. These values are once again dwarfed by the labour-dependent costs, which contribute the most sizeable portion of the process operating costs at 62.00 %. To further explore the contribution of utility costs to the total operating cost, Table 51 was also taken from the ICR.

Table 51: Utility costs for the scaled-up CBD isolate process.

Utility	Unit Cost ($/kW-h)	Amount (/year)		Cost ($/year)	%
Std Power	0,10	26033	kW-h	2603,27	100,00
TOTAL		26033	kW-h	2603,27	100,00

Utility	Unit Cost ($)	Amount (/year)		Cost ($/year)	%
Steam	32,00	916	MT	29301,28	36,99
Steam (High P)	36,00	242	MT	8727,56	11,02
Cooling Water	0,10	14611	MT	1461,10	1,84
Chilled Water	0,50	79460	MT	39729,99	50,15
TOTAL				79219,93	100,00

Table 51 indicates that the cost of electricity consumed by the scaled-up process comes to approximately $ 2600 for the scaled-up process (a minuscule 3.18 % of the total utility costs for the process), with cooling and heating agents once again contributing the vast majority of the utility costs.

With a gross margin of 98.54 % (approximately 15 % higher than in the case of a non-scaled process, which had a gross margin of 84 %), an ROI of 1344 % (an exponential 1232 % higher than that of the non-scaled process, at 102 %), and a payback time of 0.07 years (almost a full year less than that of the non-scaled process). Thus, it can be confidently said that the scaled-up CBD isolate process has the potential for great profitability if implemented. If GMP-grade enzymes are used in the process, as discussed in Section 5.2 and calculated in Appendix E,

these parameters will manifest as a gross margin of 98.03 %, an ROI of 1342 %, and a payback time of 0.07 years once again, leaving minimal financial impact.

8. Conclusions and Recommendations

This work aimed to model a theoretical enzymatic bioreactor and all the necessary surrounding processes that would be required to facilitate the bioremediation of THC into CBD to produce a CBD product with THC levels below the legal concentration limits (0.001 %). In addition, the objectives included performing a sensitivity analysis and optimisation on the modelled process, as well as an economic analysis.

The primary purpose of this was to determine whether further research into the potential biochemical remediation of THC into CBD would be worth pursuing, in terms of both functionality and potential profitability in terms of the CBD industry.

Two primary process designs are compared: one producing a CBD isolate, and another producing a full spectrum CBD blend containing other cannabinoids beyond CBD, as well as other compounds like flavonoids and terpenes. The isolate process also produces ancillary products in the form of CBG, CGN, and THC isolates. Both processes apply cold ethanol extraction, filtration, decarboxylation, and an enzyme reactor that converts THC into CBD. The isolate process also contains additional phases, namely CPC and distillation.

An additional alternative design, the production of CBD isolates using supercritical CO_2 extraction instead of cold ethanol extraction was also explored; the process closely mirrored the cold ethanol extraction isolate process, with the only major variation being the means of extraction.

Project indices (gross margin, ROI, payback period, IRR, and NPV) were compared for the three designs, where the production of CBD isolates using cold ethanol extraction emerged as the most profitable of the designs, eclipsing the production of CBD blends by a large margin and the supercritical CO_2 process by a smaller, but still definitive, amount. The relevant project indices for the CBD isolate process were as follows: a gross margin of 83.86 %, an ROI of 101.58 %, a payback time of 0.98 years, and an NPV of $ 190 458 000.

Because the isolate process proved the most profitable of the alternatives, its potential profitability when scaled up to industrial size was also assessed. The process feed rate was increased from 5148 kg to 79 200 kg of cannabis buds per year, solvent input streams were proportionally scaled up, and several equipment units were multiplied as required. Additionally, the quantity of the enzyme required for catalysing the reaction was recalculated

based on the increased plant material in the process. The NPV of the scaled-up process came to $ 3.99 billion and a gross margin of 99 % was achieved, with an ROI of 1340 % and a payback time of 0.07 years.

Therefore, from the economic analyses, the production of CBD isolates using THC-to-CBD bioremediation is a potentially profitable, as-yet-untapped production method that would, indeed, benefit from further research.

However, during the exploration of the potential applicability of this process, the lack of experimental data was a major complication. Several blind spots exist which compromise the accuracy of the model, and which would need to be thoroughly investigated before committing to the concept of THC-to-CBD bioremediation.

While this research provides valuable insights into the potential for THC to CBD conversion through enzymatic processes, several limitations must be acknowledged. One of the primary constraints is the scarcity of experimental data regarding cannabinoids, particularly concerning enzymatic conversions and substrate behaviours. Chemical data regarding CBD is incomplete, and data regarding the other cannabinoids are even more so. There is almost an entire lack of VLE data and solubility coefficients as well.

Due to this lack of empirical data, many of the parameters used in the research—such as enzyme conversion rates and partition coefficients—relied on estimations derived from theoretical models and often limited and unrelated experimental values from existing literature. Although these estimates were carefully selected to be conservative, this reliance on theoretical assumptions introduces uncertainty into the findings. This makes modelling a process such as this difficult and somewhat unreliable, and to model a truly accurate process, experimental data for each process unit would need to be gathered before the design could truly be validated.

Additionally, the simulations performed throughout the research, while informative, do not always reflect the full complexities of real-world conditions. For instance, factors such as enzyme denaturation, substrate inhibition, or unforeseen interactions during the conversion process are difficult to fully account for in a simulated environment. As a result, the outcomes of the simulations should be interpreted with an understanding that actual experimental conditions may yield different results.

Another significant limitation lies in the development of the enzyme itself. The research relies on the assumption of a specific enzyme capable of efficiently converting THC into CBD, yet

the discovery or engineering of such an enzyme remains an unknown area of study. This adds an additional layer of uncertainty to the findings, as the success of the proposed process hinges on the creation and optimization of an enzyme that, as of now, remains theoretical.

Finally, the economic feasibility and scalability of this process are dependent on future advancements in both enzyme engineering and industrial applications. Although the sensitivity analyses included in the research help assess the impact of uncertainties, it is important to recognize that further experimentation and real-world validation will be required before the proposed process can be confidently applied on an industrial scale.

In conclusion, while this research offers a promising framework for the enzymatic conversion of THC to CBD, its findings must be understood within the context of these limitations. The theoretical nature of the study, the reliance on assumptions, and the gaps in empirical data underscore the need for further research and experimentation to validate and refine the proposed process.

The enzyme, as of the date this work was completed, is entirely theoretical. Thus, before the process could be investigated in earnest, an enzyme capable of performing the bioremediation of THC into CBD would first need to be discovered or designed, likely requiring genetic modification for it to be commercially viable. The purpose of this work was to explore the potential of such an enzyme, and whether this manner of investigation into such an enzyme could be a profitable venture, so, understandably, data on the enzyme is absent. However, due to the promising outcome of these exploratory simulations, it is recommended that the theoretical enzyme be earnestly investigated before the process is truly designed.

A potential direction for finding an enzyme capable of facilitating the process (or which could be used as a baseline for further modification) would be to investigate enzymes from organisms such as aphids that feed on cannabis, which may possess relevant enzymatic activity for cannabinoid conversion or enzymes found with cannabis which are known to be capable of converting between cannabinoids.

Exploring the potential of genetic to enhance or introduce enzymes that facilitate cannabinoid conversions will also be crucial. Utilising techniques such as directed evolution or protein engineering could also aid in engineering enzymes tailored for the conversion of THC to CBD. Comparative studies of various enzyme sources to identify the most efficient and cost-effective options for cannabinoid conversion should also be considered. These recommendations aim to

provide a possible approach to advancing the research and the potential application of enzymatic cannabinoid conversion.

Additional improvements to the process could include replacing hazardous solvents (acetonitrile and TBME, specifically) with less dangerous alternatives; though, again this would require experimentation into which solvents are applicable for CBD separation. Improvement to the filtration system would also be recommended, as the batch-wise nature of the plate-and-frame system is not ideal within an otherwise continuous process; a moving simulated bed would be a valid alternative, as it has also been found effective for CBD oil production.

The specifics of considerations falling outside the scope of this work such as cannabis cultivation, enzyme production, waste disposal, cleaning, and other such matters would also need to be tackled in earnest before the process could truly come to fruition, though each of these would be a topic worthy of in-depth investigation and would require individualised attention.

Ultimately, however, the aim of this work (i.e.: to explore whether research into the potential biochemical remediation of THC into CBD would be worth pursuing) has been fulfilled. Though investigated in broad strokes due to the inherent limitations of the topic, the positive outcome of the economic analysis supports that further research into the possibility of bioremediation from THC into CBD could be potentially profitable and should be pursued.

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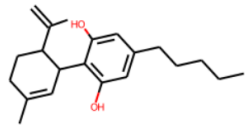
Appendix A: Chemical and Material Properties of Process Components

Chemical Properties of (-)-Cannabidiol (CAS 13956-29-1)



InChI
InChI=1S/C21H30O2
/c1-5-6-7-8-16-12-19(22)21(20(23)13-16)18-11-15(4)9-10-17(18)14(2)3/h11-13,17-18,22-23H,2,5-10H2,1,3-4H3/t17-,18+/m0/s1
InChI Key
QHMBSVQNZZTUGM-ZWKOTPCHSA-N
Formula
C21H30O2
SMILES
C=C(C)C1CCC(C)=CC1c1c(O)cc(CCCCC)cc1O
Molecular Weight[1]
314.46
CAS
13956-29-1

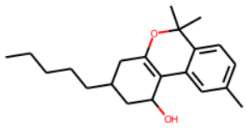
Physical Properties

Property	Value	Unit	Source
$\Delta_f G°$	35.84	kJ/mol	Joback
$\Delta_f H°_{gas}$	-410.40	kJ/mol	Joback
$\Delta_{fus} H°$	46.51	kJ/mol	Joback
$\Delta_{vap} H°$	91.79	kJ/mol	Joback
$log_{10}WS$	-6.10		Crippen
$logP_{oct/wat}$	5.846		Crippen
McVol	275.270	ml/mol	McGowan
P_c	1765.41	kPa	Joback
I_{np}	[2375.00; 2375.00]		
T_{boil}	888.36	K	Joback
T_c	1119.73	K	Joback
T_{fus}	589.51	K	Joback
V_c	0.935	m^3/kmol	Joback

Temperature Dependent Properties

Property	Value	Unit	Temperature (K)	Source
$C_{p,gas}$	[902.02; 1008.09]	J/mol×K	[888.36; 1119.73]	
$\Delta_{fus} H$	28.40	kJ/mol	340.70	NIST

Chemical Properties of Tetrahydrocannabinol (CAS 56282-24-7)



InChI
InChI=1S/C21H30O2
/c1-5-6-7-8-15-12-18(22)20-16-11-14(2)9-10-17(16)21(3,4)23-19(20)13-15/h9-11,15,18,22H,5-8,12-13H2,1-4H3
InChI Key
SJQJFSCMYCROHP-UHFFFAOYSA-N
Formula
C21H30O2
SMILES
CCCCCC1CC2=C(c3cc(C)ccc3C(C)(C)O2)C(O)C1
Molecular Weight[1]
314.46
CAS
56282-24-7

Physical Properties

Property	Value	Unit	Source
$\Delta_f G°$	90.95	kJ/mol	Joback
$\Delta_f H°_{gas}$	-384.39	kJ/mol	Joback
$\Delta_{fus} H°$	42.76	kJ/mol	Joback
$\Delta_{vap} H°$	87.45	kJ/mol	Joback
$log_{10}WS$	-6.43		Crippen
$logP_{oct/wat}$	5.323		Crippen
McVol	268.710	ml/mol	McGowan
P_c	1572.21	kPa	Joback
I_{np}	[2419.00; 2490.00]		
T_{boil}	862.36	K	Joback
T_c	1075.33	K	Joback
T_{fus}	539.58	K	Joback
V_c	1.024	m^3/kmol	Joback

Temperature Dependent Properties

Property	Value	Unit	Temperature (K)	Source
$C_{p,gas}$	[883.36; 995.53]	J/mol×K	[862.36; 1075.33]	

Similar Compounds

Figure 33: Cannabinoid chemical data (Cheméo, 2023a, 2023b).

Table 52: General chemical and material data process for the process.

	Boiling Point (°C)	**Density (kg/m^3)**	**Molar Mass (g/mol)**	**References**
Ethanol	78.37	789	46.068	(Louisiana State University, 2023)
Acetonitrile	82.00	786	41.05	(Labchem, 2013)
TBME	55.20	740	88.15	(The OSHA Standard 29, 2012)
Heptane	98.42	684	100.23	(ThermoFisher Scientific, 2021)
Water	100	1000	18.02	-
Crude Oil	187.00	980	-	(Salas-Guerrero, Buendia-Atencio and Orozco, 2023)
CO_2	-78.46	-	44.01	SuperPro Database

Appendix B: VLE Calculations

Due to the inherent scarcity of chemical data regarding cannabinoids, the Antoine constants for CBD must be calculated from literature. Component vapour pressure values (P_{sat}) for CBD at different temperatures were taken from Lovestead and Bruno (2017), as seen in Table 53. These values included experimental values, calculated 'predicted' values, and values measured on n-eicosane using concatenated gas saturation techniques. These values were used as a baseline for the VLE calculations to follow.

Table 53: Experimental and predicted Psat values for THC and CBD (Lovestead and Bruno, 2017).

	Measured THC	**Measured CBD**	**Predicted THC**	**Predicted CBD**	**Measured n-eicosane**
Temp / °C	**P_{sat} / Pa**	**P_{sat} / Pa**	**P_{sat} / Pa**	**P_{sat} / Pa**	**P_{sat} / Pa**
25			2.57E-05	2.73E-06	
30			4.87E-05	6.16E-06	2.18E-03
40			1.15E-04	2.90E-05	1.29E-02
50			5.15E-04	1.24E-04	4.30E-02
61.1	1.93E-03	5.18E-03	1.69E-03	5.60E-04	1.30E-01
81.1	8.35E-03	5.65E-03	1.19E-02	6.71E-03	
101.1	1.10E-01	8.95E-02	6.82E-02	6.16E-02	
121.1	2.20E-01	6.00E-01	3.27E-01	4.51E-01	
141.1	1.58E+00	2.24E+00	1.35E+00	2.73E+00	

Equation 7, also known as the Antoine equation, can be linearised into straight-line equations in the form of Format 1 and Format 2. Because both formats are linear expressions with two variables in the form of $y = a_0 + a_1x_1 + a_2x_2$, it becomes possible to determine the Antoine equation constants A, B, and C using Format 1 and Format 2.

$$log(Psat) = A - \frac{B}{C+T}$$

Equation 7

$\log(\mathrm{P}) = \mathrm{A} - \frac{\mathrm{B}}{\mathrm{C+T}}$

$(C+T)\log(P) = A(C+T) - B$

$Tlog(P) + Clog(P) = AT + AC - B$

$Tlog(P) = AT + AC - B - Clog(P)$

$\log(P) = A + \frac{1}{T}(\mathrm{AC} - \mathrm{B}) + (-C)\frac{\log(P)}{T}$

Format 1

$\log(\mathrm{P}) = \mathrm{A} - \frac{\mathrm{B}}{\mathrm{C+T}}$

$(C+T)\log(P) = A(C+T) - B$

$Tlog(P) + Clog(P) = AT + AC - B$

$Clog(P) = AT + AC - B - Tlog(P)$

$\log(P) = \frac{AC-B}{C} + \frac{A}{C}T + \left(-\frac{1}{C}\right)(Tlog(P))$

Format 2

Using Lovestead's measured values (Table 53), the constants A, B, and C were calculated as shown in Table 54. Similarly, the relevant calculations using Lovestead's predicted values can be found in Table 55. All pressures are reported in mmHg. To gauge the viability of these calculations, the linest function on Microsoft Excel was also applied in both cases, as seen in Table 56.

Table 54: Calculations for Antoine's constants A, B, and C using Lovestead's measured values.

Measured Value		**Format 1**			**Format 2**		
T (K)	*P (mmHg)*	*logP*	*1/T*	*logP/T*	*logP*	*T (K)*	*TlogP*
298.15			3.35E-03			298.15	
303.15			3.30E-03			303.15	
313.15			3.19E-03			313.15	
323.15			3.09E-03			323.15	
334.25	3.89E-05	-4.41E+00	2.99E-03	-1.32E-02	-4.41E+00	334.25	-1.47E+03
354.25	4.24E-05	-4.37E+00	2.82E-03	-1.23E-02	-4.37E+00	354.25	-1.55E+03
374.25	6.71E-04	-3.17E+00	2.67E-03	-8.48E-03	-3.17E+00	374.25	-1.19E+03
394.25	4.50E-03	-2.35E+00	2.54E-03	-5.95E-03	-2.35E+00	394.25	-9.25E+02
414.25	1.68E-02	-1.77E+00	2.41E-03	-4.28E-03	-1.77E+00	414.25	-7.35E+02

Format 1		
		Coefficient
A	intercept	-3.917213
AC-B	x_1=1/T	1555.8886
-C	x_2=logP/T	393.14745
A	-3.917213	
B	-15.8462	
C	-393.1474	

Format 2		
		Coefficient
(AC-B)/C	intercept	-4.380123222
A/C	x_1=T	0.010785159
-1/C	x_2=TlogP	0.002445671
A	-4.40989715	
B	12.17413266	
C	-408.8856783	

Table 55: Calculations for Antoine's constants A, B, and C using Lovestead's predicted values.

Measured Value		**Format 1**			**Format 2**		
T (K)	*P (mmHg)*	*logP*	*1/T*	*logP/T*	*logP*	*T (K)*	*TlogP*
298.15	4.62E-08	-7.34E+00	3.35E-03	-2.46E-02	-7.34E+00	298.15	-2.19E+03
303.15	2.18E-07	-6.66E+00	3.30E-03	-2.20E-02	-6.66E+00	303.15	-2.02E+03
313.15	9.30E-07	-6.03E+00	3.19E-03	-1.93E-02	-6.03E+00	313.15	-1.89E+03
323.15	4.20E-06	-5.38E+00	3.09E-03	-1.66E-02	-5.38E+00	323.15	-1.74E+03
334.25	5.03E-05	-4.30E+00	2.99E-03	-1.29E-02	-4.30E+00	334.25	-1.44E+03
354.25	4.62E-04	-3.34E+00	2.82E-03	-9.42E-03	-3.34E+00	354.25	-1.18E+03
374.25	3.38E-03	-2.47E+00	2.67E-03	-6.60E-03	-2.47E+00	374.25	-9.25E+02
394.25	2.05E-02	-1.69E+00	2.54E-03	-4.28E-03	-1.69E+00	394.25	-6.66E+02

Format 1		
		Coefficient
A	intercept	5.033161437
AC-B	x_1=1/T	182.2142009
-C	x_2=logP/T	-2353.274131
A	5.033161	
B	11662.19	
C	2353.274	

Format 2		
		Coefficient
(AC-B)/C	intercept	-4.380123
A/C	x_1=T	0.0107852
-1/C	x_2=TlogP	0.0024457
A	-4.4099	
B	12.17413	
C	-408.886	

Table 56: Linest function results for Lovestead's measured P_{sat} values and predicted P_{sat} values.

Measured	**Format 1**			**Format 2**		
	X1	*X2*	*Intercept*	*X1*	*X2*	*Intercept*
Coefficients	393.1474	1555.889	-3.917213	0.002445671	0.010785159	-4.380123222
Standard Error (SE)	56.76775	966.1799	2.1059197	0.000389151	0.004287274	2.045365568
Coefficient of determination (R^2)	0.997449	0.084511	N/A	0.997639881	0.081293301	N/A
F statistic	391.0533	2	N/A	422.7074584	2	N/A
Residual sum of squares (SS)	5.585943	0.014284	N/A	5.587009722	0.013217202	N/A
Predicted	**Format 1**			**Format 2**		
	X1	*X2*	*Intercept*	*X1*	*X2*	*Intercept*
Coefficients	182.2142	-2353.274	5.033161437	0.005055709	-0.02101	10.036093
Standard Error (SE)	25.681071	638.9994	1.546678025	0.00068253	0.010754	4.6449849
Coefficient of determination (R^2)	0.9993205	0.062853	N/A	0.998036558	0.10684	N/A
F statistic	3676.5169	5	N/A	1270.774459	5	N/A
Residual sum of squares (SS)	29.048431	0.019753	N/A	29.01110997	0.057074	N/A

Using the Antoine constants calculated using Format 1 and Format 2, P_{sat} values were calculated using Equation 7. The original P_{sat} values were then subtracted from the P_{sat} values calculated using Format 1 and Format 2, the results of which were then squared and summed. Using the Excel solver, the square error value was minimized to produce optimised Antoine constants. The calculation of the new constants for Lovestead's measured- and predicted values can be found in Table 57 and Table 58, respectively. The Antoine constants producing the smallest square error are those calculated using the measured P_{sat} – Format 1 values, where A is -3.7109; B is -14. 4896; and C is -383.6274.

Table 57: Linearisation of P_{sat} values calculated via Format 1 and Format 2 from Lovestead's measured P_{sat} values.

Temp / K	P_{sat} / mmHg	From Trendline	Psat - Format 1 (Psat/mmHg)	Square Error	Psat- Format 2 (Psat/mmHg)	Square Error
298,15		0,0037513	0,000131711		5,01235E-05	
303,15		0,0024446	0,000128556		5,07271E-05	
313,15		0,0008891	0,000121212		5,21515E-05	
323,15		0,0002415	0,000112086		5,39634E-05	
334,25	3,89E-05	3,886E-05	9,90127E-05	3,62E-09	5,66522E-05	3,16805E-10
354,25	4,238E-05	4,238E-05	6,25049E-05	4,05072E-10	6,5002E-05	5,11823E-10
374,25	6,71E-04	0,0006713	5,54595E-06	4,43236E-07	8,74166E-05	3,40926E-07
394,25	0,0045004	0,0045004	0,004499588	6,14728E-13	0,000264189	1,79452E-05
414,25	0,0168014	0,0168014	0,0005785	0,000263182	2,09237E-07	0,00028228
				4,47261E-07		1,8287E-05

	Format 1	Format 2
A	-3,710863	-4,409897
B	-14,4896	12,174133
C	-383,6274	-408,8857

Table 58: Linearisation of P_{sat} values calculated via Format 1 and Format 2 from Lovestead's predicted P_{sat} values.

Temp /K	Psat / mmHg	Psat - Format 1 (Psat/mmHg)	Squared Error	Psat - Format 2 (Psat/mmHg)	Squared Error
298,15	4,62E-08	2,71E-03	7,33704E-06	4,87E-03	2,3759E-05
303,15	2,18E-07	2,71E-03	7,33611E-06	4,87E-03	2,37573E-05
313,15	9,30E-07	2,71E-03	7,33225E-06	4,87E-03	2,37503E-05
323,15	4,20E-06	2,71E-03	7,31455E-06	4,87E-03	2,37185E-05
334,25	5,03E-05	2,71E-03	7,06717E-06	4,87E-03	2,32713E-05
354,25	4,62E-04	2,71E-03	5,04771E-06	4,87E-03	1,94686E-05
374,25	3,38E-03	2,71E-03	4,54266E-07	4,87E-03	2,22483E-06
394,25	2,05E-02	2,71E-03	0,00031569	4,87E-03	0,000243432
414,25	0,00E+00	2,71E-03	7,33729E-06	4,87E-03	2,37594E-05
			0,000364917		0,000312156

	Format 1:	Format 2:
A	2,3883363	-2,341869
B	11662,131	12,17919
C	2353,3386	-408,8855

The Antoine constants were then used to plot graphs, with P_{sat} values calculated using Equation 7. Six P_{sat} – Temperature graphs were plotted (Figure 34) – two using Lovestead's original values, one for the measured values and one for the predicted values, and graphs for both datasets were then plotted using the constants calculated using Format 1 and Format 2.

It can be seen in Figure 34 that the Antoine constants producing P_{sat} values erring most closely to the baseline values are those calculated using Format 1 from Lovestead's measured P_{sat} values; as these Antoine constants also produced the smallest square error, this is to be expected. Thus, these A, B, and C values (Table 57) are the ones that were further investigated.

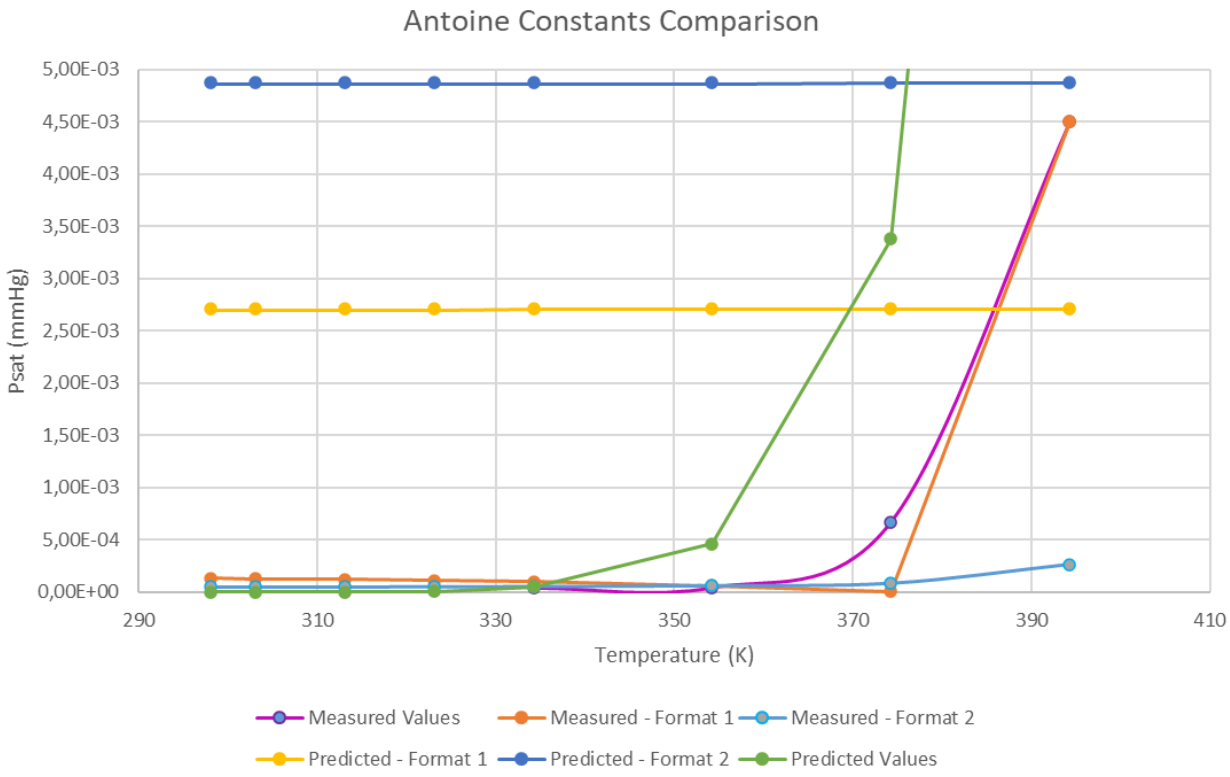


Figure 34: Psat-Temperature graphs using the baseline measured and predicted P_{sat} values, as well as P_{sat} values calculated from the Antoine constants calculated using Format 1 and Format 2.

The measured P_{sat} – Format 1 Antoine constants were input into the SuperPro-simulated CPC process to gauge whether the relevant values produced align with expectations. Immediately upon inputting the Antoine constants, there was a conflict between the constants and the boiling point for CBD (160 °C – 180 °C). Using the new Antoine's constants, the SuperPro-calculated new boiling point for CBD was reported as 112.7 °C. This is significantly lower than the commonly reported boiling point for CBD, bringing the accuracy of the calculated Antoine constants into question.

The Txy graph produced by SuperPro at atmospheric pressure between heptane and CPC, as would be relevant for the CPC process, can be seen below in Figure 35, displaying unusual behaviour which is not expected for CBD and heptane. SuperPro was unable to produce a

bubble chart for these components, citing a failure in algorithm convergence (Figure 36). It is well documented that heptane is commonly used to separate CBD from cannabis oil; thus, from these results, it must be assumed that the calculated Antoine constants are not accurate and cannot be used in the process simulation.

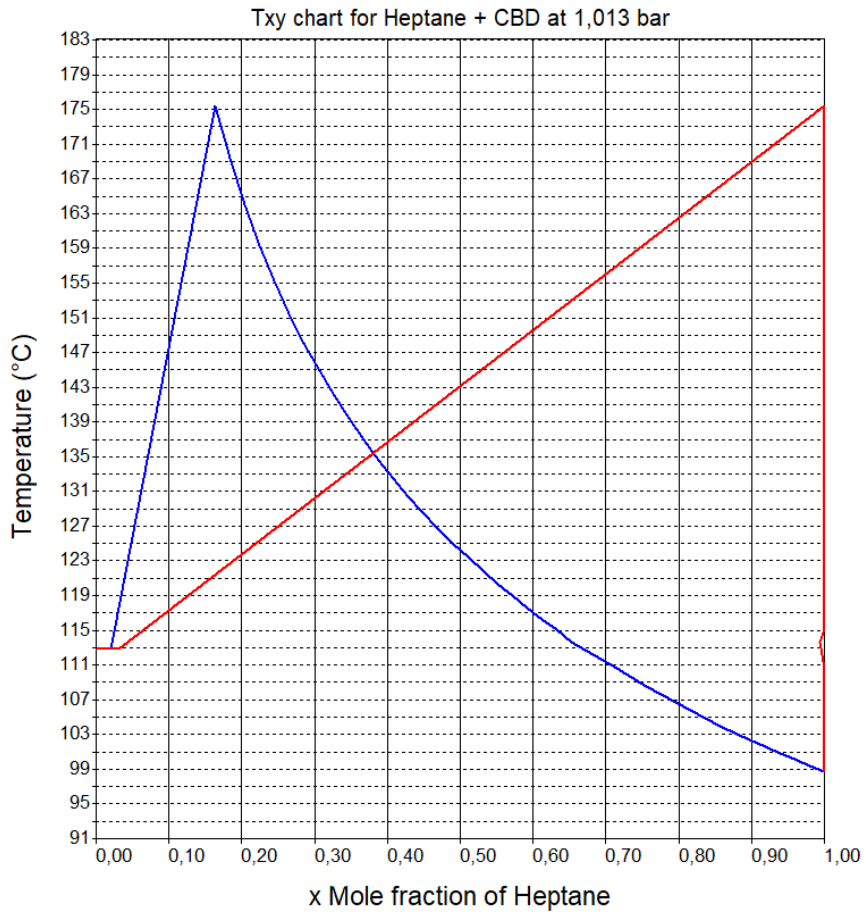


Figure 35: Txy graphs for heptane and CBD at atmospheric pressure, using the measured P_{sat} – Format 1 Antoine constants.

Binary Chart Error Messages

Error x:0,04	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,04	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,06	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,06	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,06	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,06	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,08	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,08	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,08	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,08	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,08	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,08	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,10	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,10	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...
Error x:0,10	CProFlashPointSolver::CalculateFlashPoint: The calculation of the bubble point temperature failed.
Error x:0,10	CProIMSServer::GetFlashPoint:Bubble/Dew Point calculation FAILED. Algorithm failed to converge ...

Figure 36: Error in producing a bubble point graph for heptane and CBD at atmospheric pressure, using the measured P_{sat} – Format 1 Antoine constants.

The failure in calculating applicable Antoine constants and a lack of existing VLE data for CBD, in general, necessitates the assumption of VLE properties for CBD. Using existing data, SuperPro pulled VLE information from chemically similar compounds, and based on expected splits (see Section 3.8.4), these assumptions were deemed functionally acceptable. However, the inherent uncertainty this kind of assumption introduces into the simulation must be noted.

Based on the SuperPro-assumed VLE properties, the new Txy and bubble point graphs can be seen in Figure 37; the newly calculated VLE graphs appear much more sensible than those produced using the calculated Antoine constants. Thus, due to a lack of available data, SuperPro-assumed VLE properties will be used for CBD and THC where necessary.

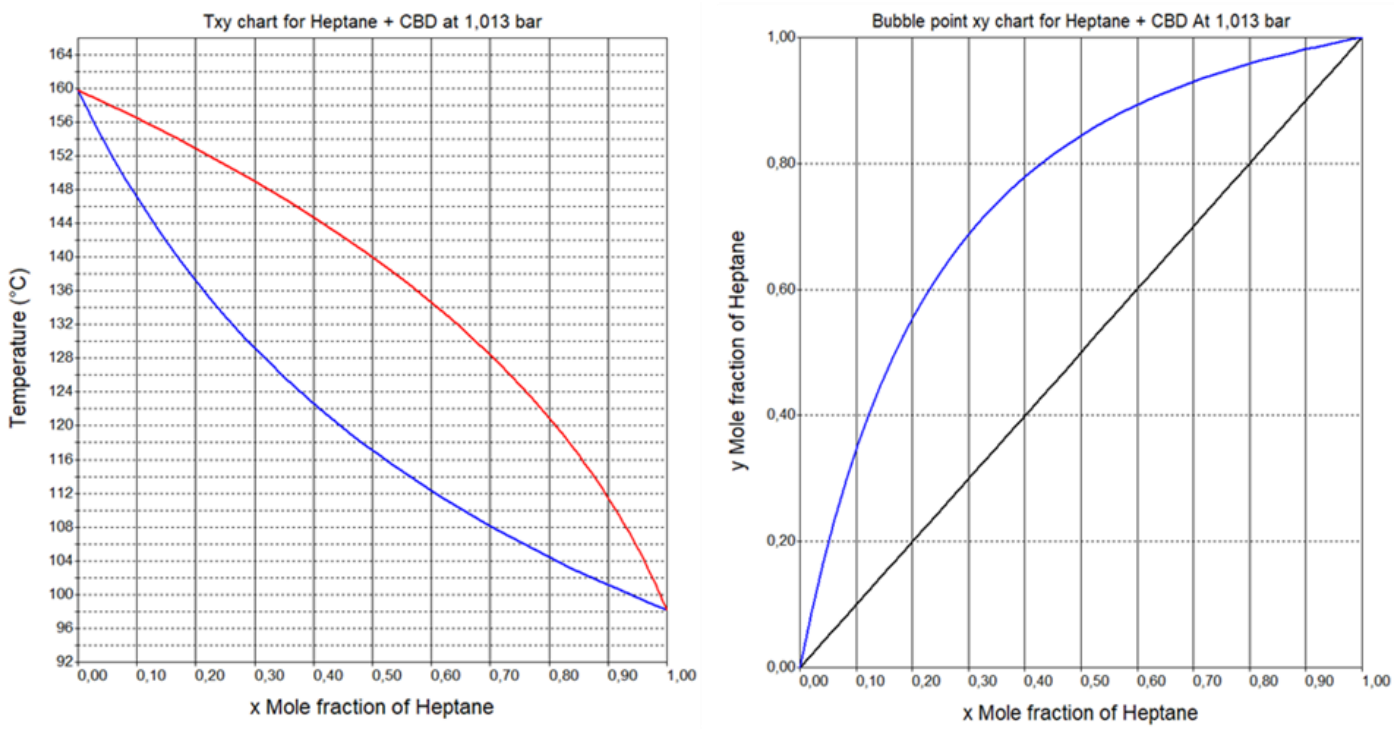


Figure 37: VLE graphs (Txy and bubble point) for heptane and CBD, using Antoine constants assumed by SuperPro.

Appendix C: Reactor Calculations

Decarboxylation

Pulling molar ratios from Equation 2 and using the component molar masses from Table 52 and Figure 33, mass ratios were calculated for a reaction equation based on component masses (Equation 3). The relevant calculations can be found in Table 59.

Table 59: Decarboxylation reaction coefficient calculations.

Reaction Ratio				
	CO2	*CBD*	*THC*	*Undesirables*
Molar	2,00	1,00	1,00	4,00
Mass	88,02	314,47	314,45	716,94
Mass Ratio	0,1228	0,4386	0,4386	1,0000

If one defined the oil segment of the decarboxylation oven input stream as only the cannabis compounds (i.e.: undesirable chemical compounds, CBD, and THC, assuming no solvent contamination), it came to a flow rate of 0.1237 kg/h. To realistically depict the decarboxylation process, approximately 2.3 % of this mass (i.e.: that of the total stream) needed to be converted into CO_2, as discussed in Section 3.6. The conversion of the decarboxylation reaction was input into the simulation and varied by trial and error until a mass percentage of 2.459 % was achieved at a conversion of 40 %. With a percentage error of 6.89 %, this was deemed acceptable, since 2.3 % was merely an approximate estimate. According to the principle of mass conservation, it is also paramount that the masses of CO_2, CBD and THC add up to that of the 'Undesirable Compounds' – i.e.: THCA and CBGA, as defined in Section 4.3. Thus, Table 59 also includes a row ensuring that the mass ratios of the product compounds add up to 1.

PFR Calculations

From Equation 8, as derived below, the mass of the enzyme catalyst can be calculated to achieve a conversion rate of 85 % within the reactor. The equilibrium constant, k, was calculated according to Equation 9, yielding a value of 1.

The following assumptions have been made:

- Negligible changes in volume
- (e=0)
- Constant pressure

$$W = Fao \int (\frac{dX}{-ra})$$

$$\frac{dX}{dW} = (\frac{kCao}{Fao})(1 - X)(\frac{To}{T})$$

$$\int dW = \int \left(\frac{Fao}{kCao}\right)\left(\frac{T}{To}\right)\left(\frac{1}{1 - X}\right) dX$$

$$W = (\frac{Fao}{kCao})(\frac{T}{To})(-\log(1 - X))$$

Equation 8

$$k = \frac{[Ccbd]}{[Cthc]}$$

Equation 9

From the SuperPro simulation:

- $F_{cbd,o}$ = 0.13837 mol/h or 0.66939 kg/h
- $C_{cbd,o}$ = 1.157038 mol/L
- X = 0.85
- T_o = 298.15 K
- T = 298.15 K

This yields a total mass of 0.09853 g/hr to achieve conversion. The process has an operating time of 7920 hours a year, and thus 780.36 grams of enzymes would theoretically be needed

per year. The process will run for 330 days a year, rounded up to 48 weeks, however, assuming the enzymes require replacement every seven days of operation, the process will require 37.46 kg of the enzyme per year.

For the scaled-up process, as described in Section 7, the required enzyme for the process increases. This new value will be calculated using the same formula, conversion rate, concentration, and temperatures as above, but with a CBD flowrate of 2.12870 mol/h, or 0.04351 kg/h, yielding an enzyme mass of 1.51581 g, or (following the same logic as above) 576.25 kg/year.

Appendix D: Ancillary Calculations, Optimisation and Sensitivity Analysis

Overall Process Data

Table 60: Overall process data.

CBD Isolate Production	
Annual Operating Time	7920,00 h
Unit Production Ref. Rate	637,05 kg MP/yr
Operating Days per Year	330,00
MP = Total Flow of Stream 'S-122'	

CBD Blend Production	
Annual Operating Time	7920,00 h
Unit Production Ref. Rate	656,11 kg MP/yr
Operating Days per Year	330,00
MP = Total Flow of Stream 'S-123'	

Component Balances

Table 61: Overall component balances.

CBD Isolate Production					
COMPONENT	**INITIAL**	**IN**	**OUT**	**FINAL**	**IN-OUT**
Acetonitrile	0	1419	1419	0	- 0
Carb. Dioxide	0	0	25	0	- 25
CBD	0	0	639	0	- 639
Ethyl Alcohol	0	3168	3168	0	- 0
Heptane	0	1188	1188	0	- 0
MthTertBtylEthe	0	165	165	0	- 0
N2	5	0	0	5	0
O2	1	0	0	1	0
Plant Material	0	5148	0	0	5148
Solid Impuritie	0	0	3089	0	- 3089
THC	0	0	54	0	- 54
Undesirable Che	0	0	311	0	- 311
Water	0	5411	6440	0	- 1030
TOTAL	6	16499	16499	6	0
				Overall Error:	0,003%

CBD Blend Production					
COMPONENT	**INITIAL**	**IN**	**OUT**	**FINAL**	**IN-OUT**
Carb. Dioxide	0	0	25	0	- 25
CBD	0	0	639	0	- 639
Ethyl Alcohol	0	3168	3168	0	- 0
N2	5	0	0	5	0
O2	1	0	0	1	0
Plant Material	0	5148	0	0	5148
Solid Impuritie	0	0	3089	0	- 3089
THC	0	0	54	0	- 54
Undesirable Che	0	0	311	0	- 311
Water	0	5411	6440	0	- 1030
TOTAL	6	13727	13727	6	0
				Overall Error:	0,002%

Table 62: Optimisation of distillation column C-103.

Distillation C-103								
Varied Component	Base Value	New Value	Actual Stages	Cooling Agent (kg/hr)	Heating Agent (kg/hr)	Capital Cost ($)	High Pressure Steam Cost for Entire Process ($/yr)	Cooling Water Cost for Entire Process ($/yr)
R/Rmin	1,25	1,20	19	44,0	0,6	99000	567,60	94,60
	1,25	1,25	19	44,0	0,6	99000	567,68	94,60
	1,25	1,30	18	44,0	0,6	94000	567,74	94,60
	1,25	1,40	18	44,0	0,6	94000	567,89	94,60
	1,25	1,50	18	44,1	0,6	94000	568,03	94,61
Column pressure	1,013	0,50	16	44,9	0,6	83000	569,41	94,60
	1,013	1,013	19	44,0	0,6	99000	567,68	94,60
	1,013	1,50	20	43,6	0,6	104000	567,13	94,61
	1,013	2,00	22	43,3	0,7	114000	567,06	94,64
	1,013	2,50	23	43,1	0,7	119000	567,44	94,67
	1,013	3,00	24	43,0	0,7	125000	567,38	94,70

Table 63: Optimisation of distillation column C-101.

Distillation C-101								
Varied Component	Base Value	New Value	Actual Stages	Cooling Agent (kg/hr)	Heating Agent (kg/hr)	Capital Cost ($)	High Pressure Steam Cost for Entire Process ($/yr)	Cooling Water Cost for Entire Process ($/yr)
R/Rmin	1,25	1,20	14	119,2	1,4	28000	566,99	94,45
	1,25	1,25	14	119,4	1,4	28000	567,68	94,60
	1,25	1,30	13	119,6	1,4	26000	568,32	94,76
	1,25	1,40	13	120	1,4	26000	569,65	95,07
	1,25	1,50	13	120,4	1,4	26000	570,98	95,38
Column pressure	1,013	0,50	13	120,3	1,3	26000	568,50	95,27
	1,013	1,013	14	119,4	1,4	28000	567,66	94,60
	1,013	1,50	14	119,4	1,4	28000	568,70	94,53
	1,013	2,00	14	119,5	1,4	28000	569,95	94,68
	1,013	2,50	15	119,9	1,4	30000	571,85	94,92
	1,013	3,00	15	120,2	1,5	30000	573,94	95,24

Figure 38: Additional sensitivity analysis graphs on distillation columns C-103 and C-101.

Sensitivity Analysis for Raw Material Costs and Labour Costs

Table 64: Sensitivity analysis of the cost of plant material on the production of CBD isolates.

Percentage of Original Value	Cost ($/kg)	Yearly Cost ($)	ROI (%)
50%	0,74	3809,50	101,59
75%	1,11	5714,25	101,59
100%	1,48	7619,00	101,58
125%	1,85	9523,75	101,58
150%	2,22	11428,50	101,57
200%	2,96	15238,00	101,56

Table 65: Sensitivity analysis of the cost of plant material on the production of CBD blends.

Percentage of Original Value	Cost ($/kg)	Yearly Cost ($)	ROI (%)
50%	0,74	3809,50	21,76
75%	1,11	5714,25	21,75
100%	1,48	7619,00	21,75
125%	1,85	9523,75	21,74
150%	2,22	11428,50	21,74
200%	2,96	15238,00	21,73

Table 66: Sensitivity analysis of the cost of labour on the production of CBD isolates.

Percentage of Original Value	Yearly Cost ($)	ROI (%)
50%	3459000	111,00
75%	5188500	106,27
100%	6918000	101,58
125%	8647500	96,94
150%	10377000	92,35
200%	13836000	83,29

Table 67: Sensitivity analysis of the cost of labour on the production of CBD blends.

Percentage of Original Value	Yearly Cost ($)	ROI (%)
50%	3459000	29,69
75%	5188500	25,7
100%	6918000	21,75
125%	8647500	17,83
150%	10377000	13,96
200%	13836000	6,31

Sensitivity Analysis for Enzyme Conversion and Input CBD Percentage

To generate values for enzyme conversion and input CBD percentage, the Inverse of the Normal Cumulative Distribution function on Excel was used. For enzyme conversion, a mean of 70 was assumed, with a standard deviation of 10. The Rand() function was used as the probability, to ensure real-life randomness within the generated values. This was inserted into Excel in the form of 'NORM.INV(RAND();70;10).'

A similar process was applied for input CBD percentage (with a mean of 5, and a standard deviation of 2.5), yielding the function 'NORM.INV(RAND(); 5; 2,5)'. The results of these functions can be found in the second rows of Table 68 and Table 69.

These variables were input into the CBD isolate process, 'simulation' output flow rates and output CBD percentage for the CBD isolate product stream. These simulation variables were plotted (see

Figure 29) and a trendline was added for each dataset. These trendline equations came to $y = 3.423x + 346.09$ in the case of enzyme conversion versus output flow, $y = 0.2295\ln(x) + 98.445$ for conversion versus output CBD percentage, $y = 7.7874x + 598.28$ for input CBD percentage versus output flow, and $y = (-6E\text{-}05)x^2 + 0.0071x + 99.432$ for input CBD percentage versus output CBD percentage. From here, output flow and input CBD percentage values were recorded off the trendlines in accordance with the randomly generated input values for conversion and input CBD percentage. These values have been labelled as 'graphical output flow' and 'graphical CBD %' in the graphs below. These values were used to measure the process model's reliability, as described in Section 5.5.

Using Equation 6, the standard deviations for each dataset were calculated and used in the sensitivity analysis carried out in Section 5.5.

Table 68: Raw data for the sensitivity analysis on enzyme conversion rate in the CBD isolate process.

Trial Conversion	Enzyme Conversion Rate %	Simulation Output Flow (kg/yr)	THC %	Simulation CBD %	Graphical Output Flow (kg/year)	Graphical CBD %	Output Square Difference	CBD Square Difference
1	69,73	584,7764	0	99,4185	584,7758	99,4191	3,7210E-07	4,1310E-07
2	70,46	587,2752	0	99,4209	587,2746	99,4215	3,8440E-07	4,0052E-07
3	74,71	601,8229	0	99,4349	601,8223	99,4350	3,2490E-07	5,5357E-09
4	60,33	552,6002	0	99,3846	552,5996	99,3859	3,7210E-07	1,7184E-06
5	64,97	568,4829	0	99,4018	568,4823	99,4029	3,4810E-07	1,2453E-06
6	63,38	563,0403	0	99,396	563,0397	99,3972	3,1360E-07	1,5118E-06
7	46,53	505,3627	0	99,3271	505,3622	99,3263	2,6010E-07	6,3629E-07
8	73,64	598,1603	0	99,4315	598,1597	99,4317	3,3640E-07	2,6807E-08
9	92,62	663,1289	0	99,4872	663,1283	99,4843	4,0960E-07	8,4569E-06
10	73,53	597,7838	0	99,4311	597,7832	99,4313	3,7210E-07	4,8689E-08
11	87,3	644,9185	0	99,4727	644,9179	99,4707	3,6000E-07	3,9365E-06
12	69,86	585,2214	0	99,4189	585,2208	99,4196	3,8440E-07	4,4917E-07
13	69,52	584,0575	0	99,4177	584,0570	99,4185	2,9160E-07	5,6329E-07
14	40,9	486,0912	0	99,3004	486,0907	99,2967	2,5000E-07	1,3658E-05
15	70,97	589,0209	0	99,4227	589,0203	99,4232	3,4810E-07	2,3818E-07
16	69,62	584,3998	0	99,4181	584,3993	99,4188	2,9160E-07	4,6295E-07
17	54,72	533,3971	0	99,3624	533,3966	99,3635	2,9160E-07	1,2357E-06
18	77,83	612,5027	0	99,4448	612,5021	99,4444	3,7210E-07	1,9015E-07
19	83,26	631,0896	0	99,4611	631,0890	99,4598	3,8440E-07	1,5833E-06
20	63,63	563,8961	0	99,3969	563,8955	99,3981	3,7210E-07	1,5203E-06

Table 69: Raw data for the sensitivity analysis on input CBD percentage rate in the CBD isolate process.

Trial CBD %	Normal Random Variable	Simulation Output Flow (kg/yr)	THC %	Simulation CBD %	Graphical Output Flow (kg/year)	Graphical CBD %	Output Variance	CBD Variance
1	8,05	660,4641	0	99,4851	660,96857	99,48526685	0,254489981	2,78389E-08
2	3,24	623,532	0	99,4546	623,511176	99,45437414	0,000433639	5,10109E-08
3	7,14	653,4769	0	99,4796	653,882036	99,47963522	0,164135178	1,24073E-09
4	8,2	661,6158	0	99,486	662,13668	99,4861856	0,271315974	3,44474E-08
5	1,09	607,0239	0	99,4398	606,768266	99,43966771	0,065348742	1,74996E-08
6	7,12	653,3234	0	99,4795	653,726288	99,47951034	0,162318741	1,06833E-10
7	4,83	635,7403	0	99,4651	635,893142	99,46489327	0,023360677	4,27389E-08
8	8,22	666,0765	0	99,4894	662,292428	99,4863079	14,3192009	9,56111E-06
9	6,1	645,4916	0	99,4732	645,78314	99,4730774	0,084995572	1,50308E-08
10	3,71	627,1407	0	99,4577	627,171254	99,45751515	0,000933547	3,4168E-08
11	1,93	613,4736	0	99,4457	613,309682	99,44547951	0,026869111	4,86176E-08
12	4,68	634,5886	0	99,4641	634,725032	99,46391386	0,018613691	3,46496E-08
13	6,83	651,0967	0	99,4777	651,467942	99,47769407	0,137820623	3,52124E-11
14	4,79	635,4332	0	99,4648	635,581646	99,46463235	0,022036215	2,81052E-08
15	9,56	672,0582	0	99,494	672,727544	99,49439238	0,44802139	1,53965E-07
16	8,07	660,6176	0	99,4852	661,124318	99,48538951	0,256763132	3,59125E-08
17	3,95	628,9835	0	99,4593	629,04023	99,45910885	0,003218293	3,65383E-08
18	2,43	617,3127	0	99,4491	617,203382	99,44889871	0,011950425	4,05193E-08
19	4,86	635,969	0	99,4653	636,126764	99,46508882	0,02488948	4,45953E-08
20	3,2	623,2249	0	99,4543	623,19968	99,4541056	0,000636048	3,77914E-08

Ancillary Calculations

Cultivating versus Purchasing Cannabis

Table 70: Calculation of the required space for a drip irrigation system and its associated cost.

1 hectare: 107639 ft2		
1 Hectare Yield (kg)	4251,74	
Required Cannabis (kg/yr)	5148,00	
Required Space (hectare)	1,21	
Cultivation Space (hectare)	4	5
Cost	79500	106000
Cost Ratio = Area Ratio*1.0667		
79500/((4/1.2108)*1.0667)		
=	22559,90	
79500/((4/1.2108)*1.0667)		
=	24063,90	
Mean Cost ($)	**23311,90**	

The cost of the drip-based irrigation system for 4 to 5 hectares of cannabis costs between $ 79 500 and $ 106 000 (The Best Grow, 2020), and the average cannabis yield per square foot is 39.5 g (Cannabis Business Times, 2016), where 1 hectare is equivalent to 107 639 ft^2, yielding 4251.74 kg per hectare. These are the values used above, in Table 70, with further calculation regarding the costs of cultivating cannabis available in Section 5.2.

Non-CBD Cannabinoids as Ancillary Products

Table 71: Output stream value for the CBD isolate process, including non-CBD cannabinoids as ancillary products.

Cannabinoid	Cannabinoid Isolate Produced per kg CBD (kg)	Net Profit per kg CBD (kg)
CBD	1,0000	64 089,93
CBN	0,3097	1012,72
CBG	0,1252	78,88
THC	0,0815	8073,88
Total	**1,5200**	**73 255,41**

Appendix E: Economic Analysis

Table 72: Cash flow analysis of the production of CBD oils (thousand $).

CBD Isolate Production

Year	Capital Investment	Debt Finance	Sales Revenues	Operating Cost	Gross Profit	Loan Payments	Depreciation	Taxable Income	Taxes	Net Profit	Net Cash Flow
1	- 26346	0	0	0	0	0	0	0	0	0	- 26346
2	- 2272	0	0	0	0	0	0	0	0	0	- 2272
3	- 2618	0	8265	7989	276	0	540	276	69	747	- 1872
4	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
5	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
6	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
7	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
8	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
9	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
10	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
11	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
12	0	0	49592	8005	41588	0	540	41588	10397	31730	31730
13	0	0	49592	7465	42127	0	0	42127	10532	31595	31595
14	0	0	49592	7465	42127	0	0	42127	10532	31595	31595
15	915	0	49592	7465	42127	0	0	42127	10532	31595	32510

IRR/NPV SUMMARY					
IRR Before Taxes	58,59 %	Interest %	7,00	9,00	11,00
IRR After Taxes	49,20 %	NPV	190210,00	161376,00	137394,00

CBD Blend Production

Year	Capital Investment	Debt Finance	Sales Revenues	Operating Cost	Gross Profit	Loan Payments	Depreciation	Taxable Income	Taxes	Net Profit	Net Cash Flow
1	- 27095	0	0	0	0	0	0	0	0	0	- 27095
2	- 3270	0	0	0	0	0	0	0	0	0	- 3270
3	- 3492	0	2876	8462	- 5586	0	777	0	0	- 4809	- 8301
4	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
5	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
6	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
7	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
8	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
9	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
10	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
11	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
12	0	0	17257	8475	8782	0	777	8782	2196	7363	7363
13	0	0	17257	7698	9559	0	0	9559	2390	7169	7169
14	0	0	17257	7698	9559	0	0	9559	2390	7169	7169
15	1039	0	17257	7698	9559	0	0	9559	2390	7169	8208

IRR/NPV SUMMARY					
IRR Before Taxes	16,48 %	Interest %	7,00	9,00	11,00
IRR After Taxes	11,90 %	NPV	13842,00	7418,00	2112,00

Alternative CBD Isolate (Supercritical CO_2) Production											
Year	Capital Investment	Debt Finance	Sales Revenues	Operating Cost	Gross Profit	Loan Payments	Depreciation	Taxable Income	Taxes	Net Profit	Net Cash Flow
1	- 26193	0	0	0	0	0	0	0	0	0	- 26193
2	- 2068	0	0	0	0	0	0	0	0	0	- 2068
3	- 2380	0	7587	7219	368	0	491	368	92	767	- 1613
4	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
5	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
6	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
7	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
8	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
9	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
10	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
11	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
12	0	0	45524	7251	38273	0	491	38273	9568	29196	29196
13	0	0	45524	6760	38764	0	0	38764	9691	29073	29073
14	0	0	45524	6760	38764	0	0	38764	9691	29073	29073
15	829	0	45524	6760	38764	0	0	38764	9691	29073	29902

IRR/NPV SUMMARY					
IRR Before Taxes	56,21 %	Interest %	7,00	9,00	11,00
IRR After Taxes	47,12 %	NPV	173178,00	146644,00	124574,00

Table 73: Profitability analyses of the production of CBD oils.

CBD Isolate Production		
A.	Direct Fixed Capital	5680000 $
B.	Working Capital	631000 $
C.	Startup Cost	284000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	31236000 $
G.	Investment Charged to This Project	31236000 $
H.	**Revenue Rates**	
	S-120 (Revenue)	494 kg/yr
	S-122 (Main Revenue)	637 kg/yr
I.	**Revenue Price**	
	S-120 (Revenue)	17749,48 $/kg
	S-122 (Main Revenue)	64089,93 $/kg
J.	**Revenues**	
	S-120 (Revenue)	8764198 $/yr
	S-122 (Main Revenue)	40828209 $/yr
	Total Revenues	49592407 $/yr
K.	**Annual Operating Cost (AOC)**	
	AOC	8005000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	12565,48 $/kg MP
	Unit Production Revenue	77847,50 $/kg MP
M.	Gross Profit (J-K)	41588000 $/yr
N.	Taxes (25%)	10397000 $/yr
O.	Net Profit (M-N + Depreciation)	31730000 $/yr
	Gross Margin	83,86 %
	Return On Investment	101,58 %
	Payback Time	0,98 years

MP = Total Flow of Stream 'S-122'

CBD Blend Production

A.	Direct Fixed Capital	8176000 $
B.	Working Capital	630000 $
C.	Startup Cost	409000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	33857000 $
G.	Investment Charged to This Project	33857000 $
H.	**Revenue Rates**	
	S-123 (Main Revenue)	656 kg/yr
I.	**Revenue Price**	
	S-123 (Main Revenue)	26302,64 $/kg
J.	**Revenues**	
	S-123 (Main Revenue)	17257386 $/yr
	Total Revenues	17257386 $/yr
K.	**Annual Operating Cost (AOC)**	
	AOC	8475000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	12917,35 $/kg MP
	Unit Production Revenue	26302,64 $/kg MP
M.	Gross Profit (J-K)	8782000 $/yr
N.	Taxes (25%)	2196000 $/yr
O.	Net Profit (M-N + Depreciation)	7363000 $/yr
	Gross Margin	50,89 %
	Return On Investment	21,75 %
	Payback Time	4,60 years

MP = Total Flow of Stream 'S-123'

Alternative CBD Isolate (Supercritical CO_2) Production

A.	Direct Fixed Capital	5170000 $
B.	Working Capital	571000 $
C.	Startup Cost	259000 $
D.	Up-Front R&D	24642000 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	30641000 $
G.	Investment Charged to This Project	30641000 $
H.	**Revenue Rates**	
	S-120 (Revenue)	432 kg/yr
	S-122 (Main Revenue)	591 kg/yr
I.	**Revenue Price**	
	S-120 (Revenue)	17749,48 $/kg
	S-122 (Main Revenue)	64089,93 $/kg
J.	**Revenues**	
	S-120 (Revenue)	7668768 $/yr
	S-122 (Main Revenue)	37855430 $/yr
	Total Revenues	45524198 $/yr
K.	**Annual Operating Cost (AOC)**	
	AOC	7251000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	12276,78 $/kg MP
	Unit Production Revenue	77073,29 $/kg MP
M.	Gross Profit (J-K)	38273000 $/yr
N.	Taxes (25%)	9568000 $/yr
O.	Net Profit (M-N + Depreciation)	29196000 $/yr
	Gross Margin	84,07 %
	Return On Investment	95,28 %
	Payback Time	1,05 years

MP = Total Flow of Stream 'S-122'

Variation in Economic Analysis Based on GMP-Grade Enzyme Costs

It is assumed enzyme costs were initially $ 22 476 /year and they are increased to $ 98330 /year, based on GMP-grade estimates. The yearly values for the CBD isolate process revenue, gross margin, ROI, payback time, IRR and NPV were, as stated in Section 5.4, $ 49 592 000, 83.86 %, 101.58 %, 0.98 years, 49.38 % and $ 19 045 800. Operating costs, fixed costs and total costs were $ 8 005 000, $ 3 087 000, and $ 1 852 000.

Change in enzyme cost = $ 98 330 – $ 22 476 = $ 75 854

New total costs = $ 1 852 000 + $ 75 854 = $ 1 927 854

Operating Costs:

Since enzyme cost is part of the operating costs, operating costs are updated:

New operating costs = $ 8 005 000 + $ 75 854 = $ 8 080 854

Gross Margin:

Gross Margin is defined as:

$$Gross\ Margin = \frac{100(Revenue - Total\ Costs)}{Revenue}$$

Equation 10

Initial gross margin: 83.86 %

New gross margin: 83.71 %

ROI:

ROI is typically calculated as:

$$ROI = \frac{100(Net\ Profit)}{Investment}$$

Equation 11

Investment is assumed to remain constant.

Net profit = Revenue - Total Costs

Initial net profit = $ 49 592 000 − $ 1 852 000 = $ 47 740 000

New net profit = $ 49 592 000 − $ 1 927 854 = $ 47 664 146

Initial ROI = (100)(47 740 000) / Investment = 101.58 %

Therefore, Investment = $ 47 000 000

New ROI = (100)(47 664 146) / (47 000 000) = 101.41 %

Payback Time:

Payback time is calculated as:

$$Payback\ Time = \frac{Investment}{Net\ Profit}$$

Equation 12

The initial payback time = 47 000 000 / 47 740 000 = 0.98 years

The new payback time = 47 000 000 / 47 664 146 = 0.99 years (essentially unchanged)

IRR:

IRR depends on the cash flows. Since the increase in enzyme cost reduces net profit, we can expect a slight reduction in IRR. Assuming a proportional decrease:

New IRR = (49.38)(47 664 146) / (47 740 000) = 49.30 %

NPV:

NPV depends on the present value of future cash flows. A reduction in net profit will reduce NPV slightly. Assuming a proportional decrease:

New NPV = (190 458 000)(47 664 146) / (47 740 000) = $ 190 153 965

Summary of New Values for the CBD Isolate Process:

Gross Margin: 83.71 % (from 83.86 %)

ROI: 101.41 % (from 101.58 %)

Payback Time: 0.99 years (from 0.98 years)

IRR: 49.30 % (from 49.38 %)

NPV: $ 190 153 965 (from $ 190 458 000)

Operating Costs: $ 8 080 854 (from $ 8 005 000)

Total Costs: $ 1 927 854 (from $1 852 000)

Now, assuming the same change in enzyme costs, new values for financial parameters can also be calculated for the CBD blend process. The initial economic analysis values for this process were, as stated in Section 5.4: Revenue: $ 17 257 000; Gross Margin: 50.89 %; ROI: 21.75 %; payback time: 4.6 years; IRR: 12.06 %; NPV: $ 14 199 000; operating costs: $8 475 000; fixed costs: $4 444 000; total costs: $7 110 000.

Using identical methods as the calculation above, for the CBD isolate process, the following values are obtained:

Summary of New Values for the CBD Blend Process:

Revenue: $ 17 257 000 (unchanged)

Gross Margin: 50.46 % (from 50.89 %)

ROI: 21.58 % (from 21.75 %)

Payback Time: 4.63 years (from 4.6 years)

IRR: 11.97 % (from 12.06 %)

NPV: $ 14 128 764 (from $ 14 199 000)

Operating Costs: $ 8 550 854 (from $ 8 475 000)

Total Costs: $ 7 185 854 (from $ 7 110 000)

Finally, a similar calculation for the scaled-up process can be performed. Assuming a change in enzyme cost from $ 345 750 to $ 1 512 656 when adhering to GMP regulations, new values for the economic analysis can be calculated. The initial values for the economic analysis were, as stated in Section 7: Revenue: $ 762 782 000; Gross Margin: 98.54 %; ROI: 1343.63 %;

payback time: 0.07 years; IRR: 226.07 %; NPV: $ 3 959 192 000; operating costs: $ 11 158 000; fixed costs: $ 8 681 000; total costs: $ 13 889 000.

Summary of New Values for the Scaled-Up Process:

Revenue: $ 762 782 000 (unchanged)

Gross Margin: 98.03 % (from 98.54 %)

ROI: 1341.57 % (from 1343.63 %)

Payback Time: 0.07 years (essentially unchanged)

IRR: 225.75 % (from 226.07 %)

NPV: $ 3 953 236 834 (from $ 3 959 192 000)

Operating Costs: $ 12 324 906 (from $ 11 158 000)

Total Costs: $ 15 055 906 (from $ 13 889 000)

Appendix F: Energy Consumption and Balances

Primary CBD Isolate Process (Cold Ethanol Process)

Table 74: Stream breakdown for the production of CBD isolates using cold ethanol.

Stream Name	S-102	S-103	S-111	S-138
Source	INPUT	P-4	P-21	P-3
Destination	P-4	P-21	P-5	P-6
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	25,00	40,82	- 35,05	- 35,05
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	2315,66	1396,55	1433,13	845,06
Total Enthalpy (kW-h)	0,03	0,03	- 0,02	- 0,18
Specific Enthalpy (kcal/kg)	37,43	37,43	- 32,89	- 20,81
Heat Capacity (kcal/kg-°C)	1,50	0,91	0,96	0,59
Component Flowrates (kg/h)				
CBD	0,00	0,03	0,03	0,03
Ethyl Alcohol	0,00	0,00	0,00	7,28
Plant Material	0,65	0,00	0,00	0,00
Solid Impuritie	0,00	0,39	0,39	0,00
THC	0,00	0,03	0,03	0,03
Undesirable Che	0,00	0,07	0,07	0,06
Water	0,00	0,13	0,13	0,13
TOTAL (kg/h)	0,65	0,65	0,65	7,54
TOTAL (L/h)	0,28	0,47	0,45	8,92

Stream Name	S-105	S-108	S-121	S-133
Source	P-6	P-6	P-10	P-10
Destination	P-10	P-13	OUTPUT	P-25
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	130,00	130,00	50,00	50,00
Pressure (bar)	1,01	1,01	2,50	2,50
Density (g/L)	1,36	974,42	4,11	766,63
Total Enthalpy (kW-h)	2,37	0,01	0,00	0,26
Specific Enthalpy (kcal/kg)	275,45	53,36	37,34	29,68
Heat Capacity (kcal/kg-°C)	0,42	0,42	0,23	0,59
Component Flowrates (kg/h)				
Carb. Dioxide	0,00	0,00	0,00	0,00
CBD	0,00	0,04	0,00	0,00
Ethyl Alcohol	7,28	0,00	0,00	7,28
THC	0,00	0,04	0,00	0,00
Undesirable Che	0,00	0,04	0,00	0,00
Water	0,13	0,00	0,00	0,13
TOTAL (kg/h)	7,41	0,13	0,00	7,41
TOTAL (L/h)	5468,67	0,13	0,87	9,66

Stream Name	S-143	S-145	S-131	S-117
Source	P-25	P-25	INPUT	P-14
Destination	P-14	OUTPUT	P-14	P-16
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	90,00	90,00	25,00	78,25
Pressure (bar)	2,50	2,50	1,01	1,01
Density (g/L)	3,81	971,01	785,89	1,66
Total Enthalpy (kW-h)	2,13	0,01	0,01	2,14
Specific Enthalpy (kcal/kg)	252,40	90,12	14,66	240,02
Heat Capacity (kcal/kg-°C)	0,39	1,01	0,59	0,39
Component Flowrates (kg/h)				
Ethyl Alcohol	7,28	0,00	0,40	7,68
Undesirable Che	0,00	0,00	0,00	0,00
Water	0,00	0,13	0,00	0,00
TOTAL (kg/h)	7,28	0,13	0,40	7,68
TOTAL (L/h)	1907,96	0,13	0,51	4618,25

Stream Name	S-101	S-116	S-115	S-127
Source	P-16	P-16	P-16	P-17
Destination	P-17	P-22	OUTPUT	P-5
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	78,25	78,25	78,25	- 35,05
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	738,41	738,41	738,41	839,45
Total Enthalpy (kW-h)	0,25	0,14	0,02	- 0,11
Specific Enthalpy (kcal/kg)	45,87	45,87	45,87	- 20,55
Heat Capacity (kcal/kg-°C)	0,59	0,59	0,59	0,59
Component Flowrates (kg/h)				
Ethyl Alcohol	4,61	2,69	0,38	4,61
Undesirable Che	0,00	0,00	0,00	0,00
TOTAL (kg/h)	4,61	2,69	0,38	4,61
TOTAL (L/h)	6,24	3,64	0,52	5,49

Stream Name	S-107	S-104	S-139	S-109
Source	P-5	P-5	P-22	P-8
Destination	P-3	P-8	P-8	P-3
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 35,05	- 35,05	- 35,05	- 35,05
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	846,35	1123,85	839,45	842,05
Total Enthalpy (kW-h)	- 0,11	- 0,03	- 0,06	- 0,07
Specific Enthalpy (kcal/kg)	- 20,95	- 27,34	- 20,55	- 20,61
Heat Capacity (kcal/kg-°C)	0,60	0,79	0,59	0,59
Component Flowrates (kg/h)				
CBD	0,02	0,01	0,00	0,01
Ethyl Alcohol	4,15	0,46	2,69	2,83
Solid Impuritie	0,00	0,39	0,00	0,00
THC	0,02	0,01	0,00	0,01
Undesirable Che	0,03	0,03	0,00	0,02
Water	0,12	0,01	0,00	0,01
TOTAL (kg/h)	4,33	0,92	2,69	2,88
TOTAL (L/h)	5,12	0,82	3,20	3,42

Stream Name	S-106	S-140	S-113	S-144
Source	P-8	INPUT	P-12	P-12
Destination	P-12	P-12	OUTPUT	OUTPUT
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 35,05	- 35,00	- 35,01	- 35,01
Pressure (bar)	1,01	1,01	1,25	1,01
Density (g/L)	1210,14	1016,57	1832,50	1011,72
Total Enthalpy (kW-h)	- 0,02	- 0,03	- 0,02	- 0,03
Specific Enthalpy (kcal/kg)	- 28,84	- 35,91	- 35,92	- 35,54
Heat Capacity (kcal/kg-°C)	0,83	1,05	1,05	1,04
Component Flowrates (kg/h)				
CBD	0,01	0,00	0,00	0,00
Ethyl Alcohol	0,31	0,00	0,00	0,02
Solid Impuritie	0,39	0,00	0,39	0,00
THC	0,01	0,00	0,00	0,00
Undesirable Che	0,02	0,00	0,00	0,00
Water	0,00	0,68	0,02	0,66
TOTAL (kg/h)	0,73	0,68	0,41	0,68
TOTAL (L/h)	0,61	0,67	0,22	0,67

Stream Name	S-112	S-125	S-128	S-114
Source	P-12	P-13	P-1	P-15
Destination	P-3	P-1	P-11	P-24
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 35,03	25,00	25,00	56,04
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	854,71	1047,01	1047,01	662,13
Total Enthalpy (kW-h)	- 0,01	0,00	0,00	0,10
Specific Enthalpy (kcal/kg)	- 20,78	10,26	10,26	29,44
Heat Capacity (kcal/kg-°C)	0,59	0,41	0,41	0,53
Component Flowrates (kg/h)				
CBD	0,01	0,04	0,08	0,08
Ethyl Alcohol	0,30	0,00	0,00	0,00
Heptane	0,00	0,00	0,00	2,84
THC	0,01	0,04	0,01	0,00
Undesirable Che	0,02	0,04	0,04	0,00
Water	0,00	0,00	0,00	0,00
TOTAL (kg/h)	0,33	0,13	0,13	2,93
TOTAL (L/h)	0,38	0,12	0,12	4,42

Stream Name	S-118	S-122	S-137	S-130
Source	P-24	P-24	INPUT	P-19
Destination	P-19	OUTPUT	P-19	P-20
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	76,15	133,10	25,00	73,58
Pressure (bar)	0,50	0,50	1,01	0,50
Density (g/L)	638,35	977,58	681,57	640,51
Total Enthalpy (kW-h)	0,13	0,00	0,00	0,14
Specific Enthalpy (kcal/kg)	40,80	20,26	13,40	39,43
Heat Capacity (kcal/kg-°C)	0,54	0,15	0,54	0,54
Component Flowrates (kg/h)				
CBD	0,00	0,08	0,00	0,00
Heptane	2,84	0,00	0,15	2,99
Undesirable Che	0,00	0,00	0,00	0,00
TOTAL (kg/h)	2,85	0,08	0,15	3,00
TOTAL (L/h)	4,46	0,08	0,22	4,68

Stream Name	S-132	S-123	S-126	S-119
Source	P-20	P-20	P-11	P-15
Destination	OUTPUT	P-15	P-15	P-7
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	73,58	73,58	55,20	56,04
Pressure (bar)	0,50	0,50	0,50	1,01
Density (g/L)	640,51	640,51	161,12	30,48
Total Enthalpy (kW-h)	0,01	0,13	0,12	0,15
Specific Enthalpy (kcal/kg)	39,43	39,43	29,61	37,70
Heat Capacity (kcal/kg-°C)	0,54	0,54	0,52	0,52
Component Flowrates (kg/h)				
Acetonitrile	0,00	0,00	3,11	3,11
CBD	0,00	0,00	0,08	0,00
Heptane	0,15	2,84	0,00	0,00
MthTertBtylEthe	0,00	0,00	0,36	0,36
THC	0,00	0,00	0,01	0,01
Undesirable Che	0,00	0,00	0,04	0,04
TOTAL (kg/h)	0,15	2,85	3,60	3,52
TOTAL (L/h)	0,23	4,44	22,32	115,36

Stream Name	S-124	S-120	S-134	S-135
Source	P-7	P-7	INPUT	P-2
Destination	P-2	OUTPUT	P-2	P-18
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	59,05	138,35	25,00	55,20
Pressure (bar)	0,50	0,50	1,01	0,50
Density (g/L)	735,84	846,24	772,13	100,75
Total Enthalpy (kW-h)	0,13	0,01	0,00	0,13
Specific Enthalpy (kcal/kg)	31,29	107,66	13,25	30,31
Heat Capacity (kcal/kg-°C)	0,53	0,79	0,53	0,53
Component Flowrates (kg/h)				
Acetonitrile	3,09	0,02	0,18	3,27
CBD	0,00	0,00	0,00	0,00
MthTertBtylEthe	0,36	0,00	0,02	0,38
THC	0,00	0,01	0,00	0,00
Undesirable Che	0,00	0,04	0,00	0,00
TOTAL (kg/h)	3,45	0,06	0,20	3,65
TOTAL (L/h)	4,69	0,07	0,26	36,26

Stream Name	S-136	S-129
Source	P-18	P-18
Destination	OUTPUT	P-11
Stream Properties		
Activity (U/ml)	0,00	0,00
Temperature (°C)	55,20	55,20
Pressure (bar)	0,50	0,50
Density (g/L)	100,75	100,75
Total Enthalpy (kW-h)	0,01	0,12
Specific Enthalpy (kcal/kg)	30,31	30,31
Heat Capacity (kcal/kg-°C)	0,53	0,53
Component Flowrates (kg/h)		
Acetonitrile	0,16	3,11
MthTertBtylEthe	0,02	0,36
Undesirable Che	0,00	0,00
TOTAL (kg/h)	0,18	3,47
TOTAL (L/h)	1,81	34,45

Table 75: Overall component balance for the production of CBD isolates using cold ethanol.

COMPONENT	INITIAL	IN	OUT	FINAL	IN-OUT
Acetonitrile	0	1419	1419	0	- 0
Carb. Dioxide	0	0	25	0	- 25
CBD	0	0	639	0	- 639
Ethyl Alcohol	0	3168	3168	0	- 0
Heptane	0	1188	1188	0	- 0
MthTertBtylEthe	0	165	165	0	- 0
N2	5	0	0	5	0
O2	1	0	0	1	0
Plant Material	0	5148	0	0	5148
Solid Impuritie	0	0	3089	0	- 3089
THC	0	0	54	0	- 54
Undesirable Che	0	0	311	0	- 311
Water	0	5411	6440	0	- 1030
TOTAL	6	16499	16499	6	0
				Overall Error:	0,002%

Table 76: Breakdown of energy usage amongst equipment for the production of CBD isolates using cold ethanol.

Procedure Name	Unit Cost ($/kW-h)	Amount (kW-h/year)	Cost ($/year)	%
P-1	0,10	0	0,00	0,00
P-5	0,10	12	1,18	0,68
P-4	0,10	257	25,74	14,93
P-8	0,10	8	0,80	0,46
P-15	0,10	4	0,36	0,21
P-21	0,10	93	9,35	5,42
P-13	0,10	14	1,39	0,81
P-17	0,10	626	62,59	36,30
P-22	0,10	365	36,51	21,18
Unlisted Equipment	0,10	86	8,62	5,00
General Load	0,10	259	25,86	15,00
TOTAL		1724	172,40	100,00

Table 77: Stream breakdown for the production of CBD blends.

Stream Name	**S-102**	**S-103**	**S-111**	**S-138**
Source	**INPUT**	**P-4**	**P-21**	**P-3**
Destination	**P-4**	**P-21**	**P-5**	**P-6**
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	25,00	40,82	- 35,05	- 35,05
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	2315,66	1396,55	1433,13	845,06
Total Enthalpy (kW-h)	0,03	0,03	- 0,02	- 0,18
Specific Enthalpy (kcal/kg)	37,43	37,43	- 32,89	- 20,81
Heat Capacity (kcal/kg-°C)	1,50	0,91	0,96	0,59
Component Flowrates (kg/h)				
CBD	0,00	0,03	0,03	0,03
Ethyl Alcohol	0,00	0,00	0,00	7,28
Plant Material	0,65	0,00	0,00	0,00
Solid Impuritie	0,00	0,39	0,39	0,00
THC	0,00	0,03	0,03	0,03
Undesirable Che	0,00	0,07	0,07	0,06
Water	0,00	0,13	0,13	0,13
TOTAL (kg/h)	0,65	0,65	0,65	7,54
TOTAL (L/h)	0,28	0,47	0,45	8,92

Stream Name	**S-105**	**S-108**	**S-121**	**S-133**
Source	**P-6**	**P-6**	**P-10**	**P-10**
Destination	**P-10**	**P-13**	**OUTPUT**	**P-25**
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	130,00	130,00	50,00	50,00
Pressure (bar)	1,01	1,01	2,50	2,50
Density (g/L)	1,36	974,42	4,11	766,63
Total Enthalpy (kW-h)	2,37	0,01	0,00	0,26
Specific Enthalpy (kcal/kg)	275,45	53,36	37,34	29,68
Heat Capacity (kcal/kg-°C)	0,42	0,42	0,23	0,59
Component Flowrates (kg/h)				
Carb. Dioxide	0,00	0,00	0,00	0,00
CBD	0,00	0,04	0,00	0,00
Ethyl Alcohol	7,28	0,00	0,00	7,28
THC	0,00	0,04	0,00	0,00
Undesirable Che	0,00	0,04	0,00	0,00
Water	0,13	0,00	0,00	0,13
TOTAL (kg/h)	7,41	0,13	0,00	7,41
TOTAL (L/h)	5468,31	0,13	0,87	9,66

Stream Name	**S-143**	**S-145**	**S-131**	**S-117**
Source	**P-25**	**P-25**	**INPUT**	**P-14**
Destination	**P-14**	**OUTPUT**	**P-14**	**P-16**
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	90,00	90,00	25,00	78,25
Pressure (bar)	2,50	2,50	1,01	1,01
Density (g/L)	3,81	971,01	785,89	1,66
Total Enthalpy (kW-h)	2,13	0,01	0,01	2,14
Specific Enthalpy (kcal/kg)	252,40	90,12	14,66	240,02
Heat Capacity (kcal/kg-°C)	0,39	1,01	0,59	0,39
Component Flowrates (kg/h)				
Ethyl Alcohol	7,28	0,00	0,40	7,68
Undesirable Che	0,00	0,00	0,00	0,00
Water	0,00	0,13	0,00	0,00
TOTAL (kg/h)	7,28	0,13	0,40	7,68
TOTAL (L/h)	1907,83	0,13	0,51	4617,95

Stream Name	S-101	S-116	S-115	S-127
Source	P-16	P-16	P-16	P-17
Destination	P-17	P-22	OUTPUT	P-5
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	78,25	78,25	78,25	- 35,05
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	738,41	738,41	738,41	839,45
Total Enthalpy (kW-h)	0,25	0,14	0,02	- 0,11
Specific Enthalpy (kcal/kg)	45,87	45,87	45,87	- 20,55
Heat Capacity (kcal/kg-°C)	0,59	0,59	0,59	0,59
Component Flowrates (kg/h)				
Ethyl Alcohol	4,61	2,69	0,38	4,61
Undesirable Che	0,00	0,00	0,00	0,00
TOTAL (kg/h)	4,61	2,69	0,38	4,61
TOTAL (L/h)	6,24	3,64	0,52	5,49

Stream Name	S-107	S-104	S-139	S-109
Source	P-5	P-5	P-22	P-8
Destination	P-3	P-8	P-8	P-3
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 35,05	- 35,05	- 35,05	- 35,05
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	846,35	1123,86	839,45	842,05
Total Enthalpy (kW-h)	- 0,11	- 0,03	- 0,06	- 0,07
Specific Enthalpy (kcal/kg)	- 20,95	- 27,34	- 20,55	- 20,61
Heat Capacity (kcal/kg-°C)	0,60	0,79	0,59	0,59
Component Flowrates (kg/h)				
CBD	0,02	0,01	0,00	0,01
Ethyl Alcohol	4,15	0,46	2,69	2,83
Solid Impuritie	0,00	0,39	0,00	0,00
THC	0,02	0,01	0,00	0,01
Undesirable Che	0,03	0,03	0,00	0,02
Water	0,12	0,01	0,00	0,01
TOTAL (kg/h)	4,33	0,92	2,69	2,88
TOTAL (L/h)	5,12	0,82	3,20	3,42

Stream Name	S-106	S-140	S-113	S-144
Source	P-8	INPUT	P-12	P-12
Destination	P-12	P-12	OUTPUT	OUTPUT
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 35,05	- 35,00	- 35,01	- 35,01
Pressure (bar)	1,01	1,01	1,25	1,01
Density (g/L)	1210,16	1016,57	1832,50	1011,72
Total Enthalpy (kW-h)	- 0,02	- 0,03	- 0,02	- 0,03
Specific Enthalpy (kcal/kg)	- 28,84	- 35,91	- 35,92	- 35,54
Heat Capacity (kcal/kg-°C)	0,83	1,05	1,05	1,04
Component Flowrates (kg/h)				
CBD	0,01	0,00	0,00	0,00
Ethyl Alcohol	0,31	0,00	0,00	0,02
Solid Impuritie	0,39	0,00	0,39	0,00
THC	0,01	0,00	0,00	0,00
Undesirable Che	0,02	0,00	0,00	0,00
Water	0,00	0,68	0,02	0,66
TOTAL (kg/h)	0,73	0,68	0,41	0,68
TOTAL (L/h)	0,61	0,67	0,22	0,67

Stream Name	S-112	S-125	S-110	S-114
Source	P-12	P-13	P-1	P-24
Destination	P-3	P-1	P-24	OUTPUT
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 35,03	25,00	25,00	153,00
Pressure (bar)	1,01	1,01	1,01	0,50
Density (g/L)	854,71	1047,01	1047,01	952,12
Total Enthalpy (kW-h)	- 0,01	0,00	0,00	0,00
Specific Enthalpy (kcal/kg)	- 20,78	10,26	10,26	118,05
Heat Capacity (kcal/kg-°C)	0,59	0,41	0,41	0,79
Component Flowrates (kg/h)				
CBD	0,01	0,04	0,08	0,00
Ethyl Alcohol	0,30	0,00	0,00	0,00
THC	0,01	0,04	0,01	0,01
Undesirable Che	0,02	0,04	0,04	0,02
Water	0,00	0,00	0,00	0,00
TOTAL (kg/h)	0,33	0,13	0,13	0,03
TOTAL (L/h)	0,38	0,12	0,12	0,03

Stream Name	S-118	S-119	S-120	S-122
Source	P-24	P-2	P-2	P-7
Destination	P-2	OUTPUT	P-7	OUTPUT
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	149,43	153,23	146,84	153,14
Pressure (bar)	0,50	0,50	0,50	0,50
Density (g/L)	962,75	949,17	966,26	950,08
Total Enthalpy (kW-h)	0,01	0,00	0,00	0,00
Specific Enthalpy (kcal/kg)	47,11	143,57	35,53	135,71
Heat Capacity (kcal/kg-°C)	0,32	0,96	0,24	0,91
Component Flowrates (kg/h)				
CBD	0,08	0,00	0,08	0,00
THC	0,00	0,00	0,00	0,00
Undesirable Che	0,02	0,01	0,01	0,00
TOTAL (kg/h)	0,10	0,01	0,09	0,01
TOTAL (L/h)	0,10	0,01	0,09	0,01

Stream Name	S-123
Source	P-7
Destination	OUTPUT
Stream Properties	
Activity (U/ml)	0,00
Temperature (°C)	143,55
Pressure (bar)	0,50
Density (g/L)	969,87
Total Enthalpy (kW-h)	0,00
Specific Enthalpy (kcal/kg)	28,53
Heat Capacity (kcal/kg-°C)	0,20
Component Flowrates (kg/h)	
CBD	0,08
THC	0,00
Undesirable Che	0,00
TOTAL (kg/h)	0,08
TOTAL (L/h)	0,09

Table 78: Overall component balance for the production of CBD blends.

COMPONENT	INITIAL	IN	OUT	FINAL	IN-OUT
Carb. Dioxide	0	0	25	0	- 25
CBD	0	0	639	0	- 639
Ethyl Alcohol	0	3168	3168	0	- 0
N2	5	0	0	5	0
O2	1	0	0	1	0
Plant Material	0	5148	0	0	5148
Solid Impuritie	0	0	3089	0	- 3089
THC	0	0	54	0	- 54
Undesirable Che	0	0	311	0	- 311
Water	0	5411	6440	0	- 1030
TOTAL	6	13727	13727	6	0
				Overall Error:	0,001%

Table 79: Breakdown of energy usage for the production of CBD blends.

Procedure Name	Unit Cost ($/kW-h)	Amount (kW-h/year)	Cost ($/year)	%
P-1	0,10	0	0,00	0,00
P-5	0,10	12	1,18	0,68
P-4	0,10	257	25,74	14,97
P-8	0,10	8	0,80	0,46
P-21	0,10	93	9,35	5,44
P-13	0,10	14	1,39	0,81
P-17	0,10	626	62,59	36,40
P-22	0,10	365	36,51	21,23
Unlisted Equipment	0,10	86	8,60	5,00
General Load	0,10	258	25,79	15,00
TOTAL		1719	171,94	100,00

Supercritical CO_2 Process

Table 80: Stream breakdown for the production of CBD isolate production using supercritical CO_2.

Stream Name	**S-102**	**S-101**	**S-109**	**S-111**
Source	**INPUT**	**P-4**	**P-5**	**P-46**
Destination	**P-4**	**P-33**	**P-46**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	25,00	40,82	78,25	78,25
Pressure (bar)	1,01	1,01	1,01	1,01
Density (g/L)	2315,66	1396,55	1,63	1,63
Total Enthalpy (kW-h)	0,03	0,03	0,29	0,06
Specific Enthalpy (kcal/kg)	37,43	37,43	35,95	35,95
Heat Capacity (kcal/kg-°C)	1,50	0,91	0,27	0,27
Component Flowrates (kg/h)				
Carb. Dioxide	0,00	0,00	6,05	1,21
CBD	0,00	0,03	0,02	0,00
Ethyl Alcohol	0,00	0,00	0,50	0,10
Plant Material	0,65	0,00	0,00	0,00
Solid Impuritie	0,00	0,39	0,00	0,00
THC	0,00	0,03	0,02	0,00
Undesirable Che	0,00	0,07	0,03	0,01
Water	0,00	0,13	0,35	0,07
TOTAL (kg/h)	0,65	0,65	6,96	1,39
TOTAL (L/h)	0,28	0,47	4274,90	854,98

Stream Name	**S-110**	**S-107**	**S-112**	**S-106**
Source	**P-46**	**P-47**	**P-48**	**P-33**
Destination	**P-47**	**P-48**	**P-33**	**P-8**
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	78,25	60,00	63,44	60,00
Pressure (bar)	1,01	1,01	251,01	200,00
Density (g/L)	1,63	1,85	424,09	323,44
Total Enthalpy (kW-h)	0,23	0,11	0,11	0,07
Specific Enthalpy (kcal/kg)	35,95	16,41	17,37	12,75
Heat Capacity (kcal/kg-°C)	0,27	0,28	0,28	0,22
Component Flowrates (kg/h)				
Carb. Dioxide	4,84	4,84	4,84	4,79
CBD	0,01	0,01	0,01	0,03
Ethyl Alcohol	0,40	0,40	0,40	0,00
THC	0,01	0,01	0,01	0,03
Undesirable Che	0,03	0,03	0,03	0,06
Water	0,28	0,28	0,28	0,00
TOTAL (kg/h)	5,57	5,57	5,57	4,92
TOTAL (L/h)	3419,92	3007,35	13,12	15,20

Stream Name	**S-104**	**S-115**	**S-117**	**S-113**
Source	**P-33**	**P-8**	**P-3**	**P-3**
Destination	**P-41**	**P-3**	**P-34**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	60,00	- 20,00	12,00	12,00
Pressure (bar)	200,00	200,00	200,00	200,00
Density (g/L)	956,94	424,76	287,04	1039,40
Total Enthalpy (kW-h)	0,07	- 0,02	0,07	0,00
Specific Enthalpy (kcal/kg)	49,55	- 4,06	530,24	6,95
Heat Capacity (kcal/kg-°C)	0,83	0,20	0,29	0,58
Component Flowrates (kg/h)				
Carb. Dioxide	0,05	4,79	0,00	0,00
CBD	0,01	0,03	0,03	0,00
Ethyl Alcohol	0,40	0,00	0,00	0,00
Solid Impuritie	0,39	0,00	0,00	0,00
THC	0,01	0,03	0,03	0,00
Undesirable Che	0,03	0,06	0,05	0,01
Water	0,41	0,00	0,00	0,00
TOTAL (kg/h)	1,30	4,92	0,11	0,02
TOTAL (L/h)	1,36	11,58	0,37	0,02

Stream Name	S-131	S-138	S-105	S-108
Source	P-34	P-34	P-6	P-6
Destination	P-5	P-6	P-5	P-13
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	520,56	520,56	130,00	130,00
Pressure (bar)	100,00	100,00	100,00	100,00
Density (g/L)	51,56	723,35	144,90	974,40
Total Enthalpy (kW-h)	0,01	0,05	0,00	0,01
Specific Enthalpy (kcal/kg)	711,62	497,67	38,65	53,43
Heat Capacity (kcal/kg-°C)	0,43	2,71	0,31	0,42
Component Flowrates (kg/h)				
Carb. Dioxide	0,00	0,00	0,00	0,00
CBD	0,00	0,02	0,00	0,03
THC	0,00	0,02	0,00	0,03
Undesirable Che	0,01	0,05	0,00	0,03
TOTAL (kg/h)	0,02	0,09	0,00	0,09
TOTAL (L/h)	0,31	0,13	0,02	0,09

Stream Name	S-127	S-103	S-116	S-125
Source	P-41	P-41	INPUT	P-13
Destination	P-5	OUTPUT	P-5	P-1
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	- 170,88	- 170,88	25,00	25,00
Pressure (bar)	50,00	50,00	1,01	100,00
Density (g/L)	119,51	1264,97	1,95	1046,96
Total Enthalpy (kW-h)	0,23	- 0,16	0,04	0,00
Specific Enthalpy (kcal/kg)	458,89	- 159,60	5,71	10,27
Heat Capacity (kcal/kg-°C)	0,38	1,21	0,23	0,41
Component Flowrates (kg/h)				
Carb. Dioxide	0,05	0,00	6,00	0,00
CBD	0,01	0,00	0,00	0,03
Ethyl Alcohol	0,00	0,40	0,50	0,00
Solid Impuritie	0,00	0,39	0,00	0,00
THC	0,01	0,00	0,00	0,03
Undesirable Che	0,02	0,00	0,00	0,03
Water	0,35	0,06	0,00	0,00
TOTAL (kg/h)	0,44	0,86	6,50	0,09
TOTAL (L/h)	3,67	0,68	3336,04	0,08

Stream Name	S-128	S-114	S-118	S-122
Source	P-1	P-15	P-24	P-24
Destination	P-11	P-24	P-19	OUTPUT
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	25,00	56,08	76,15	133,46
Pressure (bar)	100,00	1,01	0,50	0,50
Density (g/L)	1046,96	660,17	638,33	976,32
Total Enthalpy (kW-h)	0,00	0,10	0,13	0,00
Specific Enthalpy (kcal/kg)	10,27	29,63	40,81	20,59
Heat Capacity (kcal/kg-°C)	0,41	0,53	0,54	0,15
Component Flowrates (kg/h)				
CBD	0,06	0,06	0,00	0,06
Heptane	0,00	2,81	2,81	0,00
THC	0,00	0,00	0,00	0,00
Undesirable Che	0,03	0,00	0,00	0,00
TOTAL (kg/h)	0,09	2,87	2,81	0,06
TOTAL (L/h)	0,08	4,35	4,41	0,06

Stream Name	S-137	S-130	S-132	S-123
Source	INPUT	P-19	P-20	P-20
Destination	P-19	P-20	OUTPUT	P-15
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	25,00	73,56	- 201,70	73,56
Pressure (bar)	1,01	0,50	0,50	0,50
Density (g/L)	681,57	640,52	8,43	640,52
Total Enthalpy (kW-h)	0,00	0,14	0,01	0,13
Specific Enthalpy (kcal/kg)	13,40	39,42	39,42	39,42
Heat Capacity (kcal/kg-°C)	0,54	0,54	0,10	0,54
Component Flowrates (kg/h)				
CBD	0,00	0,00	0,00	0,00
Heptane	0,15	2,96	0,15	2,81
Undesirable Che	0,00	0,00	0,00	0,00
TOTAL (kg/h)	0,15	2,96	0,15	2,82
TOTAL (L/h)	0,22	4,63	17,57	4,40

Stream Name	S-126	S-119	S-124	S-120
Source	P-11	P-15	P-7	P-7
Destination	P-15	P-7	P-2	OUTPUT
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	55,20	56,08	59,04	133,39
Pressure (bar)	0,50	1,01	0,50	0,50
Density (g/L)	138,04	30,36	735,84	824,57
Total Enthalpy (kW-h)	0,12	0,15	0,13	0,01
Specific Enthalpy (kcal/kg)	29,80	37,68	31,29	100,28
Heat Capacity (kcal/kg-°C)	0,53	0,52	0,53	0,76
Component Flowrates (kg/h)				
Acetonitrile	3,11	3,11	3,09	0,02
CBD	0,06	0,00	0,00	0,00
MthTertBtylEthe	0,36	0,36	0,36	0,00
THC	0,00	0,00	0,00	0,00
Undesirable Che	0,03	0,03	0,00	0,03
TOTAL (kg/h)	3,56	3,50	3,45	0,05
TOTAL (L/h)	25,78	115,36	4,69	0,06

Stream Name	S-134	S-135	S-136	S-129
Source	INPUT	P-2	P-18	P-18
Destination	P-2	P-18	OUTPUT	P-11
Stream Properties				
Activity (U/ml)	0,00	0,00	0,00	0,00
Temperature (°C)	25,00	55,20	55,20	55,20
Pressure (bar)	1,01	0,50	0,50	0,50
Density (g/L)	772,13	100,75	100,75	100,75
Total Enthalpy (kW-h)	0,00	0,13	0,01	0,12
Specific Enthalpy (kcal/kg)	13,25	30,30	30,30	30,30
Heat Capacity (kcal/kg-°C)	0,53	0,53	0,53	0,53
Component Flowrates (kg/h)				
Acetonitrile	0,18	3,27	0,16	3,11
MthTertBtylEthe	0,02	0,38	0,02	0,36
Undesirable Che	0,00	0,00	0,00	0,00
TOTAL (kg/h)	0,20	3,65	0,18	3,47
TOTAL (L/h)	0,26	36,26	1,81	34,45

Table 81: Overall component balance for the production of CBD isolate production using supercritical CO_2.

COMPONENT	IN	OUT	IN-OUT
Acetonitrile	1419	1419	- 0
Carb. Dioxide	47520	9587	37933
CBD	0	527	- 527
Ethyl Alcohol	3960	3960	0
Heptane	1188	1176	12
MthTertBtylEthe	165	165	- 0
Plant Material	5148	0	5148
Solid Impuritie	0	3089	- 3089
THC	0	114	- 114
Undesirable Che	0	371	- 371
Water	0	1030	- 1030
TOTAL	59400	21437	37963
		Overall Error:	63,911%

Table 82: Breakdown of energy usage for the production of CBD isolates using supercritical CO_2.

Standard Power				
Procedure Name	**Unit Cost ($/kW-h)**	**Amount (kW-h/year)**	**Cost ($/year)**	**%**
P-1	0,10	0	0,00	0,01
P-4	0,10	257	25,74	48,47
P-15	0,10	4	0,36	0,68
P-13	0,10	14	1,39	2,62
P-33	0,10	5	0,53	0,99
P-48	0,10	68	6,76	12,73
P-3	0,10	43	4,31	8,12
Unlisted Equipment	0,10	35	176,90	5,00
General Load	0,10	105	530,69	15,00
TOTAL		531	53,10	100,00

High Voltage Power				
Procedure Name	**Unit Cost ($/kW-h)**	**Amount (kW-h/year)**	**Cost ($/year)**	**%**
P-8	20,60	169	3484,80	100,00
TOTAL		169	3484,80	100,00