Abstract

Lignocellulosic biomass (LCB) resources are central to modern, sustainable development of economies worldwide. They are abundant, cheap and do not contribute to net CO_2 emissions hence their refinement for energy and valuable industrial commodities production provides a necessary alternative to crude oil. LCB have been used as solid fuels since the paleolithic ages. Today, advances on bio-chemo catalysis and carbon chemistry have seen more efficient processes converting biomass to a wide range of valuable products e.g. chemicals, biofuels and other bio-based commodities. Likewise, increase in awareness of the dangers of the continuous use of non-renewable fossil have continued to drive and accentuate the potential of biomass as a sustainable source of organic carbon but the high cost of biomass processing, poses a major threat to proposals for further development and commercialisation. LCB is an abundant, cheap and sustainable resource but it is also naturally inert and recalcitrant to biocatalytic conversion. As such, biorefining processes generally require a feed activation step (pre-treatment) prior to efficient conversion. The high cost of pre-treatment have made the need for modern strategies targeting cheaper, effective, catalyst-compatible and sustainable pre-treatment techniques very imperative. Cheap, recyclable and sustainable non-derivatising zinc chloride molten hydrate salt systems was proposed in this work as a plausible alternative to acid and alkali pre-treatment by means of investigations probing a) pre-treatment efficiency at relatively mild conditions, and (b) influence on biocompatibility of fermentation medium.

This work sought to establish a holistic approach for improved techno-feasibility of large-scale biorefining by exploring the potentials of an intensified and integrated process. The proposed process involved mild pre-treatment by solvation of local waste LCB in inorganic $ZnCl_2.nH_2O$ and whole-cell microbial conversion of liberated sugar to value-added products (e.g. succinic acid). Succinic acid (BSA) is a valuable, bio-based commodity with a growing

demand and market value as a top-platform chemical. The biocommodity has a wide-range of application in several industries such as biopharmaceuticals, food and materials production. By targeting anaerobic fermentative synthesis of SA, a waste-to-value concept was explored, further contributing to the proposed strategy of cheaper, sustainable bioprocess. The efficiency and general potentials of the bio-chemocatalytic process for SA production was described by charting specific objectives such as, (a) feedstock analysis (organic and inorganic composition), (b) pre-treatment efficiency as described by biomass deconstruction in molten hydrate salt (MHS) (c) statistical optimisation of sugar yield, and (d) metagenomic analysis of media selectivity of microbial catalysts as correspondent to yield.

The suitability of locally-sourced corn cob wastes as feeds in a bioconversion system was assessed by means of the characteristic organic and inorganic composition of the materials. The high (82.5 %) percent composition of sugars in the biomass was promising for anaerobic fermentation process as explored in this study. Using zinc chloride tetrahydrate $ZnCl_2.nH_2O$ salt solvent system, this work demonstrated the efficient pre-treatment of locally sourced waste corn cobs at relatively mild conditions of 70 ^{o}C for 60 minutes. The solvation process resulted in microstructural modifications of the materials that were correlated for reduced crystallinity and improved fiber reactivity. In comparison to common techniques (such as dil. acid or alkali treatment at relatively harsher operating conditions) used in overcoming biomass recalcitrance, the effect of $ZnCl_2.nH_2O$ treatment on biomass recalcitrance was promising especially from cost (energy and solvent) and sustainability perspective. Implications for biomass susceptibility to biological digestion of inherent saccharose was also assuring as approximately 85 and 95.1 % of cellulosic and hemicellulosic fractions were recovered upon enzymatic saccharification of treated samples.

A predictive second-order polynomial model, developed using a $_{2}3$ Box-Behnken design was used to describe the effect (main and interactive) of pre-treatment process variables (time, temperature, and solvent loading) on yield towards the parametric optimisation of the process. The experimental yield of reducing sugars (Y_E) was well-fitted into the resulting model according high f-value (> p-value) obtained from the regression analysis and ANOVA tests of the model. ANOVA tests also reiterated the significance of the response model (Reducing sugar yield) with a reliability of 92.5 %, (adjusted R^2= 88. %), suggesting an error margin of < 8%. The optimal parameters (90 minutes, 107 ^oC , 18 g/g liquid-to-solid ratio) for the recovery of a total of 90 % of total sugars (based on biomass compositional data) were obtained by multi-objective numerical optimisation and cross-validated experimentally.

The fermentability of the dissolved biomass sugars at varying concentrations of $ZnCl_2$ salt was characterised by the observed synthesis of organic intermediates and target product; Succinic acid, at select salt concentrations. Anaerobic fermentation set-up was a batch simultaneous saccharification and co-fermentation (SScoF) bioprocess with feed of pre-treatment slurry at varying solvent; $ZnCl_2.4H_2O$ loading, catalysed by a mixed-consortia of rumen bacteria at standard anaerobic fermentation conditions. The mixed acid- fermentation process of the unconditioned $ZnCl_2$ MHS pre-treatment slurry, resulted in the conversion (up to 2 %) of dissolved biomass sugars to SA (10.4 g L^{-1}). SA production declined with increasing salt concentration but the original findings were promising especially considering the uncontrolled carbon channelling resulting from the use of mixed-consortium of microbial biocatalysts. Analysis of SA yield results were reconciled with bioactivity; as measured by DNA quantification, at the different salt concentration while microbial inventory analysis was done at constant slurry salt concentration.

The choice of a mixed-consortia of rumen bacteria as to single-cell cultures was utilized to broaden the sampling range for SA-producing biocatalysts (using $ZnCl_2$ salt concentration as a singular factor to model their micro-environments). Selectivity of media for microbes was profiled by characterizing the inoculum (cow dung) and fermenter samples using advanced next-generation sequencing (NGS) tools. The characteristic operational taxonomic units (OTUs) of the active species in raw cow dung were determined towards informing specific microbial activity in media. The microbiome of the cow-dung to consisted of approximately 1500 OTUs, with the detection of up to 265 genera (of which only 18 genera constituted over 1 % of relative abundance). The dominant phyla was *Firmicutes* and *Actinobacteria* both of which are notable for immense industrial applicability in organic acid and alcohol biosynthesis. The observed strains of phyla such as *thermileophilia, Acidimicrobiia* have potentials in metageneomic engineering for programmed cell-free catalysis and synthetic biomanufacturing designs which could find applicability in proposed process.

This thesis is a careful documentation of proof of concept of SA biosynthesis in an intensified bio-chemocatalytic process and experimental proof of efficient biomass deconstruction; characterised by ultra-hemicellulosic solubulization and increased bioreactivity (saccharification and digestion) of residual cellulose in $ZnCl_2.nH_2O$ solvent systems at relatively mild conditions. The findings as described in this thesis have the potentials to be fundamental to the design of advanced process for cheaper, efficient and integrated one-pot, bio-chemocatalytic pre-treatment and conversion of abundant waste LCB resources such as corn cob to valuable commodities such as bio-based succinic acid (BSA). Some of the findings as described in this dissertation have been communicated as scientific articles in reputable journals and as contributions to scientific conferences.