A Thermodynamic Analysis of Biological Systems Using Process Synthesis

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Declaration

I declare that this thesis is my own, unaided work, except where otherwise acknowledged. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

Signed this ____ day of _____ 20__

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Abstract

The steady decline in fossil fuel reserves means that renewable and sustainable alternatives are becoming increasingly important to explore. A key tool in studying biological process is thermodynamics. Thermodynamics has been successfully used to understand chemical processes and similar techniques can be applied to biological processes. Using continuous data from a chemostat the thermodynamic properties, that is the enthalpy of formation and of maintenance and Gibbs free energy of formation and of maintenance, were estimated for the bacterium *Clostridium Thermolacticum*. The benefit of this method is that the estimated properties are for the living microorganism as they are found in a biological system. The results can be used to predict the possible products based on a given substrate and the thermodynamically feasible region for the system. The feasible region is a useful tool in determining the limits of performance of the system. The estimated maintenance requirements of the microorganism can be superimposed on the feasible region as a vector to show how the requirements of the microorganism affect the product yield. A special case, the maintenance limited case where there is no formation of new biomass, is considered in light of the feasible region and maintenance vector. The maintenance limited case is used to predict the product spectrum when there is no formation of biomass. The feasible region can be extended to consider the effect that additional products and alternative feeds have on the system. For a given feed and possible products is possible to predict the the product spectrum. This approach can be used to determine the maximum amount of biomass that can be formed or how the products are affected when there is no biomass formation. The maintenance requirements of the microorganism

will limit the product spectrum as determined by the maintenance vector. A similar approach is used for the analysis of photosynthesis and combustion. It is shown that, from a thermodynamic point of view, photosynthesis can be treated as the reverse combustion process. This analysis highlights the inefficiencies in the combustion reaction based on the Carnot temperature of the process. In a similar way it is shown that the photosynthetic reaction can be operated at close to reversible due to the Carnot temperature requirements of the process and the entropy associated with light entering the system.

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Nomenclature

ATP	:	Adenosine Triphosphate
C_{cells}	:	Concentration of the cells [g/L]
e_i	:	Extent of reaction i [mmol/hr]
F	:	Flowrate [l/hr]
ΔG	:	Overall or system Gibbs free energy [kJ/mol] or [J/mmol]
ΔG_{rxn}	:	Gibbs free energy of reaction [kJ/mol] or [J/mmol]
$G_{f,i}$	:	Gibbs free energy of formation of component i [kJ/mol]
G_{in}, G_{out}	:	Gibbs free energy into and out of the system [kJ/hr]
G_{lost}	:	Gibbs free energy lost [kJ/hr]
G_m	:	Gibbs free energy of maintenance of the cells [kJ/mol] or [J/mmol]
G_{mixing}	:	Gibbs free energy of mixing [kJ/kmol.L]
hfN_A	:	Energy of 1 mole of light [kJ/mol]
ΔH	:	Overall or system Enthalpy [kJ/mol] or [J/mmol]
ΔH_{rxn}	:	Enthalpy of reaction [kJ/mol] or [J/mmol]
$H_{f,i}$	:	Enthalpy of formation of component i [kJ/mol]
H_{in}, H_{out}	:	Enthalpy into and out of the system [kJ/hr]
H_m	:	Enthalpy of maintenance of the cells [kJ/kmol.L]
H_{mixing}	:	Enthalpy of mixing [kJ/mol] or [J/mmol]
n_i	:	Molar flowrate of component i [mmol/hr]
Q_{lost}	:	Heat loss [kJ/hr]
R	:	Ideal gas constant [J/mol]
ΔS	:	Overall or system Entropy [kJ/mol.K]
S_{in}, S_{out}	:	Entropy into and out of the system [kJ/hr.K]
S_{lost}	:	Entropy lost [kJ/hr.K]
T	:	Temperature of the system [K]

T_0	:	Standard temperature [K]
T_c	:	Carnot Temperature [K]
V	:	Volume [L]
x_i	:	Composition of component i in the phase in which it is found

Chapter 1

Introduction

1.1 Overall Introduction

The Statistical Review of World Energy released by BP (BP plc, 2010) shows that fossil fuels, which account for 87.9% of the global primary energy consumption, have a very limited lifetime. Based on the proven reserves (BP plc, 2010) and current consumption oil, gas and coal are estimated to last another 45, 63 and 120 years respectively. It is therefore important to look at alternative renewable and sustainable energy supplies. In order to understand which process are renewable and which processes are sustainable, it is important to understand the processes and key factors affecting them. Using thermodynamics it is possible to define the theoretical limits of a given process. The theoretical limits give all possible process outputs that satisfy the laws of nature and, hence, all processes are described. The theoretical limits define the boundary conditions of all possible processes and define the limits of performance for these processes. Using the theoretical limits is an important benchmark in determining the feasibility of the process when compared to other systems.

Thermodynamics has been a useful tool in studying chemical processes (Patel, Hildebrandt, Glasser, and Hausberger, 2005). These methods can be used to compare different processes (Patel, Hildebrandt, and Glasser, 2010) as well as to understand and improve them (Hildebrandt, Glasser, Hausberger, Patel, and Glasser, 2009; Sempuga, Hausberger, Patel, Hildebrandt, and Glasser, 2010). Thermodynamics has also been applied to understanding microbial processes (Roels, 1983).

The microbial systems offer a higher degree of complexity and thus multiple methods (von Stockar, Maskow, Liu, Marison, and Patino, 2006) have been proposed for studying the systems. The methods for studying biological processes are typically broken into catabolic and anabolic reactions (vanBriesen, 2002). The methods are mostly based on Gibbs free energy or entropy calculations (Battley, 1992) and do not consider enthalpy. As shown by Patel, Hildebrandt, Glasser, and Hausberger (2007) it is important to consider both enthalpy and entropy as well as the mass balances when analysing processes.

Applying the laws of thermodynamics to biological systems will aid in understanding the limits of the process and the limitations when considering other systems and processes.

1.2 Aim of the Thesis

The overall aim of the thesis is to introduce a new set of tools for the analysis of the heat and work flows in biological systems. The first part of the thesis applies thermodynamics to better understand the operations and limitations of biological processes. In chemical engineering, thermodynamics has been extensively used to study the chemical processes. Process Synthesis (Patel et al., 2005, 2007; Hildebrandt et al., 2009; Patel et al., 2010; Sempuga et al., 2010) has been shown to be very useful in understanding these processes. The work in this thesis extends the ideas of Process Synthesis for use in biological systems. The last section of work looks at the conversion of cellulosic biomass using bacteria obtained from termites. There is a significant amount of research on the cellulolytic bacteria in the termite gut, however none of the literature found discussed the use of the bacteria in a continuous reactor. This work attempts to use the bacteria in the termite gut to produce useful products such as ethanol and acetic acid from cellulose containing material.

1.3 Thesis Overview

The chapters in this thesis are written as journal articles with each chapter covering a specific idea. The journal article style aids in grouping specific concepts together as well as aiding in the publication of the work. Each chapter contains its own introduction with its own literature review and th which may have some similarities to other introductions as the topics are related. The first three chapters are placed in order of the development and application of concepts. However, since the chapters are written in a journal article style

each chapter can be read independently.

Chapter 2 applies the thermodynamic methods of process synthesis to data from a typical fermentation experiment. This approach is used predict the enthalpy and Gibbs free energy of formation and the maintenance energy requirements of the bacteria as found in the system.

Chapter 3 uses the properties of the bacteria predicted in Chapter 2 to understand the thermodynamic limitations of metabolism. Chapter 3 develops a method to look at different cases of metabolism, in this case the effect of different feeds and additional products on the system. This chapter looks at the special case where maintenance is the limiting factor, i.e. where there is no growth of bacteria.

Chapter 4 applies the approach of the previous chapters to photosynthesis and combustion. This approach shows that, from a thermodynamic point of view, photosynthesis can be treated as the reverse reaction of combustion. This work highlights the intrinsic inefficiencies associated with combustion. This work also shows that the core reaction of photosynthesis is theoretically close to a reversible system.

Chapter 5 studies the feasibility of using bacteria obtained from termites to convert cellulosic material into usable products. Whereas the previous chapters are theoretically based this chapter describes experimental work. Although there is no direct link between this chapter and the previous chapters this work is important as it encouraged the development of the work in Chapter 2 and, ultimately, the work in all the previous chapters. An important goal of this line of work is to develop the analytics to a point where the concepts from the previous chapters can be applied to the data obtained from the experiments.

Chapter 6 is the concluding chapter. This chapter summarises the results from the previous chapters and makes recommendations for future work that can be pursued in this area.

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Chapter 2

On the Calculation and Use of the Thermodynamic Properties of Living Bacteria

Abstract

The thermodynamic properties, that is the enthalpy of formation ($H_{f,cell}$), and of maintenance (H_m) and Gibbs free energy of formation ($G_{f,cell}$) and of maintenance (G_m) of a bacterium cell were estimated from the mass balance alone. It must be emphasized that these thermodynamic properties are for living microorganisms as they are found in biological systems. The results allow one to predict the thermodynamically feasible region for a given substrate and products. Given the necessary information, the methods used here can be applied to any microogranism. The methods are illustrated by applying them to a set of experimental data from the literature.

Keywords: thermodynamics, fermentation reactions, process targets

2.1 Introduction

Chemists and chemical engineer's routinely use tables of Enthalpy and Gibbs free energy of formation to do calculations on chemical systems, reactions and processes. This gives them valuable information about the systems without it being necessary for them to do experiments, a very large saving in effort. If one were able to do the same for living organisms this would constitute a significant advance.

Thermodynamics is recognised as a useful tool in understanding microbial growth and has been applied by many authors (Roels, 1983; Battley, 1998; Tijhuis, van Loosdrecht, and Heijnen, 1993; Nielsen and Villadsen, 1994; Schill, Liu, and von Stockar, 1999; von Stockar and Liu, 1999; Liu, Marison, and von Stockar, 2001; von Stockar, Maskow, Liu, Marison, and Patino, 2006). An important aspect to thermodynamics is the formation properties of the individual components. As a result, different correlations for estimating thermodynamic parameters for the stoichiometry of microbial growth processes have been proposed.

The enthalpy of formation of bacterial cells is typically based on the enthalpy of combustion of cells. Gurakan, Marison, von Stockar, Gustafsson, and Gnaiger (1990) proposed a standard method for determining the elemental composition and enthalpy of combustion of biological material. The method is based on freeze-dried (lyophilized) cells. The elemental composition of cells was measured in a CHN-analyzer with the oxygen composition being assumed to make up the remaining mass of the cells. The enthalpy of combustion was measured using adiabatic combustion calorimetry. This method has been applied by other authors (Liu et al., 2001; Powers, Howell, and Vacinek, 1973). Battley (1998) determined the enthalpy of formation of yeast using combustion calorimety. The work by Battley (1998) included the elements potassium, sulphur, phosphorous, magnesium and calcium which then accounts for 99.97% of the dry mass of the yeast. The experimental results by Battley (1998) again are based on lyophilized cells. Roels (1980) used combustion data from Morowitz (1968) to calculate the enthalpy of formation of biomass.

Roels (1983) proposed a correlation for the enthalpy of combustion of organic compounds based on their degrees of reductance. The degree of reductance is the number of electrons available to transfer to oxygen during combustion. Roels (1983) showed that there is a proportional relationship between the enthalpy of combustion and the degree of reductance which was then extended to biomass.

The entropy and the Gibbs free energy of formation of the biomass are more difficult to measure. A statistical thermodynamics method was used by Grosz and Stephanopoulos (1983) to calculate the entropy of biomass. This method predicts the entropy of *E.coli* based on the partition function which considers acoustic and optical vibrations. The calculations in this work were based on the composition on dry biomass.

Similarly to methods for the enthalpy of combustion, Roels (1983) applied the correlation of the degree of reductance to Gibbs free energy of combustion of organic products. This method showed that there is a linear relationship between the degree of reductance and the Gibbs free energy of combustion.

Battley (1999) applied a correlation, similar to Thornton's rule (Thornton, 1917), to determine the entropy of formation of the cells. Battley (1999) showed that there is a good correlation between the entropy of formation of an organic product and the sum of the entropies of individual atoms of that compound. This method was applied to determining the entropy of formation of the bacterial cells.

Battley (1992), in another article, discussed two methods of calculating the Gibbs free energy of combustion for organic products. The first method used combustion data and showed that there is a relationship between the Gibbs free energy change and the available electrons. This method was applied to biomass after first accounting for the ash content. The second method by Battley (1992) assumes that the Gibbs free energy of combustion is 2.4% less than the enthalpy of combustion. Using data for the enthalpy of combustion of cells the Gibbs free energy of combustion can be calculated.

The only experimentally based method for determining the entropy of the cells was carried out by Battley, Putnam, and Boerio-Goates (1997). In this work low temperature heat capacity measurements were used to calculate the absolute entropy of yeast cells. The yeast cells were first lyophilized before the heat capacity measurements were taken from 7K up to 300K. After the heat capacity measurements the yeast cells were reconstituted and grown to test for viable cells. Only 2% of the cells were viable, however these cells responded similary to normal yeast cells. The heat capacity of the cells between 0K and 7K was assumed to follow a third power relationship ($C_p = aT^3$). The integral with respect to temperature of the heat capacity over the temperature ($S = \Delta Cp/TdT$) from 0K to 298K gives the absolute entropy of the lyophilized cells. This data was used with the molar entropies of the elements to calculate the entropy of formation of the cells.

The enthalpy, entropy and Gibbs free energy are generally based on dried or freeze-dried cells. Several papers (Battley, 1999, 1992; Battley et al., 1997) consider the hydration term but conclude that the formation properties are not significantly affected by it. The problem with all the methods used to calculate

formation properties is that they are based on forms of biomass that are not the same as the biomass in the system being studies. Firstly the biomess is always dried or freeze-dried and therefore does not account for the associated water in the formula. The hydration term is used to account for this however after hydration only 2% of the cells are viable (Battley et al., 1997) which that this process damages this cells and may not have the same structure after testing. Secondly, since the biomass does have water associated with it, the formation properties would have to include not only the hydration terms, but the formation properties of the water as well.

Integrating mass and thermodynamic properties is a useful tool in defining targets and limits for chemical processes (Patel, Hildebrandt, Glasser, and Hausberger, 2007; Glasser, Hildebrandt, Hausberger, Patel, and Glasser, 2009; Hildebrandt, Glasser, Hausberger, Patel, and Glasser, 2009; Patel, Hildebrandt, and Glasser, 2010; Patel, Hildebrandt, Glasser, and Hausberger, 2005). Unlike in chemical processes, where thermodynamic analysis has been used extensively to optimise and improve on processes (Bejan, 1996; Lems, van der Kooi, and de Swaan, 2003), the same is yet to be done on bioprocesses (von Stockar et al., 2006). Lems et al. (2003) used non-linear irreversible thermodynamics and chemical kinetics to optimise the performance of chemical energy transfer. In microorganisms the second law has been used to analyse microbial growth and metabolism (von Stockar and Liu, 1999; von Stockar et al., 2006). These methods can be applied to biological systems, however, the thermodynamic properties of the microorganisms currently available are not suitable for these systems.

We believe one can use an approach for living systems, similar to that of Patel et al. (2007), that allows us to deal with the overall system and not concern ourselves with the detailed internal workings of the cell. This is achieved by looking at the system as a whole, that is only looking at overall inputs and outputs and doing balances on them. In order to do these balances properties such as enthalpy of formation for energy balances and Gibbs free energy of formation for work balances of all the species including the cells are needed. With this very basic information, one can determine the limits

of performance, called targets, for the system. These targets are important for understanding and optimizing systems, as for example by comparing the performance of real processes to the target, it can be seen how much room for improvement there is in the process and therefore what gains it is possible to make. Furthermore one can calculate what would be possible if one changed the substrates or if other products were formed without doing the experiments, a very large saving in work for systems that can theoretically be shown to not be promising.

In this paper we show how these thermodynamic properties can be calculated for living cells from comprehensive experimental product distribution data such as is typically measured for microbiological systems. We will use readily available data from the literature to show how the method works. We will then use these values to show how such data can be used.The important point is that this extra information is available without any further experimentation. Thus if one does not do the analysis that has been done below one is not obtaining all the information that is available from the experimental results, surely a great pity.

2.2 Experimental Results

A chemostat is a continuous bioreactor maintained at steady-state (within the accuracy of the control system). The chemostat is continuously fed fresh media. The liquid volume of the chemostat is maintained by drawing off culture liquid. If there are gaseous products produced by the culture then a gas stream must also be removed in order to maintain the pressure. In order to get a complete understanding of the chemostat all the streams entering and leaving should be measured (i.e flowrate) and analysed (i.e. composition). The liquid stream should be analysed not only for the main product but also for any other potential products produced by the culture. Gaseous products such as carbon dioxide, hydrogen and methane are often produced by microorganisms and as such it should be expected that there is a gaseous product from a chemostat.

All this information is useful for analysing the mass balance over the chemostat and understanding the system being studied.

The data in Table 2.1. was chosen as it shows experimental data which is has quantified all the streams which are leaving the chemostat. This information is thus useful for further analysis. The data was generated by the steady state experimental measurements in a chemostat by Collet et al. (2004), reported for continuous hydrogen production from lactose. According to the authors, a constant 29mmol/l of lactose was fed at different dilution rates to a 2 litre chemostat with the liquid volume maintained at 1 litre and the temperature at 58°C. The bacteria grown in the chemostat was *Clostridium Thermolacticum*. The authors measured the different product spectra at different dilution rates as shown in Table 2.1.

Parameters									
Dilution rate (h^{-1})	0.013	0.028	0.04	0.058	0.082	0.105	0.13	0.15	0.19
Cell dry weight (g/l)	0.60	0.70	0.67	0.58	0.48	0.47	0.41	0.39	0.27
Lactose rem (mmol/l)	1	3	7	12	16	19	21	23	26
Acetate (mmol/l)	28	26	25	21	16	13	10	8	4
Ethanol (mmol/l)	41	36	33	26	20	17	13	10	5
Lactate (mmol/l)	22	17	8	1	1	1	0	0	0
Hydrogen (mmol/l)	65	78	61	44	30	22	17	14	8
Total CO_2 (mmol/l)	94	93	82	65	48	43	31	27	18
Carbon balance %	99	98	97	97	97	100	100	101	101
Yield H_2 on lactose (mmol/mmol)	2.3	3.0	2.8	2.7	2.4	2.1	2.1	2.3	2.5

Table 2.1: Steady state metabolites concentration for continuous cultures at pH 7 and 29 mmol lactose feed (Collet et al., 2004)

An important question one can ask is; has the maximum amount of information been extracted from the data? Experiments are difficult and take a long time, calculations are quick and relatively easy! We will show how this data can be used to obtain the required thermodynamic properties of the living cells. A characteristic of our approach is that only the pure inputs to and outputs from the system, as shown in Figure 2.1, are considered. In Figure 2.1 the experimental measurements are taken around the chemostat as shown by the dashed line. The experimental data is therefore based on the mixed streams entering and exiting the system. The approach in this work is based on the pure inputs and outputs of the system as shown by the solid boundary in Figure 2.1. These pure compounds are considered to be at their standard states and standard temperature and pressure. Cells are also considered as a bag

of chemicals having fixed overall properties. This choice of system boundary does not require an understanding of the details occurring inside the system, such as the biological pathways. The choice of boundary based on the pure compounds allows us to ignore the mixing terms that would be required by boundary for the experimental data.

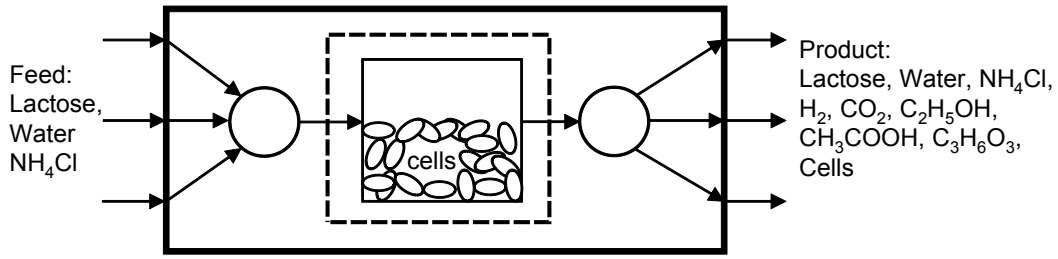

Figure 2.1: Schematic diagram of the system used in this analysis. The boundary of the experimental measurements is depicted by the dashed line. The boundary of the analysis in this work is defined by the thick, solid line. We consider all the components to be entering the system as pure compounds at there standard state of 25°C and 1 atm. The cells are considered as if they are a single species.

2.3 Mass Balance Analysis

The mass balance is a fundamental conservation law and is an important aspect of any chemical system. In particular, the correct mass flows into and out of the system are necessary to perform further, more detailed, calculations.

We first note that the authors have done a carbon balance on their results and based on this they confidently state they have measured all the carbon based species entering and leaving the system. However we wish to go further than this. We need to look at the overall mass balance, as in order to do the analysis we envisage we need to know all the quantities of all the species entering and leaving the system.

In Figure 2.2A we compare the mass flowrate of products (including the dry biomass) leaving the system to the mass flowrate of reactants or inputs to

the system. The mass balance on the system shows that, although there is a fairly good carbon balance across the process, there is a greater mass flowing out of the reactor than flowing in (Figure 2.2A). This suggests that there is a least one extra reactant which has not been measured. Since the carbon entering and leaving the system is accounted for the additional mass must be found in the other elements in the system. In the same manner as the carbon balance a hydrogen and oxygen balance can be performed as shown in Figure 2.3.

Nitrogen is not shown in Figure 2.3 as Collet et al. (2004) do not include nitrogen containing species in their analysis except for biomass. If nitrogen was not included in the biomass formula this could affect the calculation for all the elements since nitrogen can account for 12% of the mass. Private communication with Collet et al. (2004) revealed they had included nitrogen by using the chemical formula $C_5H_7NO_2$ for the bacteria. An elemental composition similar to that of $C_5H_{8.75}N_{0.75}O_{0.6}$ (von Stockar and Liu, 1999) is more commonly used while other authors such as (Akutsu, Li, Tandukar, Kubota, and Harada, 2008) have used $C_5H_7NO_2$. Either formula could have been used in the methods proposed in this work. The overall results would be similar and the methodology would remain unchanged. In fact it would be more accurate to experimentally determine the composition of the cells after each experiment. However, the additional testing is not always possible or practical hence the need for average biomass compositions and elemental balances over the system.

The elemental balance in Figure 2.3 shows that there is unaccounted for hydrogen and oxygen leaving the system. This shows that even with a good carbon balance the other elemental balances are important checks for the system. To include hydrogen and oxygen in the analysis we need to identify all the components in the system, including the components not containing nitrogen. The nitrogen source in the media was ammonium chloride (Talabardon, Schwitzguebel, and Peringer, 2000). The ammonium chloride introduces chlorine into the system. Chlorine is only a trace compound in the biomass and therefore most of the chlorine remains unconsumed. We will assume that no chlorine is consumed and it all remains unreacted. The other major component

in the system is water. Since biological systems are dilute it is impractical to measure the water accurately enough to account for any consumption or production. This further shows the importance of performing other elemental balances (hydrogen, oxygen and nitrogen) on experimental data.

The mass of the cells exiting the system are determined on a dry weight basis. Since we are analysing the bioreactor we are not dealing with dry biomass but rather living cells which contain water. A typical living cell contains 70% water by mass. The empirical formula for dry biomass is as used by Collet et al. (2004) is $C_5H_7NO_2$ and the extra water is included by means of a hydration term which results in the formula of $C_5H_7NO_2 \cdot 15\,H_2O$.

What we now do is write an independent set of mass balances for the system. The mass balances (Equations (2.1) - (2.5)) can be written for each of the measured species produced from the lactose. The enthalpy and Gibss free energy of formation for the mass balances will be discussed later with Equations (2.1) - (2.4) being calculated from standard data and Equation (2.5) calculated from results of this work. Note that these are just mass balances and do not imply that these are reactions that are actually occurring. The mass balances can be in any form as long as it can be shown that the mass balances are independent. An alternative approach to defining system mass balances is creating composition matrices as discussed by Roels (1983). The matrix approach is a comprehensive method which can easily handle large numbers of components. The results of the two methods are fundamentally the same. However, it was chosen that each mass balances would be defined for one product with lactose as the reactant to be representative of the system. The use of individual mass balances becomes useful when considering multiple products and their effect on the overall system.

The mass balances for the system (Equations (2.1) - (2.5)) show that water will be consumed in all cases. From these equations it is clear that the amount of water involved in these mass balances is quite large. Now as all the species involved are in quite dilute solution it will be virtually impossible to measure the amount of water consumed, the values will need to be calculated based on

these independent mass balances.

	ΔG_{rxn}	ΔH_{rxn}	
$C_{12}H_{22}O_{11\,(aq)} + 13\,H_2O_{(l)} \longrightarrow 12\,CO_{2\,(g)} + 24\,H_{2\,(g)}$	-81.55	1226.8	(2.1)
$C_{12}H_{22}O_{11\,(aq)} + H_2O_{(l)} \longrightarrow 4\,C_2H_6O_{(l)} + 4\,CO_{2\,(g)}$	-499.48	-208.33	(2.2)
$C_{12}H_{22}O_{11\,(aq)} + H_2O_{(l)} \longrightarrow 4\,C_3H_6O_{3\,(l)}$	-262.36	-227.65	(2.3)
$C_{12}H_{22}O_{11\,(aq)} + H_2O_{(l)} \longrightarrow 6\,CH_3COOH_{(l)}$	-411.34	-397.15	(2.4)
$C_{12}H_{22}O_{11\,(aq)} + 2\cdot4\,NH_4Cl_{(aq)} + 29\cdot8\,H_2O_{(l)} \longrightarrow$ $2.4C_5H_7NO_2{\cdot}15\,H_2O_{(s)} + 2.4HCl_{(aq)}$	517.89	432.54	(2.5)

Collet et al. (2004) measured the volumetric flowrate of the gases out of the system as well as measuring the gas composition using a gas chromatograph. These results showed that the gas consisted of only carbon dioxide and hydrogen. The gas composition shows that the hydrogen and carbon dioxide partial pressures sum to 100kPa. This shows that water in the gas leaving the system has not been accounted for. At 58°C the vapour pressure of water is 18kPa which shows that approximately 18% of the gas would be water. The water in the gas would affect the calculation of molar flowrates of hydrogen and carbon dioxide by decreasing their compositions in the gas. The decrease in the gas would reduce the molar flowrates of hydrogen and carbon dioxide leaving the system. Furthermore the water in the gas would change the partial pressure of the carbon dioxide which was used to estimate the aqueous carbon dioxide. The reduced partial pressure would reduced the amount of dissolved carbon dioxide from the henry's law. A lower dissolved carbon dioxide would also cause a lower bicarbonate concentration. The lower carbon dioxide in both the gas stream and liquid streams would decrease the carbon balance which lies between 97% and 101%. The gas measurement and the unaccounted water therefore are a potential causes for experimental error resulting in the unbalanced mass balance.

To calculate the water and ammonium chloride consumed in the reactor

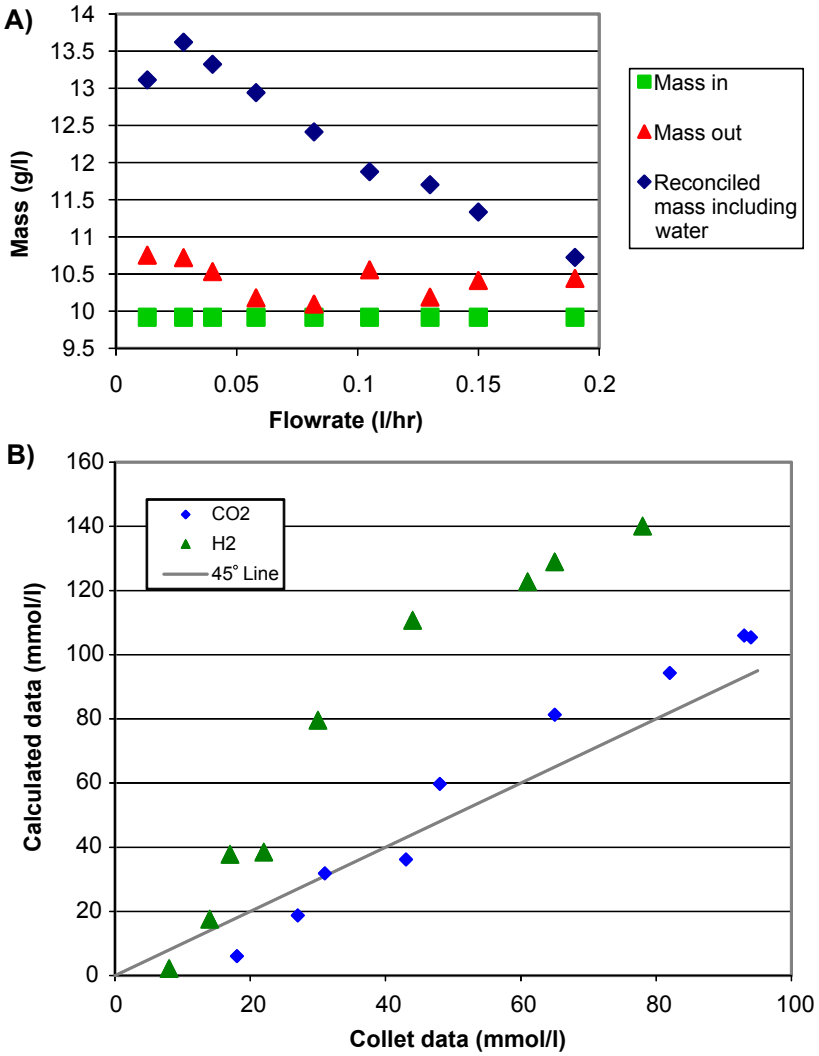

Figure 2.2: **(A)** Plot of the total mass per litre entering and leaving the reactor, based on the results in Table 1, against flowrate. Reconciled mass balance refers to the total mass entering the system and the total mass leaving the system, including reacting water, after reconciliation has been done. **(B)** Predicted carbon dioxide and hydrogen concentration in Table 2.2. versus that given by Collet et al. (2004) in Table 2.1.

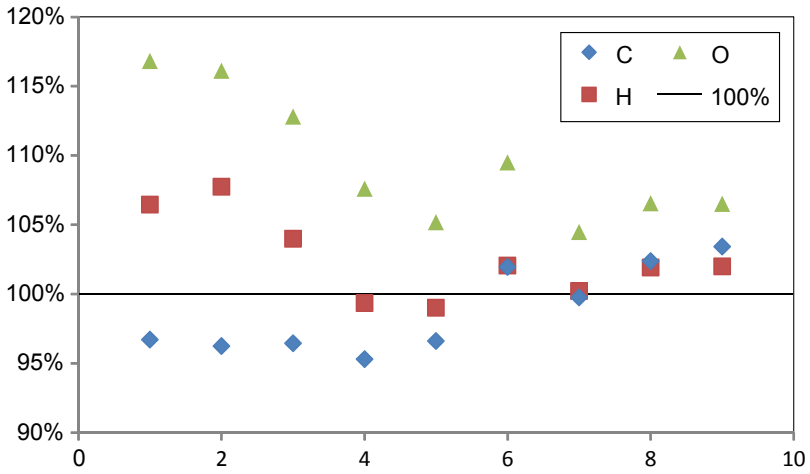

Figure 2.3: Elemental balance (moles out/moles in) around the reactor using the data from Collet et al. (2004) in Table 2.1.

we need to determine the extents of each of the mass balances. We can define molar extents of reaction e_i in terms of molar flowrates n_i for component i, for each of the mass balances given in Equations (2.2) to (2.5). As can be seen from Equations (2.6) - (2.9) the extents of Equations (2.2) to (2.5) can be determined from the measured concentrations of ethanol, lactate, acetate and cells respectively. The extent of Equation (2.10) was not calculated from the carbon dioxide or hydrogen measurements because of the uncertainty with the measurement of gas flowrates and composition. However there is a piece of information that has not yet been used. The consumed lactose not accounted for in Equations (2.2) to (2.5), must have been consumed in Equation (2.1). The extent of Equation (2.1) can thus be calculated from the consumed lactose as shown in Equation (2.10). These extents can be calculated as follows:

$$e_2 = n_{\text{ethanol}}/4 \tag{2.6}$$

$$e_3 = n_{\text{lactate}}/4 \tag{2.7}$$

$$e_4 = n_{\text{acetate}}/6 \tag{2.8}$$

$$e_5 = n_{\text{cells}}/2.4 \tag{2.9}$$

$$e_1 = (n_{\text{lactose in}} - n_{\text{lactose out}}) \cdot (e_2 + e_3 + e_4 + e_5) \tag{2.10}$$

A reconciled mass balanced can be calculated from the extents of Equations (2.1) to (2.5). Table 2.2 shows the data for the reconciled mass balance on a

molar flow basis. Appendix A contains the reconciled mass balance on a liquid volume and a molar flow basis for comparison to the data by Collet et al. (2004). Figure 2.2. shows the reconciled mass balance.

Flowrate (l/hr)	0.013	0.028	0.04	0.058	0.082	0.105	0.13	0.15	0.19
Mols In									
Lactose (mmol/hr)	0.38	0.81	1.16	1.68	2.38	3.05	3.77	4.35	5.51
NH_4Cl (mmol/hr)	0.61	1.31	1.87	2.71	3.83	4.91	6.07	7.01	8.88
Total Water (mmol/hr)	722	1556	2222	3222	4556	5833	7222	8333	10556
Water Consumed (mmol/l)	2.0	4.8	6.2	7.8	8.5	8.3	9.2	8.4	6.2
Mols Out									
Lactose (mmol/hr)	0.013	0.084	0.280	0.696	1.312	1.995	2.730	3.450	4.940
NH_4Cl (mmol/hr)	0.54	1.13	1.63	2.41	3.48	4.47	5.60	6.49	8.42
Total Water (mmol/hr)	720	1551	2216	3214	4547	5825	7213	8325	10549
CO_2 (mmol/hr)	1.37	2.97	3.77	4.72	4.90	3.80	4.14	2.81	1.15
H_2 (mmol/hr)	1.68	3.92	4.91	6.42	6.52	4.03	4.90	2.62	0.40
Ethanol (mmol/hr)	0.53	1.01	1.32	1.51	1.64	1.79	1.69	1.50	0.95
Acetate (mmol/hr)	0.36	0.73	1.00	1.22	1.31	1.37	1.30	1.20	0.76
Lactate (mmol/hr)	0.29	0.48	0.32	0.06	0.08	0.11	0.00	0.00	0.00
Cells (mmol/hr)	0.07	0.17	0.24	0.30	0.35	0.44	0.47	0.52	0.45
HCl (mmol/hr)	0.07	0.17	0.24	0.30	0.35	0.44	0.47	0.52	0.45

Table 2.2: Reconciled mass balance on reactor. Total water is the water entering or leaving the system as media. Consumed water shows the amount of water entering the system which takes part in the chemical reactions

To show the effect of our assumptions we plot in Figure 2.2B. the carbon dioxide and hydrogen we predict in Table 2.2. versus that given in Table 2.1. We can see that the carbon dioxide results agree fairly well, while those for hydrogen are different. This shows that, firstly, the new results do not affect the overall carbon balance and secondly that, this hydrogen discrepancy will not affect the subsequent calculations of the thermodynamic properties because gaseous hydrogen as an element in its standard state has zero values for its properties of formation.

2.4 Thermodynamics of a Cell

We can regard a cell that is in our chemostat (reactor) as a small chemical factory that takes a substrate such as lactose and converts it into products such as those in Table 2.1. The energy effects of this (Enthalpy) and how efficiently from a thermodynamic process (loss in entropy or gain in Gibbs free energy) they do this we do not know. However the Enthalpy and the

loss in work potential (Gibbs free energy) of these factories will depend on the number of cells in the chemostat, namely proportional to the concentration of cells (C_{cells}).

The new cells that are made and swept out of the chemostat can be thought of as bags of chemicals and so can be regarded as having an Enthalpy and Gibbs free energy of formation. The total amount of these quantities will be proportional to the number of cells made and swept out the chemostat. As the chemostat can be regarded as a perfectly stirred vessel this value will be proportional to the molar flow-rate of cells (concentration of cells times the flow-rate (F)).

As the experimental results cover a range of both cell concentrations and flow-rates one can in principle regress on both sets of quantities. These ideas will be used in what follows.

2.5 Estimation of $H_{\mathbf{f,cell}}$

With the assumptions of no shaft work (W_S) and ideal mixing (i.e. $H_{\text{mixing}} = 0$) and for a steady state constant pressure process, the energy balance equation reduces to:

$$H_{\text{out}} - H_{\text{in}} = -Q_{\text{lost}} \tag{2.11}$$

Where

H_{out} - Total enthalpy leaving the system

H_{in} - Total enthalpy entering the system

Q_{lost} - Heat loss from the system

As per Figure 2.1 the inlet and outlet enthalpies can be calculated from the individual components and equation (2.11) can be written more specifically as:

$$\left[\sum_i H_{f,i}\right]_{\text{out}} - \left[\sum_i H_{f,i}\right]_{\text{in}} = -Q_{\text{lost}} \qquad i = 1, ..., n \tag{2.12}$$

Where

$H_{f,i}$ - The enthalpy for each component entering or leaving the system

The calculation of the inlet stream enthalpy is fairly straight-forward as it contains only well known chemical species. The outlet stream, however, contains bacterial cells which will carry an enthalpy with them. It is proposed that an enthalpy of formation (per mole) can be assigned to the cells, which is denoted as $H_{\text{f,cell}}$. The primary heat loss is from the reactor being maintained isothermally but this will be supplied by the external heater so it need not be included in the energy balance. The heat production of the cells to keep them viable (the enthalpy of the maintenance requirements of the cells, H_{m}) must be included with this heat lost as Q_{lost}. This will clearly be proportional to the amount of cells in the reactor. Thus we can write $Q_{\text{lost}} = H_m C_{\text{cells}} V$. The energy balance for the data by Collet et al. (Collet et al., 2004) can be expanded and rewritten to give

$$\left(\left[\sum_i H_{f,i}\right]_{\substack{\text{out}\\ \text{excluding}\\ \text{cells}}} - \left[\sum_i H_{f,i}\right]_{\substack{\text{in}\\ \text{excluding}\\ \text{cells}}}\right) / C_{\text{cells}} = -H_{\text{f,cell}} F - H_{\text{m}} V$$
$$i = 1, ..., n-1 \tag{2.13}$$

Where

C_{cells} - Concentration of cells leaving the system

F - Flowrate out of the chemostat

H_{m} - Enthalpy of maintenance requirements for the cells

V - Volume of the system

The left side of equation (2.13) can be plotted against the flowrate of the liquid. If Equation (2.13) is a reasonably good model, this should give a straight line with the gradient being the enthalpy of formation of the bacterial cells and the intercept H_m. The reconciled data previously calculated is used

with literature values of the Enthalpies of formation of the species (Alberty (1998) at 298.15K, 1 bar) to give Figure 2.4A . Note the reconciled data allows us to include the water consumed in the reactor.

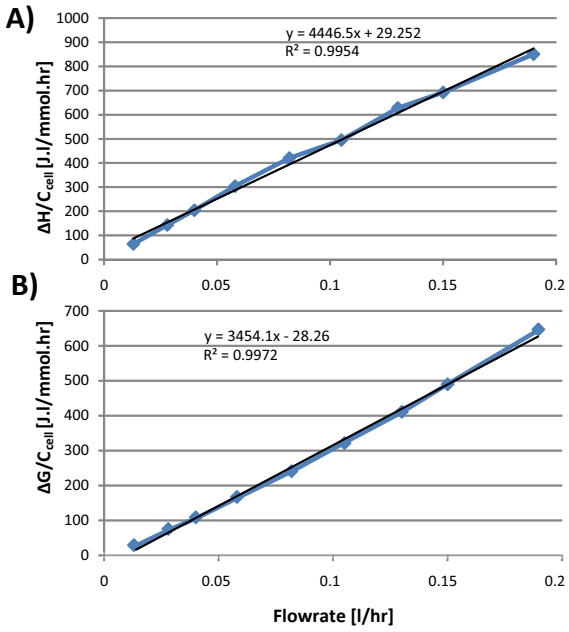

Figure 2.4: Estimation of the thermodynamic properties of the cells **(A)** Plot of left hand side of Equation (2.13) versus flowrate used to estimate $H_{f,cell}$ **(B)** Plot of left hand side of Equation (2.16) versus flowrate to estimate $G_{\mathrm{f,cell}}$

A simple linear regression shows that a straight line fits fairly well with the data in Figure 2.4A. This shows that Equation (2.13) is a reasonable model of the system though there does seem to be a slight curvature. The slight curvature shows that even for a good model there may be parameters that have been omitted. Given the goodness of fit for the data in Figure 2.4 this model will give us the information that we require. If a higher degree of accuracy is required, more parameters could be included in the analysis. Additional parameters would increase the time and potentially the amount of data required to get the desired answer.

The negative of the gradient calculated by the simple linear regression gives the enthalpy of formation of the cells and the associated variance as -4447 ± 114kJ/mol. This is the enthalpy of formation of the bacterial cells including any associated water with it. The enthalpy of formation can be

used to calculate the enthalpy of combustion of cells. This typically how the enthalpy and Gibbs free energy of biomass is quoted (Roels, 1983). Table 2.3 shows data for the enthalpy of combustion for various methods including this work. From Table 2.3 it can be seen that this method shows good correlation to other works matching the results by Morowitz (1968) and Roels (1983). In a similar manner the intercept calculated by the simple linear regression gives us the enthalpy of maintenance of the cells (H_m) as -29 ± 12kJ/kmol.L. We will discuss these values at a later stage.

Method	**Cell formula**	**-Gibbs free energy of combustion (kJ/C-mol)**	**-Enthalpy of combustion (kJ/C-mol)**	**Author**
Statistical thermodynamics	$CH_{1.8}O_{0.5}N_{0.2}$	541.2	560	Morowitz (1968)
Statistical mechanics	$CH_{1.77}O_{0.49}N_{0.24}$	553-563	—	Grosz and Stephanopoulos (1983)
Thermodynamics	$CH_{1.59}O_{0.374}N_{0.263}$	527.6	519.02	Battley (1993)
Low-temperature calorimitry	$CH_{1.613}O_{0.557}N_{0.158}$	515	503.893	Battley et al. (1997)
Roels' correlation	$CH_{1.613}O_{0.557}N_{0.158}$	541.2	560	Roels (1983)
Battley's empirical method	$CH_{1.613}O_{0.557}N_{0.158}$	515.2	509.37	Battley et al. (1997)
	$CH_{1.4}O_{0.4}N_{0.2} \cdot 3H_2O$	581.1	561.8	This work

Table 2.3: Comparison of enthalpy and Gibbs free energy of combustion of biomass to other published work. (Table originally produced by von Stockar and Liu (1999) and updated to include enthalpies of combustion and this work

2.6 Estimation of $G_{\mathbf{f,cell}}$

In exactly analogous way as for the energy balance we can write a Gibbs free energy balance, namely

$$G_{\text{out}} - G_{\text{in}} = -G_{\text{lost}} \tag{2.14}$$

Where

G_{out} - Total Gibbs free energy leaving the system

G_{in} - Total Gibbs free energy entering the system

G_{lost} - Gibbs free energy lost by the system as an irreversibility

As in the energy balance above we assumed a steady state process with no

shaft work and ideal mixing. The Gibbs free energy of mixing for each phase entering or leaving the system is given by:

$$G_{\text{mixing}} = \sum_i n_i RTln(x_i) \tag{2.15}$$

By taking all the unknowns to the right hand side the Gibbs free energy balance gives:

$$\left(\left[\sum_i G_{f,i} \right]_{\substack{\text{out} \\ \text{excluding} \\ \text{cells}}} + G_{\text{mixing,out}} - \left[\sum_i G_{f,i} \right]_{\substack{\text{in} \\ \text{excluding} \\ \text{cells}}} - G_{\text{mixing, in}} \right) / C_{\text{cells}}$$
$$= -G_{\text{f,cell}} F - G_{\text{m}} V \qquad i = 1, ..., n-1 \tag{2.16}$$

Where

$G_{f,i}$ - The Gibbs free energy for each component entering or leaving the system

G_{m} - Gibbs free energy of maintenance requirements for the cells

G_{mixing} - Gibbs free energy of mixing

The Gibbs free energy of formation of products and reactants was again obtained from Alberty (Alberty, 1998). A plot of the left hand side of equation against the molar flowrate of cells is shown in Figure 2.4B. A simple linear regression again shows that a straight line is a good fit for the data. From the gradient and intercept calculated by the simple linear regression the Gibbs free energy of formation and Gibbs free energy of maintenance of the living cells is -3454 ± 69kJ/mol and 28 ± 7kJ/kmol.L respectively. The fit of the enthalpy plot is marginally better than the fit for Gibbs free energy though they have the virtually the same R^2 value. The data in Figure 2.4B shows a slight curvature as before, this shows that additional parameters would be required to achieve a better fit. As with the enthalpy calculation the goodness of fit shows that the model will give us the data we require with a reasonable degree of accuracy.

The data for the Gibbs free energy of combustion of biomass determined by

various methods is shown in Table 2.3. The Gibbs free energy of combustion for this work is approximately 10% higher than other methods used. Gibbs free energy is difficult to calculate as there are no direct methods for its determination. The methods used in Table 2.3 are based on measurements and calculations of dry biomass. The results in this work have treated the biomass as a living organism as it is found in the bioreactor. The differences in the Gibss free energy could be accounted for by the hydration of the cells. If the hydration term is related to the Gibbs free energy of mixing which can be estimated using ideal mixing ($RTx_i\ln(x_i)$). The ideal mixing term is 2.8kJ/mol which would account for some of the difference. The main cause for the difference could due to the differences in the states of the cells of the different methods and the different empirical formulae used. The other methods used also do not consider the effects of creating concentration gradients within the cells.

2.7 Application to Biochemical Reactions

We have developed a general method to look at complex systems using a G-H diagram as shown in Figure 2.5. This is particularly useful for biological reactions where many species may be produced. The strength of this plot is that no matter what the number of the number of species involved the diagram is two-dimensional. The labelled "vertices" (we think by chance the ethanol and the lactate vertices appear to lie on other straight lines) on the graph represents the complete conversion of 1 mole of lactose to that species and the line from the origin to this point has a length proportion to the extent of that mass balance. Thus a point on this line but closer to the origin will have a smaller extent proportional to the length of the line. The convex hull of all the points for all the species represents the mass balance feasible region. Any point within the region thus can be represented by a linear combination of all the vertices and thus an infinity of constrained mass balances. In this case however when we take into account the maintenance term the resultant must lie at $\Delta H = 0$ and $\Delta G = 0$ and thus we can in principle calculate all product

distributions that are feasible by taking the appropriate vector sums.

From Figure 2.5 it can be seen that we cannot only make cells. The production of only cells requires both work and heat input into the system. The heat could be supplied by an external source but the work is only supplied by the chemical potential of the products. Therefore in order to produce cells the microorganism must produce liquid products. Figure 2.5 shows that making acetate is the most irreversible product on a per mole of lactose basis. In other words producing acetate supplies the microorganism with most work potential.

There are many other ways in which Figure 2.5 may be used but we will not go into more details in this paper.

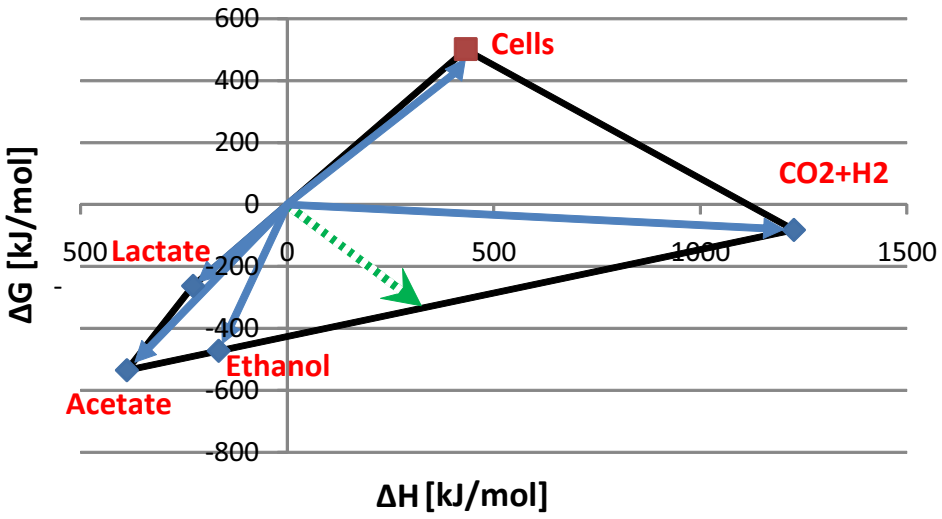

Figure 2.5: G-H diagram for for lactose fermentation, reactions (2.1) - (2.5), per mole of lactose consumed. The associated maintenance vector (dashed arrow) is also plotted

2.8 Conclusion

From fundamental calculations of energy and entropy balances using only mass balances, the estimation of some thermodynamic properties ($G_{\mathrm{f,cell}}$, $H_{\mathrm{f,cell}}$, G_m, H_m) of a living cell from reported experimental data is shown to be possible. It must be emphasised that these predicted properties are valid for living cells

as they would be found in a biological system. Obviously the experimental data must have a reasonable degree of accuracy in order to obtain reasonable results. It is possible, however, to reconcile the data to improve its reliability.

It was shown how these values could be used in a G-H plot that we have developed to get valuable information about the limits of performance of the bacterial system.This approach allows us to calculate a thermodynamically feasible region in which microorganisms can grow. This data allows us to determine what products and product limits are achievable. This information gives a better understanding of bioprocesses, such as how close to the thermodynamic limit the process is operating.

While the estimation of thermodynamic properties of $G_{\mathrm{f,cell}}$, $H_{\mathrm{f,cell}}$, G_m and H_m were done for *Clostridium thermolacticum* bacterium the techniques described above can be applied to any microorganism.

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Chapter 3

A Thermodynamic Approach to Predicting the Product Spectrum Of Metabolism In A Maintenance Limited Case

Abstract

Thermodynamics is an important tool for understanding the chemical processes and what compounds they produce. Thermodynamics has also been applied to biological systems in order to understand these complex systems. One application in this area is predicting biomass and fermentation product yields. These methods are typically based on a catabolic and anabolic reaction approach and do not allow for the consideration of multiple products in the system. In this work a new method is proposed for analysing a microbial system with multiple products. The anabolic reaction and all of the catabolic reactions are plotted on a Gibbs free energy-Enthalpy (ΔG-ΔH) diagram which produces an attainable region. The maintenance requirements of the microorganism is considered with respect to the attainable region. The maintenance vector produced is used to predict the fermentation products produced in the maintenance limited case, when no biomass is formed in the system.

3.1 Introduction

Thermodynamics can be applied to chemical processes to calculate the limits of performance as shown by Patel, Hildebrandt, Glasser, and Hausberger (2007). The limits of performance of the process gives a theoretical number to which the performance of real processes can be compared. The work by Patel et al. (2007) illustrates that the thermodynamics can be used to determine the product spectrum likely to be produced by the process. There have been many thermodynamically based methods proposed to determine biomass yield for biological systems (Heijnen and van Dijken, 1992). The products of the biological systems are often overlooked with most analyses considering only one product.

Attempts to predict the biomass yield have been made for many decades. Mayberry, Prochazka, and Payne (1967) grew bacteria on selected organic compounds to determine the important parameters in predicting growth yields.

Growth yields based on the amount of substrate consumed, the amount of carbon consumed, the amount of oxygen consumed and available electrons were tested. The growth yield based on available electrons gave the most consistent results and was chosen as the best parameter to determine the growth yields.

The principle of available electrons is still used in thermodynamic studies of biological systems (Roels, 1983). The analysis of the microbial metabolism is typically broken into catabolic and anabolic reactions. This is done because division of cell behaviour into simple reactions, while generally accurate, hides some of the complexity of the behaviour of the organisms (vanBriesen, 2002). The Gibbs free energy of the equations of the electron acceptor and donor (or anabolic and catabolic reactions) and thus the second law of thermodynamics was used by vanBriesen (2002) to determine the bacterial yield.

Battley (1993) and Battley (1995) looked at the thermodynamics of growth by studying the anabolic and catabolic processes. Glucose, ethanol and acetic acid were used as feeds and the products of the catabolic process were only carbon dioxide and water. The stoichiometries of the reactions were determined experimentally. Battley (1995) also included the use of a non-conservative process which was used as the thermodynamic limit for the microorganisms.

Splitting the metabolic process into anabolism and catabolism was also the approach taken by Schill, Liu, and von Stockar (1999). In this analysis different system boundaries were used for the enthalpy and Gibbs free energy balances. Schill et al. (1999) also looked at the relationship of the biomass yield and the ATP yield. The use of ATP requires an understanding of the metabolic paths of the microorganism.

Heijnen and van Dijken (1992) looked at the required information for a "black box" analysis of biomass yields. Although some of the methods analysed met the requirements, none of the methods offered the opportunity for the determination of product distributions.

Rodriguez, Kleerebezem, Lema, and van Loosdrecht (2006) modelled the

product formation of an anaerobic mixed culture. The model chosen looked at the following multiple products: ethanol, acetate, propionate, butyrate, lactate, hydrogen, carbon dioxide and biomass. The authors used a "grey box" approach and included some biological information in order to reduce the degrees of freedom. Various parameters of the model were set and the model was solved by optimising the biomass yield at those conditions. The results show that carbon dioxide, hydrogen and biomass are always formed. Generally only two liquid products were formed and were primarily acetate, butyrate or ethanol.

The use of the catabolic and anabolic reactions is not limited to a single fermentation product. A multiple product approach can be combined with the anabolic and catabolic processes without requiring detailed biological information and thus remaining a "black box" approach. This information can be used to understand what products are likely to be formed.

3.2 Process Synthesis

Process synthesis uses three tools. The first two of these are mass and energy balances which are based on conservation laws. The third tool is entropy which is an inequality as defined by the second law of thermodynamics. As shown by Patel et al. (2007) these three tools can be used to set targets for the system and define the limits of performance by considering a reversible process. The mass, heat and work integration showed that a non-spontaneous reaction can be coupled with a spontaneous reaction. The spontaneous reaction supplies work and energy to the non-spontaneous reaction and enables the non-spontaneous reaction to proceed.

The enthalpies and Gibbs free energies of reaction can be calculated for a given reaction or mass balance. Sempuga, Hausberger, Patel, Hildebrandt, and Glasser (2010) showed that the data can be plotted on Cartesian coordinates with enthalpy on the x-axis and Gibbs free energy on the y-axis as shown in

Figure 3.1. In this way multiple, related reactions can be analysed together. This type of plot is useful in understanding the heat and work requirements of the reactions or mass balances under consideration. As shown by Patel, Hildebrandt, and Glasser (2010) the Carnot temperature is related to the enthalpy and Gibbs free energy of reaction and therefore that information could be extracted from a plot such as Figure 3.1. The heat and work requirements will be the only properties considered in this paper. Figure 3.1 shows the Cartesian coordinates with the classified regions in terms of the heat and work requirements.

The enthalpy of reaction (ΔH) determines the heat requirements of the system. Regions I and IV of Figure 3.1 show where ΔH is positive. This region is endothermic and requires heat input for the process to proceed. Conversely in the regions II and III the process is exothermic. In the exothermic process the heat needs to be rejected to the surroundings. The work requirements of the process are determined by the Gibbs free energy of reaction (ΔG). A positive ΔG shows that the process requires work added to the system to proceed. The endogenic, positive ΔG is found in regions I and II. The exogenic process is thus found in regions III and IV. The negative ΔG behaves slightly differently to the negative ΔH. The enthalpy of the system is based on the conservation of energy and therefore any excess heat must be transferred to the surroundings. The Gibbs free energy is based on the second law of thermodynamics which is an inequality. The negative ΔG shows that the process has the potential to do work, however if the process does not have a means to reject the work to the surroundings the work potential is lost. The lost work manifest as entropy generation and therefore the process becomes irreversible.

The use of thermodynamics has largely been used for analysis of chemical reactions. Thermodynamics is based on the laws of nature and as such does not exclude any system to which is applies. Biological systems are just complicated chemical reactions where the microorganism can be treated as bags of chemicals (Chapter 2). Therefore under any experimental conditions the use of thermodynamics can be applied to biological systems. An important as-

pect for applying thermodynamics is to ensure a system region is well defined. Without a well defined system region it is impossible to account for the heat and work flows in order to apply the laws of thermodynamics.

As discussed by von Stockar and Liu (1999), the high metabolic rate of the microorganism is associated with the maintenance limited case when there is zero yield of biomass. In the case of a theoretical maximum yield of bacteria the metabolic rate becomes zero. The growth of the microorganism is therefore a compromise between these two limiting cases. In order to understand the growth of the microorganism it is important to understand the theoretical limits of growth. In this work the maintenance limited case, when there is no biomass formed, will be analysed.

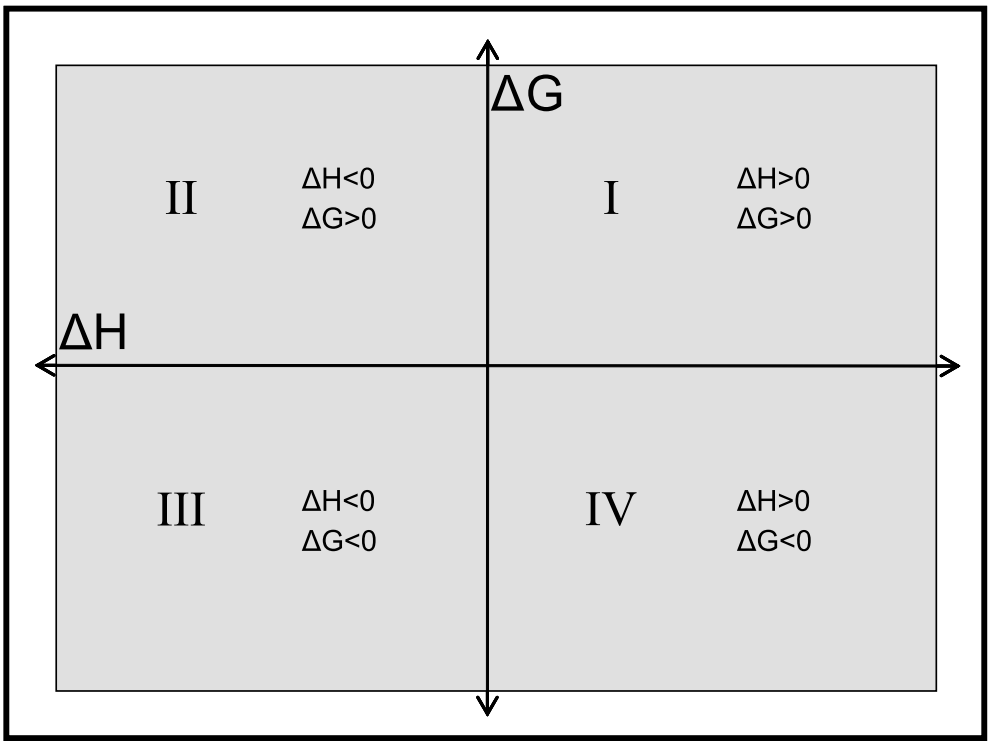

Figure 3.1: Classified regions of heat and work flows on a Gibbs free energy-Enthalpy plot. When $\Delta H > 0$ the system requires heat (regions I and IV) and when $\Delta H < 0$ the system rejects heat (region II and III). The system requires work when $\Delta G > 0$ (region I and II) and the system has the potential to do work when $\Delta G < 0$ (regions III and IV). Regions III and IV are associated with a spontaneous or feasible process

3.3 Fermentation

Fermentation is the process in which an organism breaks down large molecules into smaller, simpler products. Fermentation is most commonly performed on sugars but it is not limited to sugar. Typical fermentation products include carbon dioxide, hydrogen, ethanol and acetate; however many different products can be formed depending on the microorganism used. Equations (3.1) - (3.5) can be used to represent the independent mass balances for fermentation. These mass balances do not show the actual reactions but show the degrees of freedom of the system. The typical fermentation products are represented by Equations (3.1) - (3.4). These reactions are the catabolic reactions and are mainly exothermic and exogenic and supply energy to the organism. Equation (3.5) is the mass balance for the formation of biomass (anabolism). This reaction is endothermic and endogenic and therefore requires energy to proceed. The organism can use Equations (3.1) - (3.4) to supply the energy required to produce biomass. The production of bacterial cells is catabolic and is thermodynamically infeasible as a reaction by itself. The microorganism uses the energy from the anabolic reactions to supply the requirements to the catabolic reaction. The microogorganism can use the anabolic reactions to produce any products that would otherwise be infeasible. The thermodynamic constraint is not on which products can be formed but rather on the relative quantities. If there are no anabolic reactions available then there would be no energy to supply catabolism. In the case of photosynthesis, carbon dioxide and water are reactants. These reactants do not react to produce exothermic and exogenic reactions. In order for a microorganism to use these materials as reactants an alternative, external source of energy is required. In the photosynthesis case the external energy is supplied by light. The enthalpy and Gibbs free energy of the reactions (or more accurately the mass balances) can be calculated using standard thermodynamic formation data. As shown in Chapter 2 the $G_{f,cells}$ and $H_{f,cells}$ can be measured for a microorganism.

	ΔG_{rxn}	ΔH_{rxn}	
$C_{12}H_{22}O_{11\,(aq)} + 13\,H_2O_{(l)} \longrightarrow 12\,CO_{2\,(g)} + 24\,H_{2\,(g)}$	-81.52	1226.75	(3.1)
$C_{12}H_{22}O_{11\,(aq)} + H_2O_{(l)} \longrightarrow 4\,C_2H_6O_{(l)} + 4\,CO_{2\,(g)}$	-499.48	-208.33	(3.2)
$C_{12}H_{22}O_{11\,(aq)} + H_2O_{(l)} \longrightarrow 4\,C_3H_6O_{3\,(l)}$	-262.36	-227.65	(3.3)
$C_{12}H_{22}O_{11\,(aq)} + H_2O_{(l)} \longrightarrow 6\,CH_3COOH_{(l)}$	-411.34	-397.15	(3.4)
$C_{12}H_{22}O_{11\,(aq)} + 2.4NH_4Cl_{(aq)} + 29.8H_2O_{(l)} \longrightarrow$ $2.4C_5H_7NO_2{\cdot}15\,H_2O_{(s)} + 2.4HCl_{(aq)}$	517.89	432.54	(3.5)

3.4 Maintenance Requirements

Microorganisms do not only produce chemicals (the fermentation products and biomass formed) when breaking down the carbon source, they also release energy to or absorb energy from the surroundings. The energy exchange between the microorganism and the surroundings forms part of the maintenance requirements of the microorganism. The energy balance over the system including the maintenance energy is shown in Equation (3.6). Chapter 2 shows a method for calculating the maintenance requirements of a microorganism from experimental data.

$$\Delta H = -H_m C_{cells} V \tag{3.6}$$

Where

ΔH - Overall enthalpy change of the system

H_m - Enthalpy maintenance requirements of the cells

C_{cells} - Concentration of the cells

V - Volume of the system

Similarly to the energy requirements of the cell there are also Gibbs free energy requirements. Equation (3.7) shows the Gibbs free energy balance for the microorganism. The maintenance requirements for the microorganism can also be calculated from experimental data as shown in Chapter 2.

$$\Delta G = -G_m C_{cells} V \tag{3.7}$$

Where

ΔG - Overall Gibbs free energy change of the system

G_m - Gibbs free energy maintenance requirements of the cells

C_{cells} - Concentration of the cells

V - Volume of the system

Although Equations (3.6) and (3.7) are mathematically similar, the nature of the maintenance terms are not. When $\Delta H > 0$ in the energy balance the microorganism can absorb energy from the surroundings whereas this is not possible when $\Delta G > 0$. The microorganism therefore needs to maintain $\Delta G \leq 0$. This is shown by Heijnen and van Dijken (1992) where the Gibbs energy dissipation is negative for chemotrophic growth.

Equation (3.6) and (3.7) can be combined to produce Equation (3.8) in order to represent the maintenance requirements of a reversible process on a ΔG-ΔH diagram. From the equations in Chapter 2 it can be shown that the reversible system follows Equation (3.8). The left hand side is the energy requirements of the system. The right hand side is the energetics of the maintenance of the cells and is defined as the maintenance vector. Equation (3.8) shows that the energy requirements of the reversible process will follow the direction of the maintenance vector.

$$\begin{bmatrix} \Delta G_{overall} \\ \Delta H_{overall} \end{bmatrix} = -K \begin{bmatrix} G_m \\ H_m \end{bmatrix} \tag{3.8}$$

According to Equation (3.8) the system can operate at any point along the maintenance vector. The further the system lies from the origin the larger the effect the maintenance requirements have on the system. The maintenance requirements are limited by the attainable region. At the intersection of the system region and the maintenance vector the system is operating at the maximum maintenance energy requirement or the maintenance limited case. On the level of the microorganism the maintenance limited case is when all the chemical energy from fermentation is being used to keep the microorganism alive. In the maintenance limited case none of the chemical energy is used for

the anabolic reaction and thus there is no new biomass formed.

The maintenance limited case is the primary focus of the work in this chapter. As mentioned above, the maintenance limited case is when the microorganism is only consuming the substrate to produce energy to keep itself alive. None of the energy or material supplied by the substrate is being used to produce new cells. This mode of operation is an unfavourable mode for the microorganism. Without the production of new cells the microorganism would not be able to thrive in the environment and would ultimately be overtaken by another microorganism. This is evident by the all the works in the introduction which only analyse the maximum biomass yields and do not study the case of zero biomass production. The maintenance limited case, however, is important for analysis since is shows the maximum yield of products which the microorganism can theoretically achieve. Since none of the available energy, work, atoms or electrons are being used to produce biomass they are all being used to produce the products. It should be noted that the overall consumption of the substrate would most likely be very different between normal growth and the maintenance limited growth. If the analysis is done relative to the total substrate fed to the system the differences in conversion would be important to include. By looking at the yields per mole of substrate consumed the change in overall yields does not affect the results. As such, using the maintenance limited case gives a useful benchmark for testing the efficiency of a given system.

The data for the maintenance vector in this chapter is taken from Chapter 2. The maintenance vector used in this work does not necessarily apply to all biological systems. Different bacteria under similar conditions could have different maintenance requirements. The same bacteria under different conditions could also have different maintenance requirements depending on their response to changing conditions. In order to determine the effect of these changes a large set of experiments and analyses would have to be performed. Chapter 2 shows one method in which the data for the maintenance vector could be calculated for desired system. The work in this chapter assumes a constant maintenance requirement for the cells.

3.5 Attainable Region

Figure 3.2 shows the Equations (3.1) - (3.5) on the Gibbs free energy-Enthalpy (ΔG-ΔH) plot. As discussed previously, the data points were calculated using only formation properties for each of the components. The use of the formation properties should account for the major terms and give a good understanding of the overall system behaviour. However, Figure 3.2 is not limited to the use of only formation properties. The use of terms, such as mixing terms and non-idealities, can be included to meet the desired accuracy.

For convenience the analysis will consider the consumption of 1 mole of lactose. This does not imply that the microorganism only consumes 1 mole of lactose, rather the results will show what products are produced for every mole of lactose consumed. Similarly it is not implied that the microorganism will consume all of the available lactose. By applying the overall, systems approach only the consumed lactose has an effect on the energetics of the system and the unconsumed lactose leaves the system in the same form as it enters. The extent of Equations (3.1) - (3.5) therefore sum to 1 mole as shown in Equation (3.9). The overall ΔH and ΔG can thus be shown to be given by Equation (3.10) and (3.11) respectively. Given the conditions in Equation (3.9)-(3.11) the data from Equations (3.1) - (3.5) forms a convex hull. The convex hull is the area that contains the set of all the convex combinations for the points in the system. The convex combinations are linear sets of combinations of all the points with all the coefficients great than or equal to zero and where all the coefficients sum to 1 as shown in Equation (3.9). The system region shows the loci of all the Gibbs free energies (ΔG) and enthalpies (ΔH) of the process achievable by linear combinations of the mass balances which consumes 1 mole of lactose. The system region and the convex hull are equivalent and as such the convex hull forms the system region. Figure 3.2 also includes the

maintenance vectors as defined in Equation (3.8).

$$\sum_i e_i = 1 \text{ mol of lactose consumed} \tag{3.9}$$

$$\text{where} \quad e_i \geq 0 \text{ mol of lactose consumed}$$

$$\Delta H_{overall} = \sum_i e_i \Delta H_i \tag{3.10}$$

$$\Delta G_{overall} = \sum_i e_i \Delta G_i \tag{3.11}$$

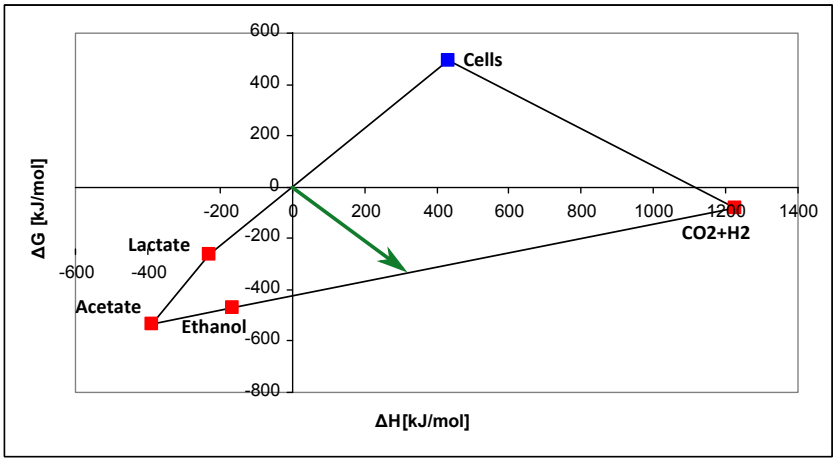

Figure 3.2: System region for the fermentation of lactose with the maintenance vector(solid arrow)

As discussed above, the larger the maintenance energy requirements the less chemical energy there is available for the production of cells through the anabolic process. The direction of the maintenance vector in Figure 3.2 illustrates this effect. The direction of the maintenance vector is directed away from the anabolic reaction.

A linear combination of mass balance can achieve any point within the convex hull, however the microorganism is limited by the energy requirements of growth. The energy requirements as shown in Equation (3.8) can be shown by an enthalpy and Gibbs free energy balance over the microorganism. Equation (3.8) shows that the energy requirements of the microorganism will follow the line defined by the enthalpy of maintenance (H_m) and Gibbs free energy of

maintenance (G_m) of the bacteria with a zero intercept. The line on which the locus of the operating point lies is the maintenance vector. The microorganism can operate at any point along this vector by varying the product spectrum produced based on 1 mole of lactose.

It is important to note that the maintenance vector is not a constant vector and could be calculated for any microorganism, or any consortium of microorganims if required. The work in this paper could therefore be applied to any microorganism if the required information is available.

The locus of the operating point can follow the maintenance vector until it reaches the boundary of the convex hull. In Figure 3.2 the ethanol mass balance lies on the boundary formed by CO_2 and acetate. It is thought that this is a coincidence however it does offer an extra degree of freedom in the limiting case. By tracing the maintenance vector in Figure 3.2 it can be seen that the locus approaches the CO_2-Acetate boundary. This boundary is formed by linear combinations of Equation (3.1) and (3.4). No points along this boundary are associated with the formation of new cells. The intersection of the CO_2-Acetate boundary and the maintenance vector is a unique case where no new biomass is formed. This unique case is the maintenance limited case since the maintenance term is at a maximum. Any further increase in the maintenance term would extend the maintenance vector out of the convex hull. For any point lying outside the convex hull one of the extents (e_i) in Equation (3.9) would have to be negative. A negative extent would imply the product is being consumed, however the feed into the system does not contain any products. Therefore the boundary of the convex hull defines the attainable region and any point outside of the boundary has no real interpretation. The mass balance for the point of intersection between the attainable region boundary and the maintenance vector can be calculated by applying the lever arm rule. Equation (3.12) shows the mass balance associated with this maintenance limited fermentation of lactose.

$$C_{12}H_{22}O_{11\,(aq)} + 6\cdot02\,H_2O_{(l)} \longrightarrow 3\cdot49\,CH_3COOH_{(l)} + 5\cdot02\,CO_{2\,(g)} + 10\cdot05\,H_{2\,(g)} \tag{3.12}$$

Equation (3.12) shows that the maximum acetate that can be produced when no biomass is formed is 3.49mols per mol of lactose. Hydrogen would also be produced at 10.05mols per mol of lactose. As discussed previously, this lactose system has the ethanol mass balance lying on the intersected boundary. This means that instead of using acetate as the product for maintenance limited growth ethanol can be used as the product. The corresponding mass balance is shown in Equation (3.13).

$$C_{12}H_{22}O_{11\,(aq)} + 5\cdot66\,H_2O_{(l)} \longrightarrow 2\cdot45\,C_2H_6O_{(l)} + 7\cdot11\,CO_{2\,(g)} + 9\cdot32\,H_{2\,(g)} \tag{3.13}$$

The maximum amount of ethanol that can be produced is 2.45mols per mol of lactose. In other cases where the boundary is only formed by 2 mass balances there will only be one mass balance associated with the maintenance limited growth. However, in this case the are two independent mass balances associated. These independent mass balances can be combined in any linear combination to produce the same point of intersection. Thus for the case of maintenance limited growth on lactose both ethanol and acetate could theoretically be produced.

The maximum values for ethanol and acetate growth on lactose can be used to show the level of performance of a given system. The results from fermentation are typical based on glucose or quoted on a glucose basis. However, the results discussed above are based on lactose, to convert them to a glucose basis they must be halved since lactose is made up of two monosaccharides. Therefore the maximum ethanol production is 1.23mol/mol of glucose and the maximum acetate production is 1.75mol/mol of glucose. The maximum hydrogen production is 5.03mol/mol of glucose. In calculations done by Rodriguez et al. (2006) they showed that the maximum ethanol production for normal fermentation was 1.1mol/mol of glucose and 3.0mol hydrogen/mol of glucose. At these conditions Rodriguez et al. (2006) achieve 89% of the ethanol limit and 60% of the hydrogen limit. By changing conditions Rodriguez et al. (2006) achieved an acetate yield of 1.6mol/mol of glucose with a hydrogen yield of 3.5mol/mol of glucose. These yields equate to 91% acetate and 70% hydrogen.

In both cases the "loss" of efficiency is a result of the energy and mass being used by the microorganism to produce biomass.

3.6 Additional Products

Figure 3.2 shows the fermentation of lactose to produce the known products of ethanol, acetate, lactate, hydrogen and carbon dioxide. One of the benefits of this plot is that it is not constrained by the choice of products. In a similar way to Figure 3.2 the system region can be extended to include alternative products. This can be useful in considering different types of systems such as different microorganisms or a mixed consortium which have different fermentation products. Another potential application could be for genetically modified organisms. The genetically modified organisms are still limited by the heat and work requirements. The ΔG-ΔH plot offers a method to better understand the implications of removing or including metabolic pathways in the GM-organism. The modification of genetic pathways can add or remove possible products by the organism.

Even without genetic modification and depending on the microorganism other products can be found during fermentation. Another typical product found in anaerobic fermentation is methane. In the same way as the previous products the mass balance for methane can be written as shown in Equation (3.14). Since ΔG-ΔH plot is not limited by the choice of products, Equation (3.14) can be included as shown in Figure 3.3

$$\mathrm{C_{12}H_{22}O_{11\,(aq)}} + \mathrm{H_2O_{(l)}} \longrightarrow 6\,\mathrm{CH_{4\,(g)}} + 6\,\mathrm{CO_{2\,(g)}} \tag{3.14}$$

The methane addition has increased the system boundary and affected the intersection of the maintenance vector and the convex hull. Therefore the inclusion of methane has created a new maintenance limited case. Equation (3.15) shows the new mass balance associated with the maintenance limited

growth.

$$C_{12}H_{22}O_{11\,(aq)} + 7{\cdot}11\,H_2O_{(l)} \longrightarrow 2{\cdot}94\,CH_{4\,(g)} + 9{\cdot}05\,CO_{2\,(g)} + 12{\cdot}22\,H_{2\,(g)} \tag{3.15}$$

The addition of methane as a product has increased the overall Gibbs free energy (ΔG) and thus increased the irreversibility of the maintenance limited case. According to von Stockar and Liu (1999) the irreversibility yields a high metabolic rate. It is beneficial for the microorganism to couple the slow, high yield anabolic reaction with the fastest catabolic reaction available. In this case if the microorganism has the ability (i.e. the available metabolic pathways) to produce methane then methane is the preferred product. This can be seen in anaerobic digesters where the product is biogas primarily composed of methane. The inclusion of additional products on the ΔG-ΔH plot allows

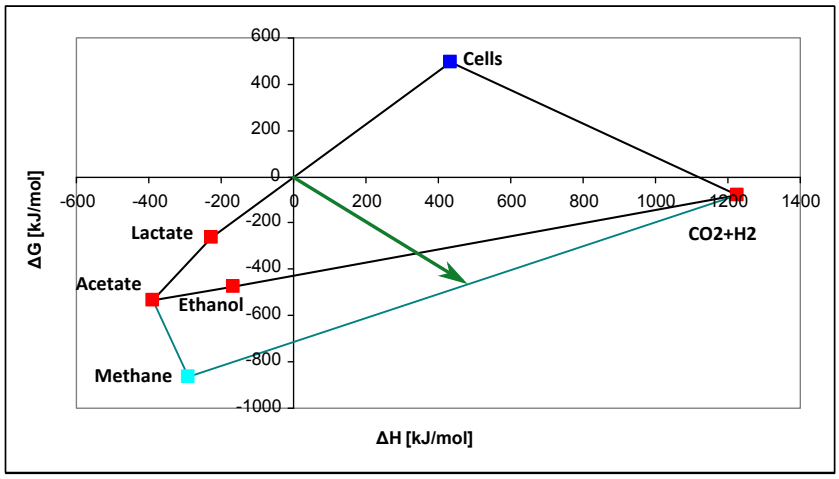

Figure 3.3: System region for the lactose fermentation with methane as an additional product

many products to be easily scanned and to understand what effects they would have on the system.

3.7 Alternative Feeds

In the previous sections the fermentation was performed on sugar with lactose as the example. Fermentation, however, is not limited to fermentation of sugars. There are many microorganisms that have the ability to ferment polysaccharides (such as starch and cellulose). Another potential feed for fermentation is glycerol. Bacteria such as *E.Coli* have the ability to ferment glycerol into alcohols and organic acids. Equations (3.16) - (3.20) show the independent mass balances for the fermentation of glycerol to some of the typical fermentation products. As in the lactose example, the enthalpies and Gibbs free energies of reaction for the mass balances can be calculated using standard formation data. The thermodynamic data for *E.Coli* is required to calculate Equation (3.20). If there is data available for *E.Coli* such as used in Chapter 2 then a similar method can be used to calculate the formation properties. Without such information available it can be assumed that the formation properties for *E.Coli* are the same as for *C.Thermolacticum* and the methods introduced in this chapter will remain unchanged. This system yet again has energy producing and energy consuming reactions as well as work producing and work consuming reactions.

	ΔG_{rxn}	ΔH_{rxn}	
$C_3H_8O_{3\,(aq)} + 3\,H_2O_{(l)} \longrightarrow 3\,CO_{2\,(g)} + 7\,H_{2\,(g)}$	25.97	353.51	(3.16)
$C_3H_8O_{3\,(aq)} \longrightarrow C_2H_6O_{(l)} + CO_{2\,(g)} + 1\,H_{2\,(g)}$	−71.68	5.34	(3.17)
$C_3H_8O_{3\,(aq)} \longrightarrow C_3H_6O_{3\,(l)} + H_{2\,(g)}$	−19.24	−10.09	(3.18)
$C_3H_8O_{3\,(aq)} \longrightarrow 1\,\frac{1}{2}\,C_2H_4O_{2\,(l)} + H_{2\,(g)}$	−87.37	−50.2	(3.19)
$C_3H_8O_{3\,(aq)} + 7{\cdot}9\,H_2O_{(l)} + 0{\cdot}5\,CO_{2\,(g)} + 0{\cdot}7\,NH_4Cl_{(aq)}$ $\longrightarrow 0{\cdot}7\,C_5H_7NO_2{\cdot}15\,H_2O_{(s)} + 0{\cdot}7\,HCl_{(aq)}$	200.80	121.86	(3.20)

The enthalpies and Gibbs free energies of reaction can be plotted on a ΔG-ΔH plot as shown in Figure 3.4. Figure 3.4 shows a similar behaviour tos the lactose system. In the glycerol fermentation there is, again, a mass balance lying on the system boundary. In this instance, similar to the lactose fermentation, there will only be two products in the maintenance limited case. The maintenance vector can be traced to the system boundary and the lever arm rule can be applied to determine the associated mass balance. Equation (3.21) shows that the maximum ethanol that can be produced via the fermentation of glycerol is 0.85mols per mol of glycerol. The maximum acetate produced is 1.10mols

per mol of glycerol as shown by Equation (3.22)

$$C_3H_8O_{3\,(aq)} + 0\cdot46\,H_2O_{(l)} \longrightarrow 0\cdot85\,C_2H_6O_{(l)} + 1\cdot31\,CO_{2\,(g)} + 1\cdot92\,H_{2\,(g)} \tag{3.21}$$

$$C_3H_8O_{3\,(aq)} + 0\cdot81\,H_2O_{(l)} \longrightarrow 1\cdot10\,C_2H_4O_{2\,(l)} + 0\cdot81\,CO_{2\,(g)} + 2\cdot62\,H_{2\,(g)} \tag{3.22}$$

This system has similar properties as for the lactose region and, as such,

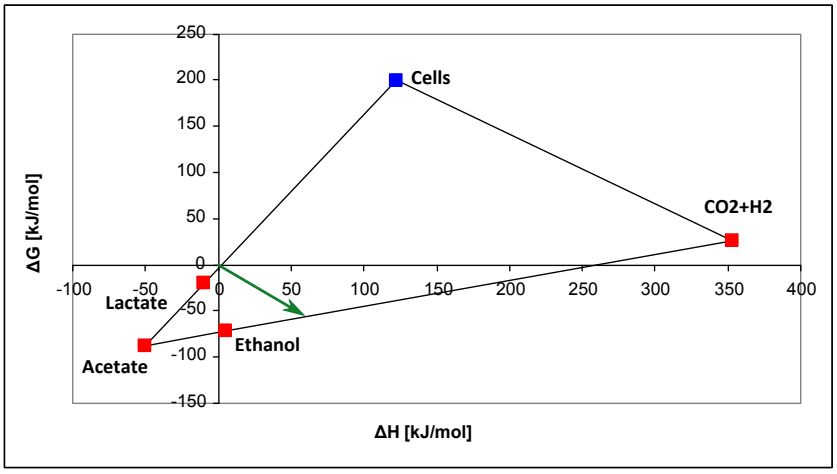

Figure 3.4: System region with the maintenance vector for the fermentation of glycerol

additional products can also be included. Methane again will be included as a possible fermentation product. The mass balance for methane is given by Equation (3.23). Figure 3.5 shows how the system region for glycerol changes with the inclusion of methane as an additional product. The convex hull, as a result of the methane, is increased. The mass balance for the maintenance limited growth on glycerol is given by Equation (3.24). This shows that the maximum amount of methane that can be produced is 0.98mol per mol of glycerol.

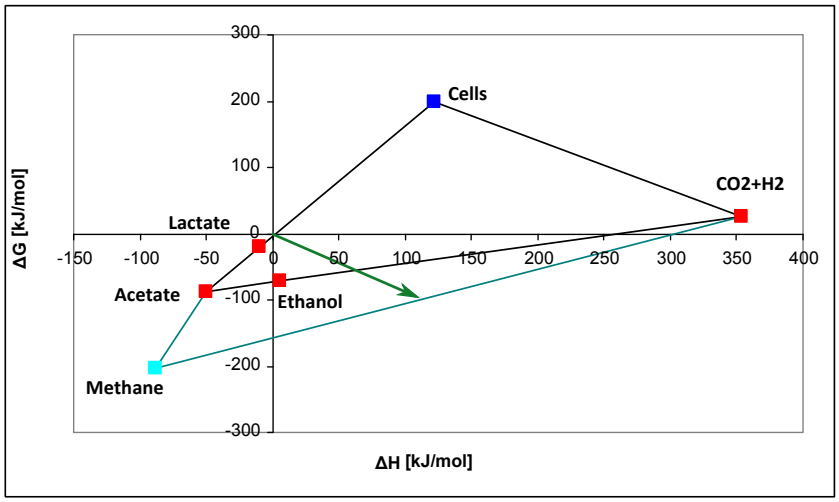

Figure 3.5: System region with the maintenance vector for the fermentation of glycerol with methane as an additional product

$$C_3H_8O_{3\,(aq)} \longrightarrow 1\cdot75\,CH_{4\,(g)} + 1\cdot25\,CO_{2\,(g)} + 0\cdot5\,H_2O_{(l)} \tag{3.23}$$

$$C_3H_8O_{3\,(aq)} + 1\cdot32\,H_2O_{(l)} \longrightarrow 0\cdot98\,CH_{4\,(g)} + 2\cdot02\,CO_{2\,(g)} + 3\cdot36\,H_{2\,(g)} \tag{3.24}$$

3.8 Conclusion

A biological system can be treated as a chemical system since the thermodynamics is defined for any consistent and well defined system boundary. The ideas fundamental to process synthesis can therefore be applied to any biological system such as fermentation as discussed in this work. This work has shown methods that use fundamental thermodynamic to gain insights into the operation of the process. Fundamental to the ideas discussed above is the use of the ΔG-ΔH plot which provides a graphical means to display the important information of the system. This graphical approach will aid in understanding the behaviour of the system and to understand the limitations of the system.

The maintenance vector offers a quick and simple way to determine the theoretical maximum products of fermentation of a chosen system.

The methods for analysing the thermodynamics in biological system currently only account for the anabolic reaction coupled with one catabolic reaction. The benefit of this work is that it introduces methods that consider all the possible products in the system and not just the main product from microbial growth.

The ΔG-ΔH diagram can be used to quickly determine the effect additional (or alternative) products would have on the system. The benefit of this type of diagram is that it is always a two-dimensional Cartesian plane regardless of the number of products being considered. In a similar manner the ΔG-ΔH plot can be used to consider the effects a different feed would have on such a process.

The power of the method discussed above is that it can be applied to any biological system which the inputs and outputs are known. A detailed understanding of the mechanism of the process is not required. These ideas could also incorporate higher degrees of accuracy if so required.

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Chapter 4

A Thermodynamic Perspective on Photosynthesis and Combustion

Abstract

Thermodynamics is an important tool in chemical engineering. The application of thermodynamics for the integration of work and heat in a process has proven to be useful in understanding the limits of performance of the system. These methods are based on a system boundary which only considers the inputs and outputs of the process."Black boxing" the system is useful since a detailed understanding of the process is not required and fundamental insights that give information on the limits of performance can be obtained by using minimal data. In this paper the heat and work integration of photosynthesis and combustion are considered. For the process to be reversible, the heat and work integration shows that the heat needs to be transferred at a Carnot temperature of 10 071K(+1 263K,-1 010K) for both processes. Combustion using the currently available equipment is unable to achieve the Carnot temperature and must be operated at a lower temperature and therefore is inherently irreversible. Photosynthesis differs from combustion in that it receives energy in the form of light. The entropy associated with light is a somewhat controversial and debated point. However, the two major theories on the entropy of light are that light is a form of non-entropic energy and light has a form of heat based on a Carnot cycle. Both of these theories show that an ideal photosynthesis process can operate close to reversible. A real photosynthetic process consumes more energy or photons than is required for the photosynthesis reaction. This system can be shown to contain an ideal system internally with all the excess energy being converted to heat and the excess work being lost as an inefficiency.

4.1 Introduction

Thermodynamics has proven to be a useful tool in chemical engineering for understanding chemical processes. Patel, Hildebrandt, Glasser, and Hausberger (2005) applied the second law of thermodynamics to develop a new method to analyse chemical systems. Using Gibbs free energy and the second law of

thermodynamics it was possible to define a reversible process by integrating the work and energy requirements. The work integration shows that there is a temperature at which the heat transfer must take place in order for the process to be reversible. This reversible temperature was denoted as the Carnot temperature by Patel, Hildebrandt, and Glasser (2010). An important and useful aspect of the developed method is that it does not require detailed information of the process. Patel, Hildebrandt, Glasser, and Hausberger (2007) extended the method to the integration of mass, energy and work of a chemical process. Since these methods are based on the inputs and outputs of the process only, and not the internal details, they can be applied to any chemical system such as photosynthesis.

A large amount of literature on thermodynamics of photosynthesis has been amassed over the years. An important aspect of the thermodynamics of photosynthesis is the role that light plays in affecting the entropy of the reaction. There are many conflicting opinions on the entropy of light absorbed by the system. Knox and Parson (2007a) treated the light entering a system as heat at the radiation temperature. The entropy associated with the heat flowing into the system is thus calculated using a Carnot cycle. The thermodynamic analysis performed by Albarrán-Zavala and Angulo-Brown (2007) also treated light as heat and therefore the entropy of the light appeared in the form of the Carnot cycle. In the work by Jennings, Engelmann, Garlaschi, Casazza, and Zucchelli (2005) and Jennings, Belgio, Casazza, Garlaschi, and Zucchelli (2007) light was treated as a form of high grade energy. High grade energy has the ability be converted completely into work and carries no entropy with it into the system. The entropy changes associated with light are not only found in literature pertaining to photosynthesis. There are many system involving light which can be analysed thermodynamically and therefore the conflicting opinions on the entropy of light are found in many fields of research. In a paper on the conversion of solar heat into work Laptev (2005) treated the absorbed radiant heat as a Carnot cycle. A similar paper by Markvat (2008) on photovoltaic conversion by solar cells considered the light as pure heat entering the system. As previously mentioned the entropy of heat flowing into the system can be described by a Carnot cycle. Suppan and Vauthey (1989)

studied photoinduced electron transfer and, in combination with fluorescence experiments, concluded that light is a non-entropic form of energy. Gudkov (1998) applied the results obtained from Suppan and Vauthey (1989) to look at the thermodynamics of chemiluminescence. Kirwan (2004) attempted to use the Gibbs-Duhem equation to calculate the entropy of a photon. Treating the volume of a photon as proportional to the cube of the wavelength, Kirwan (2004) showed that the entropy of a single photon is a constant related to Boltzmann's constant. The entropy of light is an important and highly debated topic. A complete thermodynamic description of photosynthesis would require a true understanding of entropy of light. Using the methods described above a thermodynamic analysis can be performed on photosynthesis and the implications of the different theories of light will be considered.

4.2 The Photosynthetic Reaction

Photosynthesis is the process in which light is absorbed and used to convert carbon dioxide and water into organic material as shown in Figure 4.1A. The system is the blackbox defined by the feeds to and the products from ,in this case, the photosynthetic organism. The generalised form of this reaction is given by equation (4.1a). Combustion is the reaction of typically an organic compound with oxygen to produce carbon dioxide and water. In this paper only combustion of organic products is considered. Again the system is defined by the feeds and products to the process. It can be seen from this Equation (4.1a) and (4.2) and from Figure 4.1 that photosynthesis is equivalent to the reverse reaction of combustion. The enthalpies of reaction for photosynthesis and combustion are related by $\Delta H_{\text{photosynthesis}} = -\Delta H_{\text{combustion}}$. Similarly the Gibbs free energies are given by $\Delta G_{\text{photosynthesis}} = -\Delta G_{\text{combustion}}$.

It should be noted that Equation (4.1a) is not a complete description of photosynthesis since is does not include the many other elements that are typically found in photosynthesis. Nitrogen is an important element in biological systems and is typically included in mass balances. From the formulas typi-

cally used for microorganisms it can be shown that the nitrogen accounts for about 10% of the mass of the cells. Equation (4.1b) incorporates nitrogen into the reaction. This reaction differs slightly from a typical photosynthetic reaction in that it uses elemental nitrogen instead of a fixed form of nitrogen (such as an ammonium or a nitrate). This assumption simplifies the calculation and also allows the results to be directly compared to the reverse combustion reaction. The calculations in this work can be repeated using an alternative form of nitrogen however, assumption of elemental nitrogen does not affect the overall results. The major terms on the total heats and Gibbs free energies of reaction (ΔH_{rxn} and ΔG_{rxn}) are carbon dioxide and water.

$$a\mathrm{CO_2} + b/2\mathrm{H_2O} \longrightarrow \mathrm{C}_a\mathrm{H}_b\mathrm{O}_c + (c/2 - a - b/4)\mathrm{O_2} \tag{4.1a}$$

$$a\mathrm{CO_2} + b/2\mathrm{H_2O} + d/2\mathrm{N_2} \longrightarrow \mathrm{C}_a\mathrm{H}_b\mathrm{O}_c\mathrm{N}_d + (c/2 - a - b/4)\mathrm{O_2} \tag{4.1b}$$

$$\mathrm{C}_a\mathrm{H}_b\mathrm{O}_c + (c/2 - a - b/4)\mathrm{O_2} \longrightarrow a\mathrm{CO_2} + b/2\mathrm{H_2O} \tag{4.2}$$

Equation (4.1a) and (4.1a) can be used to calculate the ΔH_{rxn} and ΔG_{rxn} for a range of chemical species. The thermodynamic data was taken from Sandler (1999) and included alkanes, alkenes, aromatics, cycloalkanes, alcohols, organic acids and a few other compounds. A list of the compounds used and the calculated properties can be found in Table C.1 in Appendix C. Chapter 3 used the ΔG vs ΔH plot to show the feasible region by microbial fermentation. Figure 4.2 shows the relationship between the ΔH_{rxn} and ΔG_{rxn} for photosynthesis and combustion. It can be seen that there is a strong relationship between the ΔH_{rxn} and ΔG_{rxn} for photosynthesis and the reverse reaction of combustion involving carbon compounds. Figure C.1 in Appendix C shows an annotated version of Figure 4.2.

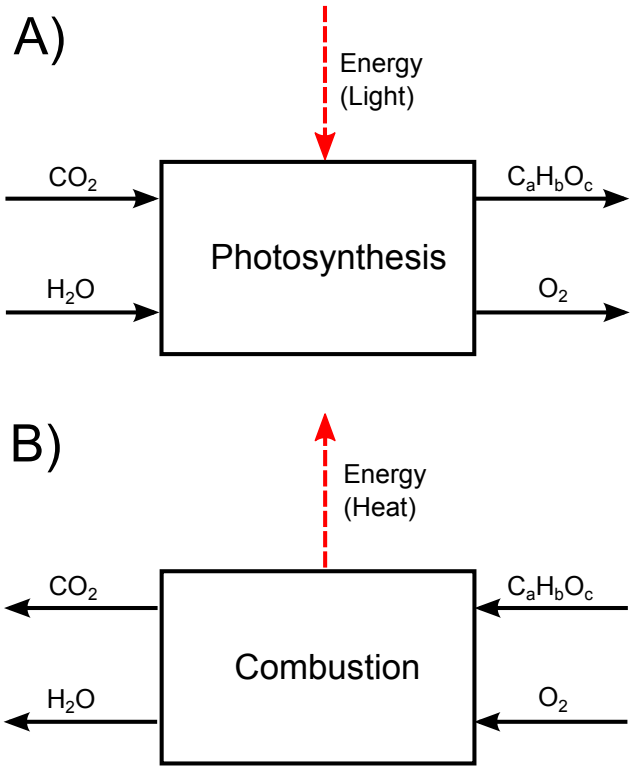

Figure 4.1: Generalised a) Photosynthesis and b) Combustion Processes illustrating the similarity between the two processes. The overall combustion process can be treated as a reversed overall photosynthesis process

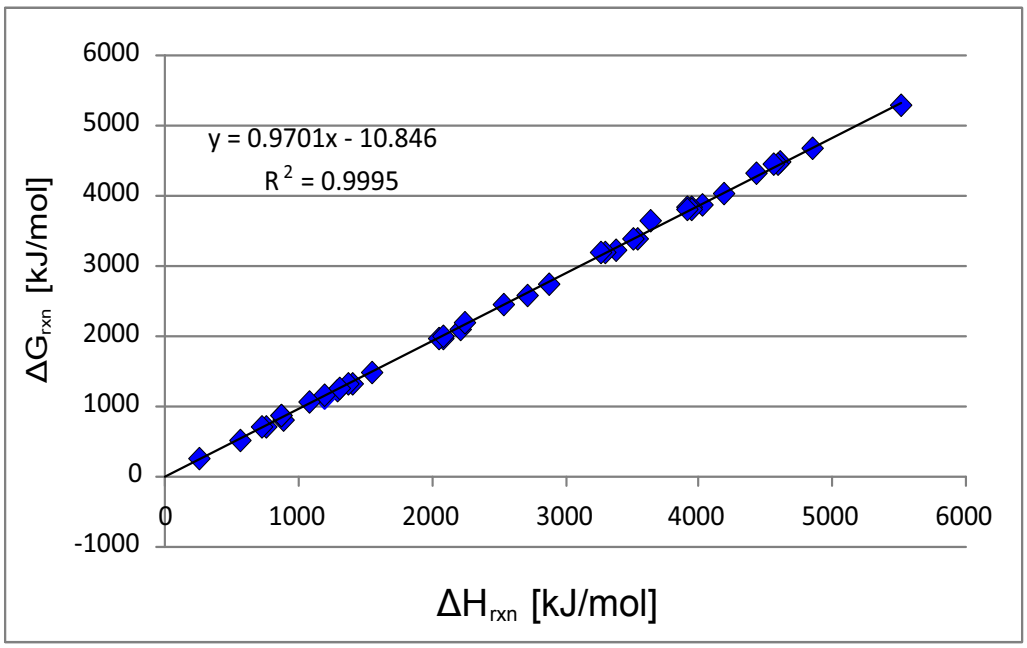

Figure 4.2: Gibbs free energy of reaction versus enthalpy of reaction for the production of organic compounds by photosynthesis

4.3 False Compensation

A similar plot to Figure 4.2 is the entropy-enthalpy plot. Liu and Guo (2001) list occurrences of this type of plot in various fields. The entropy-enthalpy plot has received much attention since it is used to show entropy-enthalpy compensation between similar reactions differing in a single constituent or the same reaction in several different solvents. Liu and Guo (2001) reviewed this topic and covered the relationship between entropy-enthalpy compensation and the isokinetic or isoequilibrium temperature. Entropy-enthalpy compensation is a term used to describe a strong statistical correlation between entropy and enthalpy. Liu and Guo (2001) also discussed the appearance of false compensation in which errors in experimental measurements cause what seems to be a entropy-enthalpy compensation. Cornish-Bowden (2002) showed that false compensation is a statistical artefact by using a set of random data to produce an "entropy-enthalpy compensation" effect. It is evident from Cornish-Bowden (2002) that high correlations should be accepted with caution since experimental errors can cause two seemingly different variables to be the same variable viewed in different ways. Sharp (2000) analysed the entropy-enthalpy effect and showed that little extra-thermodynamic information can be determined from a system and that the the extra-thermodynamic information can be difficult to distinguish from the other factors that cause the effect.

Cornish-Bowden (2002) states that the major problem arises when the data (enthalpy and entropy) is estimated from the same temperature dependant data. This is typically done using the transition state theory and an Arrhenius plot. The data in Figure 4.2, however, has not been derived from temperature dependant data from a single experiment. This in its own right should not discount the possibility of a false compensation type effect. Figure 4.3 shows the same data as in Figure 4.2 but in the form of enthalpy and entropy. As can be seen by Figure 4.3 there is no strong correlation between entropy and enthalpy for the series of reactions. An annotated version of Figure 4.3 can be found in Appendix C in Figure C.2.

Figure 4.3 shows that the entropy is not a major component of the Gibbs

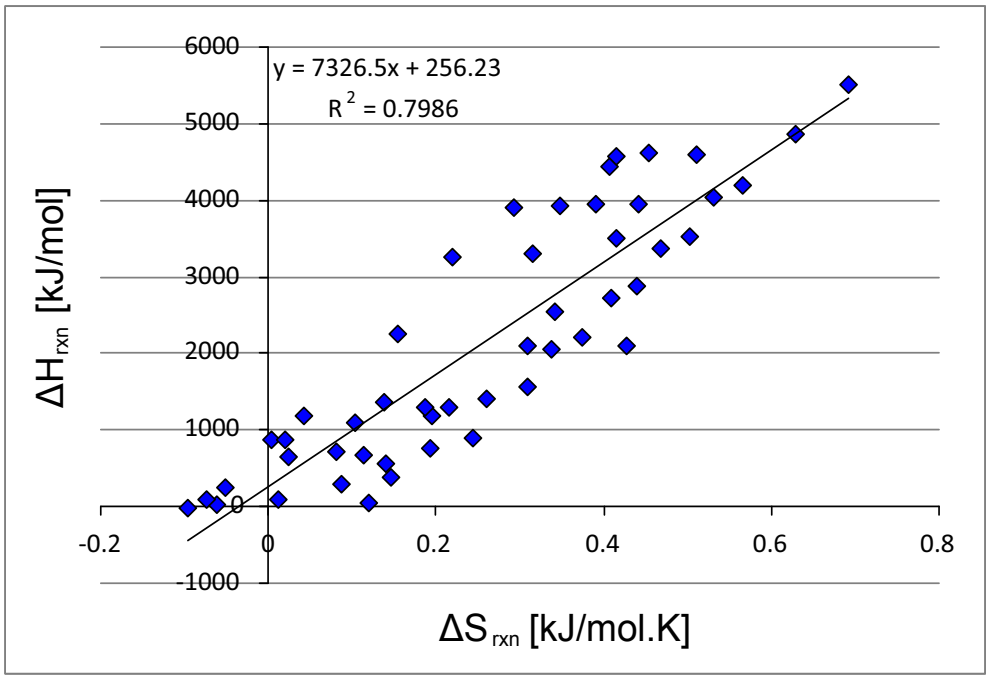

Figure 4.3: Enthalpy of reaction versus entropy of reaction for the production of organic compounds by photosynthesis

free energy. Since the data is produced from formation data only, the large enthalpies and Gibbs free energies of reaction must come from either the products, the reactants or both. The enthalpies and Gibbs free energies of the reactant and products can be plotted separately on the ΔG-ΔH plot as shown in Figure 4.4. Figure 4.4A shows the left hand side of Equation (4.1b) (the CO_2, H_2O and N_2). Comparing Figure 4.2 with Figure 4.4A shows that removing the products from the equation only has a small effect on the system. The gradient of the fitted data decreases by approximately 3.5% while the goodness of fit has not significantly changed. Figure 4.4B shows a similar plot using only the right hand side of the equation. From this figure it is obvious that the right hand side does not follow the same trend. The data in Figure 4.4B is more scattered and clustered closer to the origin. The fact that the data is clustered around the origin shows that it does not significantly add to the overall enthalpy and Gibbs free energy of reaction. The corresponding annotated plots to Figure 4.4 are found in the appendix as Figure C.3 and Figure C.4.

From the results above it is clear that carbon dioxide and water are the

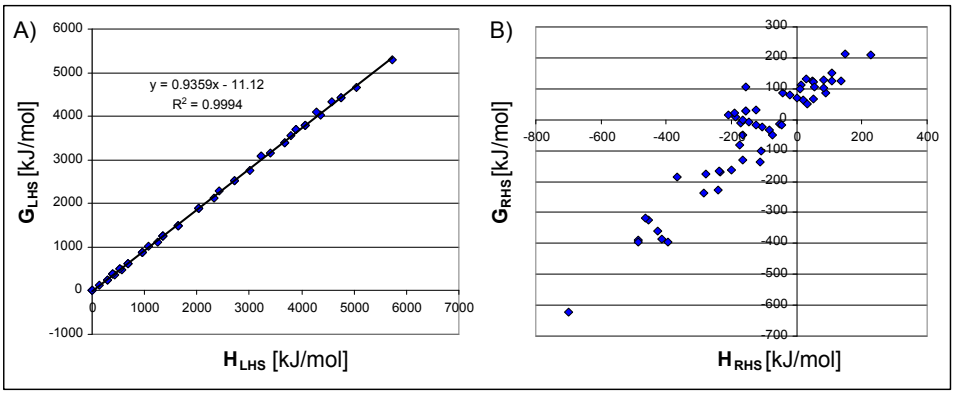

Figure 4.4: Gibbs free energy-Enthalpy plot for A) components on the left hand side of reaction (4.1) and B) components on the right hand side of reaction (4.1)

components that primarily affect the enthalpy (heat) and Gibbs free energy (work) of photosynthesis and combustion. The data in Figure 4.2 is largely affected by the ratio of $(G_{\mathrm{CO_2}} + G_{\mathrm{H_2O}})$:$(H_{\mathrm{CO_2}} + H_{\mathrm{H_2O}})$ which can be seen from the small intercept. The ratio of Gibbs free energy to enthalpy for carbon dioxide is 1.00 and for water is 0.83. The gradient of the fitted line should therefore lie in this range. One of the important factors that determines where in the range the gradient lies is thus the ratio of carbon dioxide to water. From Equation (4.1a) and (4.1b) it can be seen that the ratio of carbon dioxide to water is determined from the ratio of carbon to hydrogen in the organic compound. For most organic compounds the ratio of carbon to hydrogen is approximately 2:1. This ratio, however, does vary, especially for highly oxygenated and unsaturated compounds. The enthalpy and Gibbs free energy of carbon dioxide are approximately 1.5 times larger than for water. These two factors combined, on average, make the gradient of the slope 0.970($\pm$0.003).

These calculations have been greatly simplified but can easily be modified to include dilution terms or, in the case of the photosynthesis reaction, a different nitrogen source. However, as was shown in Figure 4.4A, these modifications should only have a minor effect on the result because of the large enthalpies and Gibbs free energies of formation of carbon dioxide and water.

4.4 Process Synthesis

As shown by Patel et al. (2010) there is a temperature at which the heat must be supplied or rejected for the process to be reversible. This temperature is known as the Carnot temperature for the reaction (T_c) and is calculated using Equation (4.3). The derivation of Equation (4.3) can be found in Appendix B. From the derivation of the Carnot temperature when the heat input is at the Carnot temperature (i.e. $T = T_c$) then $S_{lost} = 0$ and the process is reversible.

$$T_c = \frac{T_0}{(1 - \frac{\Delta G}{\Delta H})} \tag{4.3}$$

It has been shown in general for a photosynthetic or combustion reaction that ΔG_{rxn}:ΔH_{rxn} is 0.97:1. This shows that the Gibbs free energy is less than the enthalpy of reaction. Patel et al. (2005) showed that this implies that all the work can be supplied or rejected in the form of heat.

From Equation (4.3) is can be seen that the Carnot temperature is a function of the ratio of $\frac{\Delta G_{rxn}}{\Delta H_{rxn}}$. Figure 4.5 shows the relationship. There is a vertical asymptote at $\frac{\Delta G_{rxn}}{\Delta H_{rxn}} = 1$. At values greater than 1 the Carnot temperature is negative. This shows that when the Gibbs free energy of reaction is greater than the enthalpy of reaction it is impossible to supply (or reject) all the work to the process in the form of heat. However, as shown by Patel et al. (2005), when $\frac{\Delta G_{rxn}}{\Delta H_{rxn}}$ is less than 1 all the work requirements can be met by transfer heat at the Carnot temperature since this temperature is positive. For the generalised process of Equation (4.1b) the Carnot temperature is 10 071K(+1 263K,-1 010K).

The operation temperature of typical combustion processes is currently limited by the materials of construction. The current materials of construction cannot operate near the Carnot temperature and therefore transfer the energy at a lower temperature. The energy transferred at a lower temperature transfers less of the available work. The remainder of the potential work is lost as an inefficiency. This lost work is in the form of entropy generation and

therefore makes the process irreversible.

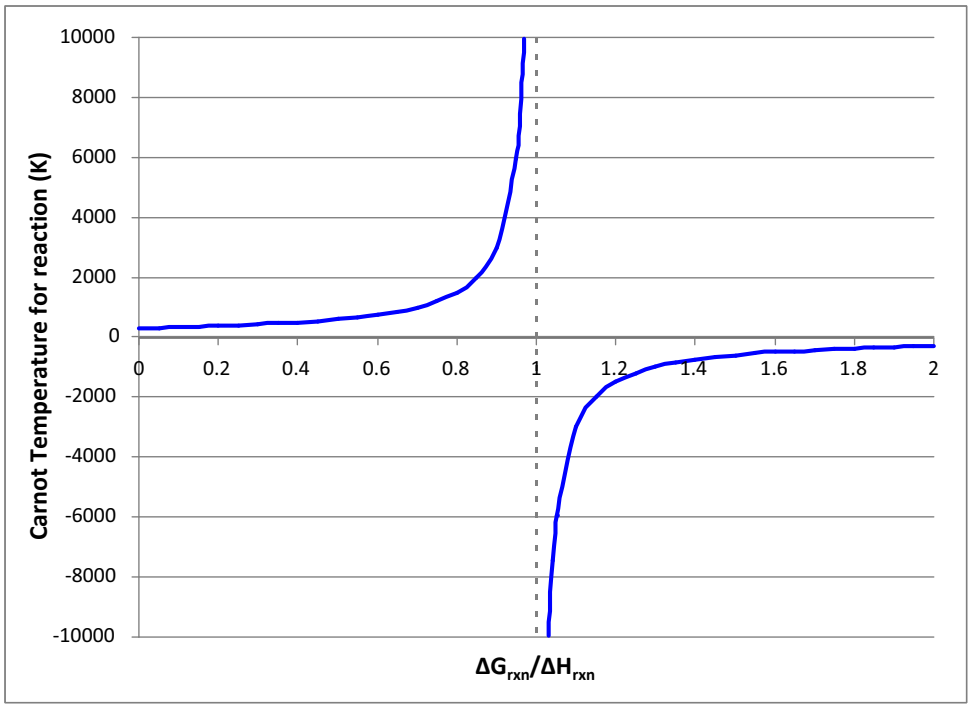

Figure 4.5: Carnot temperature as a function of $\Delta G_{rxn}/\Delta H_{rxn}$

4.5 Photosynthesis

Photosynthesis is the reverse process of combustion and requires heat and work to be supplied to the process. It is well understood that the energy for the system is supplied by light. Unlike in combustion which produces or rejects work, photosynthesis requires a work input to the process. For the reaction to proceed the process requires a minimum amount of work input determined by ΔG_{rxn}. Similar to combustion, if excess work (i.e. more than ΔG) is added to the process and not used or removed it is lost as an irreversibility or an inefficiency.

The work required for the photosynthesis reaction is supplied in the form of light. It is still not clearly understood what the exact role light plays in photosynthesis. It is understood that light is a high quality form of energy, i.e. it has a high potential to do work or alternatively it has a small or zero en-

tropy associated with it. However the exact effect of light on a photosynthetic system, whether it is photosynthesis or photochemistry, is not clearly understood. This uncertainty is demonstrated in the series of papers by Jennings *el al.* and Knox *et al.*. Jennings and co-workers (Jennings et al., 2005; Jennings, Casazza, Belgio, Garlaschi, and Zucchelli, 2006; Jennings et al., 2007) show that there is a zero entropy generation in photosynthesis. The work by Knox and co-workers (Lavergne, 2006; Knox and Parson, 2007a,b) argue against this supposition treating light as a Carnot engine. The different cases are used by different authors in differing fields as shown below. For the analysis in this work both cases, Carnot engine and non-entropic light, will be discussed for the generalised photosynthesis reaction developed above.

4.5.1 Light as Non-entropic Energy

Suppan and Vauthey (1989) concluded that light is a form of high grade, non-entropic energy. Gudkov (1998) used the supposition that light is a high grade form of energy and does not have any associated entropy to thermodynamics does not constrain the quantum yield of chemiluminescence. Using the assumption that there is no entropy associated to the absorbtion of light yields the following:

$$\Delta H_{light} = hfN_A \tag{4.4}$$

$$\Delta S_{light} = 0 \tag{4.5}$$

Given $G = H - T_0S$

$$\Delta G_{light} = hfN_A \tag{4.6}$$

Therefore

$$\frac{\Delta G_{light}}{\Delta H_{light}} = 1 \tag{4.7}$$

Therefore for non-entropic light the ratio $\frac{\Delta G_{light}}{\Delta H_{light}} = 1$ which corresponds to an

infinite Carnot temperature (Equation (4.3)). In this photosynthetic system all the energy that is carried through the system boundary is available as work. The ratio for the light (=1) is larger than the ratio for the process (=0.97). When the energy requirements of the process are met:

$$\Delta H_{light} = \Delta H_{rxn} \tag{4.8}$$

Since the light ratio is greater than the process ratio

$$\Delta G_{light} > \Delta G_{rxn} \tag{4.9}$$

Therefore

$$W_{lost} = \Delta G_{light} - \Delta G_{rxn} \tag{4.10}$$

The higher ratio shows that more work is being carried in by the light than is required by the process. The system is therefore heat limited. The unused work entering the process is therefore lost as an inefficiency or an irreversibility. The inefficiency is a 3% loss of the available work entering the system.

4.5.2 Light as a Carnot Engine

In a thermodynamic analysis of solar cells Markvat (2008) showed that the light entering the system can be treated as a Carnot engine. Laptev (2005) studied the conversion of solar heat into work by treating the light entering the system as a Carnot cycle. The work by Lineweaver and Egan (2008) shows that for photosynthesis the entropy generation is caused by the high energy, high temperature photons from the sun being absorbed into the low temperature photosynthetic system. Albarrán-Zavala and Angulo-Brown (2007) performed a simple thermodynamic analysis of photosynthesis. Using non-equilibrium thermodynamics Mészena and Westerhoff (1999) showed that the Gibbs free energy of light is the light energy multiplied by the Carnot efficiency. In this

analysis light was treated as a heat source flowing from a warm reservoir to a cooler reservoir. The sun was treated as a thermal reservoir with a temperature of 5762K and the photosynthetic organism was treated as the cold reservoir with a temperature of 298K. In this way a Carnot temperature of 5762K can be assigned to light. Equation (4.3) can be used to show that the ratio for light $\frac{\Delta G_{light}}{\Delta H_{light}} \approx 0.95$. In this case the ratio for the light is slightly less than the ratio for the process (with a Carnot temperature of 10 071K(+1 263K,-1 010K)). When the energy requirements of the process are met:

$$\Delta H_{light} = \Delta H_{rxn} \tag{4.11}$$

Since the light ratio is less than the process ratio

$$\Delta G_{light} < \Delta G_{rxn} \tag{4.12}$$

This shows that the light does not carry in all the required work for the process to proceed. In order for the process to proceed it would need to absorb more photons to account for the extra work requirements:

$$\Delta G_{light} = \Delta G_{rxn} \tag{4.13}$$

Since more light is absorbed

$$\Delta H_{light} > \Delta H_{rxn} \tag{4.14}$$

Therefore

$$Q_{lost} = \Delta H_{light} - \Delta H_{rxn} \tag{4.15}$$

In this case the energy requirements dictate the requirements of the system and thus the system is work limited. In order to meet the work requirements of the process extra light would have to be absorbed and thus extra energy would be absorbed by the process too. Unlike the case of the heat limited process, the extra energy absorbed by the work limited process cannot just be

lost. The extra heat added to the process would have to be rejected to the surroundings. The extra heat that needs to be emitted to the surroundings is an inefficiency which is approximately 2%. In order for the process to be reversible it would require a Carnot temperature of 5762K (i.e. the temperature of the surface of the sun) and a ΔG:ΔH ratio smaller than predicted by Figure 4.2. As discussed, the effects of the nitrogen source and other effects such as mixing have not been accounted for and could decrease the Carnot Temperature. However, without taking into account these effects, the analysis shows that the core reaction of photosynthesis is close to reversible.

4.6 Real Photosynthetic Systems

The analysis above was done by matching the heat and work requirements of photosynthesis by the heat and work supplied by the light i.e. $\Delta H_{rxn} \leq \Delta H_{light}$ and $\Delta G_{rxn} \leq \Delta G_{light}$. As discussed in Section 4.5, when the heat and work requirements of photosynthesis are matched, the process is close to reversible ($S_{lost} \approx 0$). The reversible process is the theoretical minimum amount of light required for the reaction to proceed. Photosynthetic organisms however, require more light than the theoretical minimum. The data in Table 4.1 shows the energy per mole of CO_2 consumed entering the photosynthetic system (in this case algae) based on measurements of the number of photons absorbed by the system. The efficiencies in Table 4.1 are based on the minimum energy required to consume one mole of CO_2. The data shows that all of these systems are all less than 33% efficient. The analysis done by Albarrán-Zavala and Angulo-Brown (2007) showed that the efficiency of the photosynthetic organisms is less than 30% with many of the pathways analysed being less than 10% efficient.

The question that arises is where does this additional energy go? When

Work	Energy Input (kJ/mol CO_2)	Efficiency
Albarrán-Zavala and Angulo-Brown (2007)	1759	27%
Raven and Johnston (1991)	1407	33%
Raven and Johnston (1991)	2674	18%
Raven and Geider (2003)	1407	33%
Warburg, Krippahl, and Lehman (1969)	2111	22%
Minimum Energy (this work)	469	100%

Table 4.1: Photosyhtesis efficiencies compared to the theoretical minimum requirement

more light enters the system than is required it can be shown that:

$$\Delta H_{light} > \Delta H_{rxn} \tag{4.16}$$

$$\Delta G_{light} > \Delta G_{rxn} \tag{4.17}$$

Which gives:

$$Q_{lost} = \Delta H_{light} - \Delta H_{rxn} \tag{4.18}$$

$$W_{lost} = \Delta G_{light} - \Delta G_{rxn} \tag{4.19}$$

This shows that the excess energy entering the system must leave the system. In a real microorganism the energy is lost in several stages as the metabolic reactions proceed. In order to assess the overall efficiency of the system it is unreasonable to do the calculations at such a level of detail. Using a high-level, macroscopic approach the systems can be rapidly analysed and the detailed analyses can be applied to a more select group. Figure 4.6 represents a model which can be used to model the real photosynthetic organism. The photosynthetic organism can be considered as two parallel processes. The first process is the reversible photosynthesis reaction as described above. The remaining light absorbed by the organism is converted by the second process into heat. The excess heat absorbed by the organism is rejected to the environment at the temperature of the organism. The heat can be rejected to the environment in two ways, via heat transfer to the environment or via latent heat of water. For systems in environments where water is scarce, such as

cacti in deserts, the photosynthetic system would have to rather rely on heat transfer only. In aqueous systems, such as with aglae, the use of heat loss via latent heat is not possible. In both cases the photosynthetic system would have to rely on heat transfer to the environment. In order to achieve heat transfer these systems would have to operate at a temperature slightly higher than the ambient temperature. The heat loss from the system also affects the thermodynamic efficiency of the system. Since the organism will be at approximately the temperature of the surroundings the heat rejected is at ambient conditions therefore:

$$T \approx T_0 \tag{4.20}$$

Equation 4.3 becomes

$$T_0 = \frac{T_0}{(1 - \frac{\Delta G}{\Delta H})} \tag{4.21}$$

Which yields:

$$\Delta G = 0 \tag{4.22}$$

Since $G = H - T_0 S$

$$0 = \Delta H - T_0 \Delta S \tag{4.23}$$

$$\Delta S = \frac{\Delta H}{T_0} \tag{4.24}$$

Equation (4.24) has an entropy generation associated with it and the process that converts the energy to heat is an irreversible process. The available work associated with the excess light is lost when the heat is rejected to the surrounding at the ambient temperature. It would be beneficial for the system to minimise the amount of excess light absorbed.

In photosynthesis there are complicated metabolic pathways and many different types of enzymes involved. It is important to note that the model in Figure 4.6 is a "black box" approach and therefore the internal details of the

system are unimportant. This is useful since the technique is valid whether the system contains a photosynthetic organism or a complicated chemical process. This information can be used to determine the limit of performance of the chosen system.

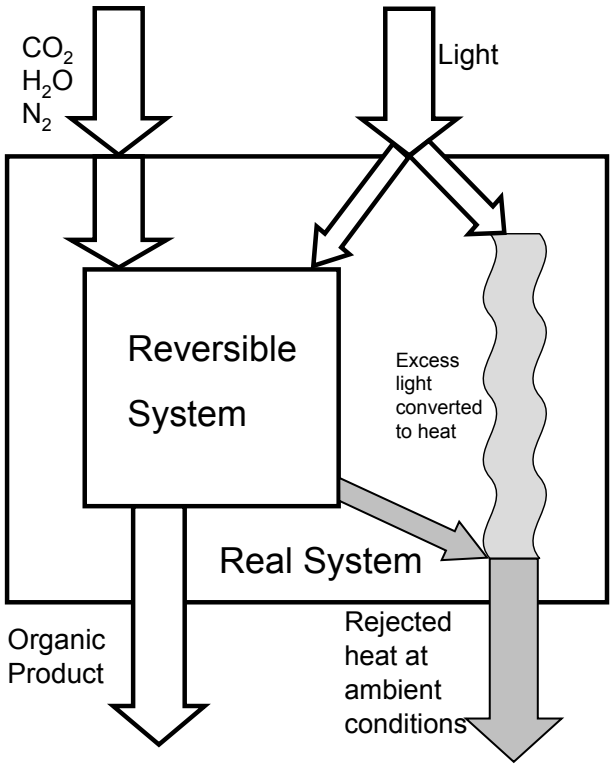

Figure 4.6: Schematic of an Ideal Photosynthetic System inside a Real Photosynthetic System

4.7 Conclusion

A simplified model of combustion and photosynthesis can be used to analyse both processes using thermodynamics. It is possible to generalise photosynthesis and combustion into a single reaction since it was shown that the components that largely influence the thermodynamics are carbon dioxide and water. The methods used, however, are not limited to this simplification. Both photosynthesis and combustion could be analysed separately in as much detail as desired. The generalised form of the reactions was used to get a simple but useful understanding of the implications of the reactions. The heat

and work integration of the process can be used to produce a reversible process. The reversible process would have to absorb or emit heat at the Carnot Temperature. The Carnot temperature for the generalised photosynthesis and combustion processes was calculated to be approximately 10 071K(+1 263K,-1 010K). This value is sensitive to the Gibbs free energies and enthalpies of reaction.

The heat and work integration showed that combustion could theoretically be operated reversibly. Reversible combustion would have to be operated at the Carnot temperature of 10 071K(+1 263K,-1 010K). The temperature required to operate combustion reversibly is unachievable by the current material available. Therefore the combustion process has to be operated at lower temperatures which introduces irreversibilities into the system.

In the same way that combustion can theoretically be operated reversibly, photosynthesis can also theoretically be performed reversibly. Again the Carnot temperature is 10 071K(+1 263K,-1 010K) but in this case the process is receiving the energy. Photosynthesis receives its energy from light. The light also carries in the work necessary to allow the reaction to proceed. There is no unanimous theory of how entropy (and thus work) is assigned to light, however two of the more popular methods show that for ideal photosynthesis the process can run close to reversible. In literature it is accepted that photosynthetic organisms absorb more light than is required to meet the heat and work constraints of photosynthesis. This extra light is converted into heat and the potential work it carries into the process is lost to the environment as an inefficiency or irreversibility.

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Chapter 5

Cellulose Degradation Using a Mixed Consortium from a Termite Gut

Abstract

The global supply of fossil fuel energy is declining. Oil reserves have been estimated to last 45 years while coal is estimated to last 120 years (BP plc, 2010). The use of renewable fuels is becoming more important in the global energy sector. Bioethanol can be produced from cellulosic biomass however the technology is different to the production of bioethanol from food crops. Termites are well known for their ability to consume large amounts of wood. The bacteria in a termite's gut are an important part of the termite's metabolism and have been shown to break down cellulose. The initial batch work was inoculated with a mixed consortium of bacteria from the termite *Termitidae Macrotermitinae Odontotermes*. The bacteria was shown to break down both pure cellulose and a natural source of cellulose (lucerne hay). Several modifications of the media were tested with the mixed consortium and the liquid products were analysed. In all cases ethanol was formed as a product of the fermentation. In most cases acetic acid was also produced as a fermentation product. The mixed consortium was also tested in continuous operation using a fluidised bed bioreactor. The consortium was able to grown in the continuous system and break down cellulose. The pH control on the reactor was an important parameter to control and maintain the operation. When the consortium was grown on pure cellulose and there was pH control on the reactor the mixed consortium produced flocs. The formation of flocs is an important result as the flocs increase the biomass density in the reactor and thus increase the overall rate of reaction.

5.1 Introduction

BP compiles a statistical review every year (BP plc, 2010) of the state of global energy. In the report the amount of proven reserves and consumption of a given fuel can be used to approximate the lifetime of the fuel's reserves. The fossil fuel oil reserves are estimated to last 45 years. Natural gas is estimated to last another 63 years whilst coal is given as 120 years. A renewable

source of energy is therefore necessary to sustain global energy demands in the future. Brazil in 2004 produced 15.28 billion litres of ethanol from sugar cane and the USA produced 12.9 billion litres from starch rich grains such as corn (Rosillo-Calle and Walter, 2006). It is possible to produce ethanol from cellulose but according to Lynd, Cushman, and Nicholls (1991) the technology is fundamentally different from that for the production of ethanol from food crops.

Termites are well known for their ability to consume large amounts of wood. Brune (1998) showed that termites have a complicated system of stomachs. The termite has three main sections in its stomach. The hindgut is the largest of these three sections and is itself highly compartmentalised (Schmitt-Wagner and Brune, 1999). Brune (1998) showed that there are steep oxygen gradients in the hindgut which render portion of it anaerobic and others aerobic. The gut of the termite offers a large variety of microorganisms as shown by Shinzato, Muramatsu, Matsui, and Watanabe (2005) and Shinzato, Muramatsu, Matsui, and Watanabe (2007). There are various aerobic and facultative anaerobic bacteria in the hindgut which consume oxygen and render it anaerobic (Adams and Boopathy, 2005; Boga and Brune, 2003; Brune, 1998). In a review article Slaytor and Chappell (1994) discussed how some bacteria are important for nitrogen fixing. Brune and Friedrich (2000) showed compelling evidence that termites produce cellulase in their salivary glands as well as in their midgut. Nakashima, Watanabe, Saitoh, Tokuda, and Azuma (2002) demonstrated that there are two cellulose digesting systems. The first in midgut using endogenous cellulase and the other in the hindgut possibly using symbiotic flagellates

Many authors (Ohkuma, 2003; Brune and Friedrich, 2000; O'Brien and Slaytor, 1982) discuss the termite digestive system and conclude that bacteria do not play a significant role in the digestion of cellulose. Schultz and Breznak (1978) looked at the hindgut of the termite *reticulitermes flavipes* but did not find any cellulolytic bacteria. Similar work on Australian termites (Eutick, O'Brien, and Slaytor, 1978) did not produce any cellulolytic bacteria. In a review article by O'Brien and Slaytor (1982), they discuss the role of bacteria in the metabolism of bacteria. They claim that the works containing cellu-

lolytic bacteria should be taken with caution since the methods for surface sterilization were not described or evaluated and since the cellulolytic bacteria isolated were not identified or counted. Since the publication of that review article there has been a significant amount of work done on termites and their symbiotic microorganisms.

Paul, Sarkar, and Varma (1986) studied *Staphlococus Saprophyticus* from the hindgut of a termite and showed that it can depolymerise cellulose. Hethener, Brauman, and Garcia (1992) isolated 3 anaerobic cellulolytic bacteria, one of which was a novel strain *Clostridium Termitidus*. In a review article by Breznak and Brune (1994) they discuss that the gut bacteria have the ability to degrade lignocellulose, however the cellulose degradation is not significantly influenced by the bacteria. Bacteria and yeast with the ability to degrade hemicellulose were isolated by Schäfer, Konrad, Kuhnigk, Kämpfer, Hertel, and König (1996) while Bakalidou, Kämpfer, Berchtold, Kuhnigk, Wenzel, and König (2002) isolated cellulolytic and xylanolytic bacteria. In a study by Wenzel, Schönig, Berchtold, Kämpfer, and König (2002) they isolated 119 cellulolytic bacteria. This work showed that bacteria may play a role in cellulose degradation in addition to the cellulolytic flagellates and endogenous cellulases. In a study comparing the crystalline- and carboxymethyl-cellulose activity in xylophagous termites, Tokuda, Lo, and Watanabe (2005) showed evidence that bacteria are involved in the digestion of cellulose. Antibiotic treatment of termite by Köning (2006) reduced cellulose activity in the hindgut which suggested symbiotic bacteria contribute to the degradation of cellulose. A review article on *Bacillus* (Tokuda and Watanabe, 2007) states that this is an important bacteria in termites (and other invertebrates) which has cellulolytic and hemicellulolytic activities. Ramin, Alimon, Sijam, and Abdullah (2008) and Ramin, Alimon, and Abdullah (2009) show that bacteria from local termites can degrade solid sources of cellulose (Whatman filter paper). Bacteria in termite gut has also been shown to be able to degrade lignin (Kuhnigk, Borst, Ritter, Kämpfer, Graf, Hertel, and König, 1994; Kato, Kozaki, and Sakuranaga, 1998). Lignin degradation requires oxygen as shown by (Kuhnigk et al., 1994).

Odelson and Breznal (1983) looked at the volatile fatty acid (VFA) production in termites. The products were primarily acetate but also included C3-C5 VFA. Breznak and Switzer (1986) showed that the bacteria in the gut produce acetate from CO_2 and H_2. According to Bignell (2000) acetate is the major product of the microbial metabolism. Breznak (2000) states that acetate is a major source of energy for termite. In their gut there are methanogens and acetogens which convert the excess hydrogen and carbon dioxide into methane and acetate respectively. Leadbetter and Breznak (1996) studied the gut of the termite and showed it contains two distinct methanogenic bacteria. Ramachandran, Wrana, Cicek, Sparling, and Levin (2008) studied the cellulolytic bacteria *Clostridium termitidis* and showed that it produced hydrogen, acetate, ethanol, lactate and formate under anaerobic conditions.

Nitrogen metabolism is not very well understood is termites (Slaytor and Chappell, 1994). Slaytor and Chappell (1994) showed that the primary source of nitrogen for most termites is from nitrogen-fixing bacteria. The nitrogen is converted into uric acid which is store in the termites body fat. It is not conclusive whether termites have the ability to mobilise the uric acid (Slaytor and Chappell, 1994).

Much publicity in the news has been targeted at the use of termites as a novel source of enzymes. Enzymes are expensive to produce and therefore enzyme reactors are expensive to operate. Chemostats or bioreactors offer a more cost effective alternative to enzyme reactors. Kivaisi and Eliapenda (1994) used rumen microorganisms to degrade bagasse and maize bran in a continuously stirred tank reactor (CSTR). Gijzen, Zwart, Van Gelder, and Vogels (1986) used rumen microorganisms to test a grass-grain mixture. Lynd, Wolkin, and Grethlein (1986) used the bacteria Clostridium Thermocellum to degrade Avicel and pretreated hardwood in a CSTR. The immobilised bacteria Zymomonas mobilis was used by Krishnan, Taylor, Davison, and Nghiem (2000) in a fluidised-bed reactor to degrade corn starch. None of the research in literature has used the bacteria obtained from termite in a continuous bioreactor.

The reactor design chosen was a fluidised bed bioreactor based on the work by Thompson, Gray, Kalala, Lindsay, Reynolds, and von Holy (2008). Fluidised bed bioreactors offer high heat transfer coefficients and simplify the task of scale-up. The work by Thompson et al. (2008) showed the production of Flocs in a fluidised bed bioreactor which increase bacterial loading in the reactor and therefore increases the overall rate of the reactor.

5.2 Aim

The research done on bacteria from termites up to the present has been on determining the strains of bacteria and their activity and function within the termite. This work has been performed *in situ* or after extraction form the termite and in batch systems. The aim of this work is to show that a mixed consortium of bacteria from a termite can be used in a continuous reactor. The mixed consortium will be used to convert cellulose into useful products . The reactor design typically used for fermentation reactions is a continuously stirred tank reactor (CSTR). It is intended to show that a fluidised bed bioreactor can be used for fermentation with the bacteria obtained from termites.

5.3 Material

5.3.1 Medium

The medium contained in $g.L^{-1}$: 0.6 $MgSO_4 \cdot 7\,H_2O$; 0.1 $MnSO_4 \cdot H_2O$; 0.02 $ZnSO_4 \cdot 7\,H_2O$; 0.002 $CuSO_4 \cdot 5\,H_2O$; 0.002 $AlK_2(SO_4)_3$; 0.1 $NaHCO_3$; 0.011 $NaMoO_4 \cdot 2\,H_2O$; 0.016 $CoCl_2 \cdot 6\,H_2O$; 0.026 $CaCl_2$; 0.002 $NaSeO_3$; 0.02 $NiSO_4 \cdot 6\,H_2O$; 0.002 $Na_2WO_4 \cdot 2\,H_2O$; 0.3 KH_2PO_4; 0.84 Na_2HPO_4; 0.002 H_3BO_3; 0.02 $FeSO_4 \cdot 7\,H_2O$; 0.018 Na_2EDTA; 0.1 NH_4Cl; 0.1 $(NH_4)_2SO_4$; 2 yeast extract. The pH was adjusted to 7 using NaOH and HCl.

5.3.2 Isolation

Termites were collected from a field in Roodepoort, Gauteng, South Africa. The termites were identified by The Agricultural Research Council(ARC) as *Termitidae Macrotermitinae Odontotermes*. Under sterile conditions five worker castes were surface sterilised with 70% ethanol and then rinsed with sterilised distilled water. The termites were homogenised with a small amount of media and then diluted up to 10ml. It was chosen to use the whole termite instead of a distinct part of the termite (such as the hindgut) since the aim was to use cellulolytic bacteria from termites. Most of the cellulose activity is found in the termites hindgut with some activity shown in the mid- and foregut. If there are any other areas of activity containing more active bacteria then by using the whole termite those bacteria would also be introduced to the media. The only carbon source in the media was cellulose and therefore the cellulolytic bacteria would thrive while the non-cellulolytic bacteria would not. The medium was incubated at 37°C (Hethener et al., 1992). Growth and cellulolytic activity of the consortium was determined by visual inspection.

5.4 Batch

The consortium was initially grown on pure cellulose (Whatman filter paper or Avicel). After a couple of days the media would turn a dark black colour as can be seen in Figure 5.2A. After about a week of incubation the filter paper would become soft and begin to break apart. These results showed that the consortium obtained from the termites was degrading the cellulose. The culture was maintained in a 100ml of media by inoculating it with 2ml of the culture. This was the stock culture used for all experiments.

The cultures were tested under aerobic and facultatively anaerobic conditions. Designing a process which is strictly anaerobic is difficult and adds complexity to the process which can increase the costs of the project. A process than can handle the presence of oxygen is a more robust process. As seen in

literature the consortium of bacteria from the gut of termites contains aerobic and facultative anaerobic bacteria in their gut. The role of these bacteria are to remove the oxygen from the system. These bacteria will help in maintaining a anaerobic conditions for the anaerobic bacteria.

The stock culture was used to inoculate 10ml of media in a 100ml serum bottle sealed with a butyl-rubber stopper. The headspace was gassed with nitrogen for 5 minutes. The carbon source used was filter paper. After 4 weeks of incubation the head space was tested using a gas chromatograph. The hydrogen composition was $6.29 \pm 0.39\%$ and the carbon dioxide composition was $15.57 \pm 0.34\%$. No methane was detected in the samples. The higher composition of carbon dioxide compared to the hydrogen is likely a product due to oxygen in the system. However, the increased concentration of carbon dioxide can also be attributed to ethanol formation.

The consortium was also tested on a natural form of cellulose in the form of lucerne hay. Lucerne was chosen because it is a high quality feed for horses and should contain many of the nutrients required for growth. Therefore it should be possible to reduce the amount of minerals added to the media. The lucerne will show how the bacterial consortium will handle unrefined cellulose. This is important since reducing the amount of pre-processing required and chemicals added can positively affect the economic feasibility of the chemical plant.

A similar trend to the pure cellulose in salts medium was observed with the lucerne hay in the salts media, after a couple of days after inoculation the media would turn cloudy but did not change colour as dramatically as with the pure cellulose as can be seen in Figure 5.2B.

A comparison was done on the growth of the culture on the two different sources of cellulose. For each substrate, 30ml of media with approximately 0.12g of cellulose was inoculated with 1ml of stock culture. Once a week 3 samples for each type of cellulose were picked at random. The samples were sterilised in an autoclave and then their pH was measured. Figure 5.1A shows

the pH for each substrate over the period of 4 weeks. The pH of both samples increased, however the lucerne showed a larger increase in pH.

Each sample was filtered through Whatman filter paper and then the filter paper was dried. The percentage consumption was calculated from the change in mass of the filter paper and the initial mass of cellulose in the sample. Figure 5.1B shows how the consumption changed over the period of the experiment. After two weeks both sources of cellulose had a similar consumption rate. After another two weeks the consumption for the lucerne decreased which is not possible. The error bars on this point show that this is likely an artefact of experimental error. However, the trend of the graph for the lucerne shows a decrease in consumption rate. This may be accounted for by the bacterial biomass attaching to the surface of the hay. Since hay is less refined than the cellulose it could offer a better surface on which the biomass could grow on. The tests with the lucerne did show a higher variability which could be caused by the heterogeneous nature of the cellulose. From these results it can be seen that a mixed consortium of bacteria from a termites gut can be used to degrade both natural and pure sources of cellulose.

To further characterise the usefulness of the mixed consortium to degrade cellulose an experiment was run to measure the liquid fermentation products. Each sample was 100ml and was grown in a 250ml glass vial sealed with a rubber septum. Each sample was inoculated with 2ml of the stock culture. The experiment was run for 3 weeks after which liquid samples were taken. The samples were tested in a gas chromatograph and the residence time of the peaks were compared to the residence times of pure samples.

This experiment also tested the effect of available nutrients in the feed. Lucerne hay was specifically chosen since it is considered a high quality horse feed. Two other media were tested with the hay feed to determine if the bacteria could use the available nutrients. The first one did not include any of the minerals of the standard media but included yeast extract. The second media had no additional chemicals added and only contained the hay and the water. A third set of flasks containing the standard media and hay was also

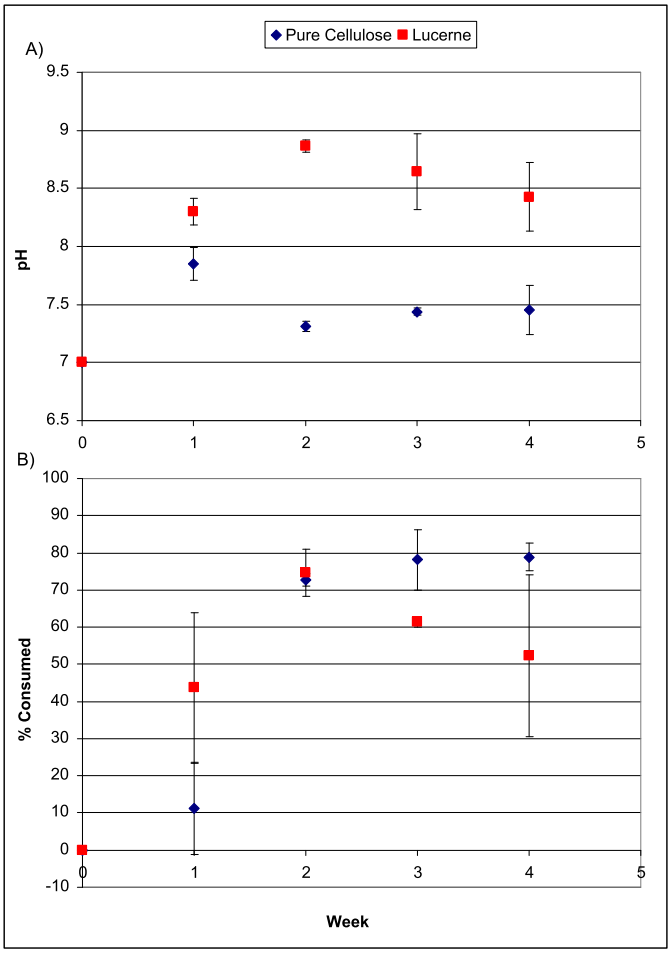

Figure 5.1: Growth Curves on the A)pH and B)the consumption of cellulose for the mixed bacterial culture on pure and natural cellulose

inoculated but left open to the atmosphere. This sample would show what effect oxygen would had on the system.

Figure 5.2 shows the samples grown on A)avicel and B)lucerne hay. In the figure it can be seen that the consortium growing on the avicel turns a dark black colour whereas the media with lucerne hay remains much lighter in colour.

Table 5.1 shows the summary of the GC data of the liquid samples. The qualitative results are based on the peak areas obtained from the GC. The ethanol peaks showed that ethanol was produced in all samples. However, much smaller signs of ethanol production showed in the media without a ni-

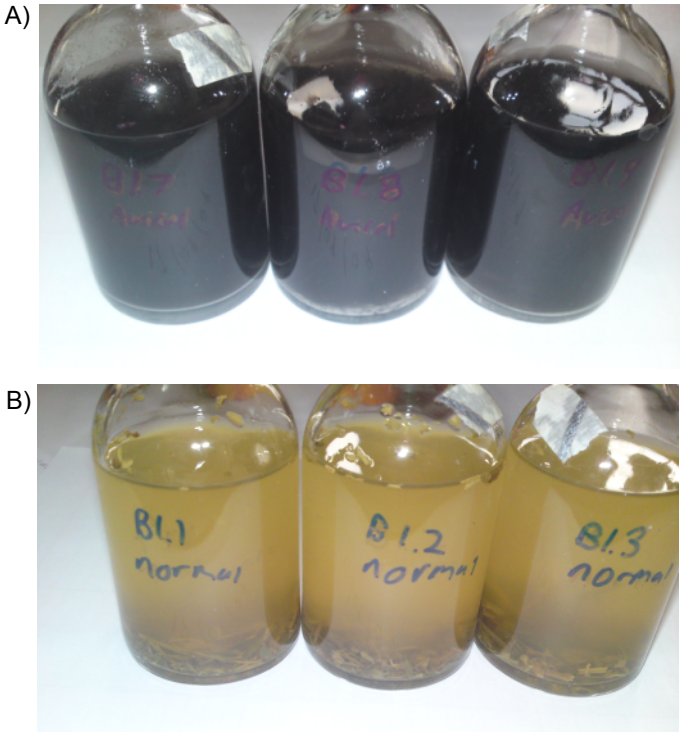

Figure 5.2: Flasks with the consortium growing anaerobically on A) Avicel and B) lucerne hay

trogen source. It is believed that the lower amounts of ethanol produced in this sample can be attributed to the lack of nitrogen containing compounds in the system since the bacteria are not able to fix nitrogen.

The standard media with both types of cellulose showed the production of organic acids other than acetic acid. When the liquid samples were taken both the samples showed signs of gas build up. However the consortium growing on the avicel had a much higher pressure build up in the flasks. From the previous gas analyses this can be attributed to the build-up of carbon dioxide and hydrogen.

Table 5.1 shows qualitative results from various batch experiments. If further analysis such as thermodynamic calculations is intended the results should be quantified in order to be able to determine the mass balance. Firstly the liquid measurements should be as concentrations and the liquid volume measured. Secondly the gas composition and gas volume for each experiment should be measured. This was not done because the aim was to test the

Media	Ethanol	Acetic Acid	Formic Acid	Propionic Acid	Butyric acid	Gas production
Avicel and Standard Media	Obvious	Small		Trace	Trace	Obvious production
Hay and Standard Media	Obvious	Small	Trace	Small		Slight production
Hay and Aerobic Media	Obvious					N/A
Hay and Yeast Extract	Moderate	Small				Not observed
Hay and Water only	Small	Trace				Not observed

Table 5.1: Effect of the media on the fermentation products. Products determined from the gas chromatography peak areas

viability of different media for growth. Further experimental work should include this information.

5.5 Continuous

The batch experiments showed that the bacterial consortium could successfully degrade pure and natural sources of cellulose into usable products. The consortium was then tested in a continuous reactor. The bioreactor for the continuous experiments was based on the jacketed, fluidised-bed bioreactor design of Thompson et al. (2008). Minor modifications to the design of the reactor, such as the volume and orientation of filters, were made but the basic principle was unchanged. Figure 5.3 shows the setup of the bioreactor. The temperature of the reactor jacket was set such that the media inside the reactor was maintained at 37°C. The system contains two pumps, one feed pump and one recycle pump, which allows the feed rate and the recycle rate to be decoupled. The recycle flowrate was set by determining which flowrate would allow the bed to be fluidised. From experimentation before incoluation the recycle flowrate was set at $1l$/min and the feed rate at $150ml$/min. The total reactor system hold-up volume was 6 litres.

The reactor was initially tested on both the media containing only yeast extract and on the pure water only using lucerne hay as the carbon source. The

Figure 5.3: Setup of the bioreactor

hay was chopped into small pieces approximately $1\,cm$ in length before being added to the reactor. In both instances the bacteria would begin to break down the hay and eventually form a plug that would travel up the length of the reactor. Figure 5.4 shows a plug that was formed and has begun travelling up the reactor. The plug eventually blocks the filters in the system and causes failure.

In response to the plug formation the recycle flowrate was increased to increase the fluidisation of the bed. The higher recycle flowrate caused elutriation of the smaller particle of the bed and ultimately also caused blockages and failure. On removal of the hay from the reactor it was noticed that the centre of the hay had been consumed. This showed that even though continuous operation was not achieved, the bacteria in the system was consuming material from the lucerne.

After sterilizing the reactor it was run again using Avicel as the cellulose source. Avicel is a pure, powdered form of cellulose and would not cause the

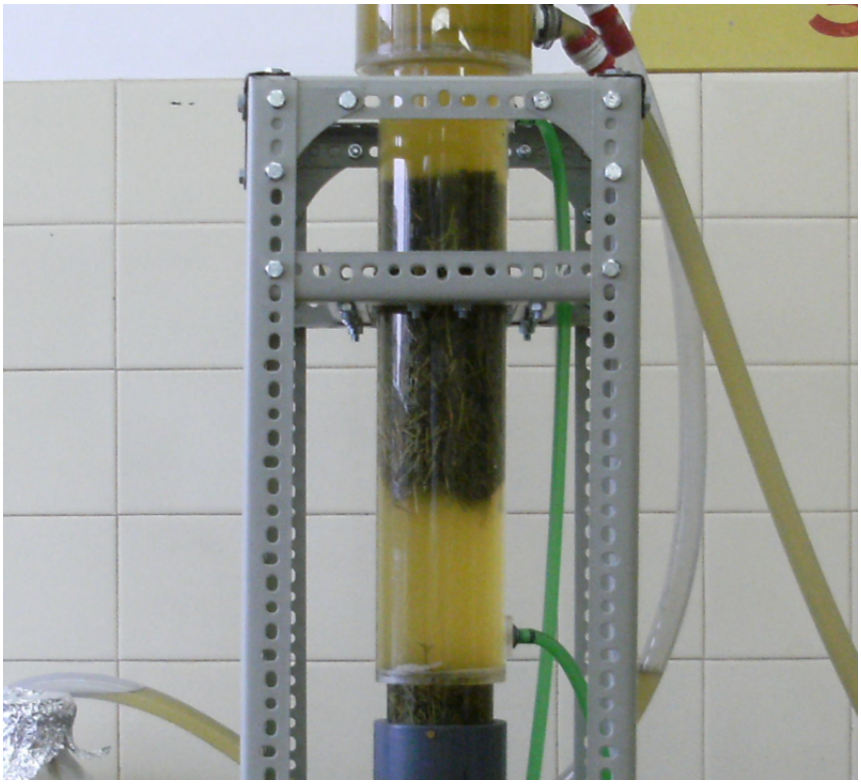

Figure 5.4: Plug of lucerne travelling up the length of the reactor

same blockage problems as the hay. Since the Avicel is pure cellulose the full salts media was used in the runs.

In first run with Avicel the pH of the reactor was not controlled. Initially the reactor showed the colour change to a dark black similar to the batch experiments. However the pH would drop within 2 days until the pH reached 4 at which point the media would turn light brown. This showed that pH control is vitally important in this type of reactor.

The experiment was reset with Avicel. The reactor pH was controlled using NaOH and was corrected daily. As expected the media turned black initially. With the pH control implemented the colour of the media did not change for the duration of the test. Liquid samples were taken during the test which showed that similar products to the batch experiment were produced. Ethanol was the primary product but acetic acid, propionic acid and butyric acid were also produced in the reactor. During the operation of the bioreactor

it was observed that the Avicel and bacteria had formed clumps or flocs in the bed as shown in Figure 5.5. This is an important and desirable observation since the formation of flocs increases the bacterial density in the reactor.

Figure 5.5: Continuous reactor showing the formation of flocs grown on Avicel

5.6 Conclusion

Along with the many articles in literature, this work shows that termites are a source of cellulolytic bacteria. Unlike the literature which focuses primarily on the isolating bacteria from specific parts of the termite (such as the middle- and hind-gut) this work was based on a mixed consortium obtained from the termite as a whole. The mixed consortium was shown to be cellulolytic when grown on both pure and natural forms of cellulose. The use of a mixed consortium simplifies the design and operation of bioreactors negating the need for strict sterile technique to ensure only a single strain of bacteria. Brune (1998) highlight the importance of the interaction between different bacteria in the termite gut and how certain microorganisms are resistant to isolation as a result of their adaptation to their microhabitats. This shows that the use of a

mixed consortium could have significant benefits over the use on monocultures.

Growth of the natural cellulose did not require the full growth medium however it did require the addition of yeast extract. This is most likely supplying a nitrogen source to the consortium since the bacteria are not able to fix nitrogen.

The mixed consortium was used in a continuous bioreactor with the aim to produce data to aid in scaling up the design. The fluidised bed bioreactor did not operate successfully with the lucerne hay as the feed material. The hay requires more processing with respect to milling. The reactor design should account for the formation of plugs, such as higher turbulence in the fluidised bed or a change in diameter prior to the filters

The operation of the bioreactor using Avicel as the cellulose feed was successful. Avicel is a fine, powdered material and therefore did not have the problem of forming plugs as in the case of the hay. The mixed consortium and the Avicel showed the formation of flocs in the reactor. The formation of flocs offers a significant advantage in a bioreactors as it increases the concentration of biomass in the reactor and as such helps improve the overall rate of reaction. During the run ethanol, acetic acid and butyric acid were measured in liquid samples taken from the reactor.

A mixed consortium obtained from termites has the potential to be used in a bioreactor for cellulose degradation. Further work on this system would include a more detailed mass balance and investigation into different operating conditions in the reactor. The reactor design should include a means to add and remove cellulose from the bed, most likely in the form of addition and removal as a slurry. The major obstacle with using hay as a feed source was the heterogeneous nature of it. This could be solved by milling the hay to a fine powder. The downside of this solution is the energy requirements to operate the mill. Another option for the using hay would be to separate the light leaves from the hard stalks and operate two parallel reactors. The two-reactor option would offer additional room for optimisation but would require

additional capital expenditure.

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Chapter 6

Concluding Remarks

6.1 Thesis Results

The work presented in this thesis has shown a new way to analyse biological systems with emphasis on the chemical engineering aspects. These methods offer an important insight into the overall workings and limitations of the biological processes without requiring a detailed understanding of the internal reactions and pathways.

As discussed in Chapter 2, there are many methods for calculating the thermodynamics properties of bacteria, however these methods are based on dry cells. Chapter 2 describes a method of using data from a typical fermentation experiment to predict the properties of the bacterial cells. This approach thus calculates the properties of cells as they are found in their natural environment. This method also predicts the maintenance energy requirements of the cells.

Chapter 3 uses the properties calculated in Chapter 2 to analyse the fermentation process. This chapter uses a simple approach to determine the limit of possible products that could be produced by fermentation. The important aspect of this method is that it is not limited to fermentation to a single product by can consider multiple products. In a similar manner the method can also be applied to consider the effects of fermentation on different feed materials. This method uses the ΔG-ΔH plot which helps understand the process in a visual way.

Chapter 4 extends the use of the ΔG-ΔH to the cases of photosynthesis and combustion. This chapter shows that photosynthesis and combustion can be treated as the same reaction with one being the reverse reaction of the other. In this analysis the irreversible nature of combustion is highlighted. In a similar manner it is shown that the core reaction in photosynthesis (i.e. the reverse combustion reaction) is close to reversible.

The work in this thesis was primarily built on data from the bacterium *Clostridium Thermolacticum* but given the sufficient experimental data the

methods can be applied to other systems. The main benefit of this work lies in the simplicity of the calculations and the diversity of the systems that it could incorporate.

The work in Chapter 5 shows that bacteria from termites can be used to degrade cellulosic material to produce fuel products or fuel precursors. The consortium of bacteria from the termite was used in a bioreactor which has not been done previously. The use of termite bacteria and other cellulosic microorganisms in bioreactors is an important step in the large scale processing of waste cellulose to fuel products.

6.2 Recommendations and Future Work

As discussed, the methods described in this work are not limited to one type of microorganism. Therefore it would be useful to produce experimental data for a wide range of microorganisms under a range of conditions and apply the analysis described in this work. The methods described also allow for higher degrees of accuracy in the calculations. Extending the methods with more sophisticated thermodynamic models of the system and more detailed mass balances will aid in the understanding of the system. However, the "bigger" picture should not be forgotten in favour of the detailed analysis.

Appendix A

Mass Balance Tables For a Continuous Reactor Using *Clostridium Thermolacticum*

Chapter 2 used experimental data by Collet, Adler, Schwitzguebel, and Peringer (2004) for the analysis of the continuous bioreactor. The data published by Collet et al. (2004) is shown in Table A.1. The data in Table A.1 can also be converted, using dilution rate and reactor volume, into molar flowrates as shown in Table A.2.

Flowrate (l/hr)	0.013	0.028	0.04	0.058	0.082	0.105	0.13	0.15	0.19
Mols In									
Lactose(mmol/l)	29	29	29	29	29	29	29	29	29
Mols Out (mmol/hr)									
Lactose (mmol/l)	1	3	7	12	16	19	21	23	26
Acetate (mmol/l)	28	26	25	21	16	13	10	8	4
Ethanol (mmol/l)	41	36	33	26	20	17	13	10	5
Lactate (mmol/l)	22	17	8	1	1	1	0	0	0
Hydrogen (mmol/l)	65	78	61	44	30	22	17	14	8
Total CO_2 (mmol/l)	94	93	82	65	48	43	31	27	18
Cells (mmol/l)	5.3	6.2	5.9	5.1	4.2	4.2	3.6	3.5	2.4

Table A.1: Mass balance per total liquid volume for a continuous reactor using *Clostridium Thermolacticum* as reported by Collet et al. (2004)

Flowrate (l/hr)	0.013	0.028	0.04	0.058	0.082	0.105	0.13	0.15	0.19
Mols In									
Lactose(mmol/l)	0.377	0.812	1.16	1.682	2.378	3.045	3.77	4.35	5.51
Mols Out									
Lactose (mmol/hr)	0.013	0.084	0.28	0.696	1.312	1.995	2.73	3.45	4.94
Acetate (mmol/hr)	0.364	0.728	1	1.218	1.312	1.365	1.3	1.2	0.76
Ethanol (mmol/hr)	0.533	1.008	1.32	1.508	1.64	1.785	1.69	1.5	0.95
Lactate (mmol/hr)	0.286	0.476	0.32	0.058	0.082	0.105	0	0	0
Hydrogen (mmol/hr)	0.845	2.184	2.44	2.552	2.46	2.31	2.21	2.1	1.52
Total CO_2 (mmol/hr)	1.222	2.604	3.28	3.77	3.936	4.515	4.03	4.05	3.42
Cells (mmol/hr)	0.07	0.17	0.24	0.30	0.35	0.44	0.47	0.52	0.45

Table A.2: Molar flow mass balance for a continuous reactor using *Clostridium Thermolacticum* calculated using data from Collet et al. (2004)

The data in Table A.2 was used in Chapter 2 to produce a reconciled mass balance as shown in Table A.4. For comparison to the original data by Collet et al. (2004) in Table A.1 the data in Table A.4 can be converted into a per total liquid volume basis as shown in Table A.3.

Flowrate (l/hr)	0.013	0.028	0.04	0.058	0.082	0.105	0.13	0.15	0.19
Mols In									
Lactose (mmol/l)	29	29	29	29	29	29	29	29	29
NH_4Cl (mmol/l)	47	47	47	47	47	47	47	47	47
Water (mmol/l)	55556	55556	55556	55556	55556	55556	55556	55556	55556
Mols Out									
Lactose (mmol/l)	1	3	7	12	16	19	21	23	26
NH_4Cl (mmol/l)	41.4	40.5	40.8	41.6	42.5	42.6	43.1	43.3	44.3
Water (mmol/l)	55399	55385	55401	55422	55452	55476	55485	55499	55523
CO_2 (mmol/l)	105	106	94	81	60	36	32	19	6
H_2 (mmol/l)	129	140	123	111	80	38	38	17	2
Ethanol (mmol/l)	41	36	33	26	20	17	13	10	5
Acetate (mmol/l)	28	26	25	21	16	13	10	8	4
Lactate (mmol/l)	22	17	8	1	1	1	0	0	0
Cells (mmol/l)	5.3	6.2	5.9	5.1	4.2	4.2	3.6	3.5	2.4
HCl (mmol/l)	5.3	6.2	5.9	5.1	4.2	4.2	3.6	3.5	2.4

Table A.3: Reconciled Mass balance calculated per total liquid volume for a continuous reactor using *Clostridium Thermolacticum*

Flowrate (l/hr)	0.013	0.028	0.04	0.058	0.082	0.105	0.13	0.15	0.19
Mols In									
Lactose (mmol/hr)	0.38	0.81	1.16	1.68	2.38	3.05	3.77	4.35	5.51
NH_4Cl (mmol/hr)	0.61	1.31	1.87	2.71	3.83	4.91	6.07	7.01	8.88
Water (mmol/hr)	722	1556	2222	3222	4556	5833	7222	8333	10556
Mols Out									
Lactose (mmol/hr)	0.013	0.084	0.280	0.696	1.312	1.995	2.730	3.450	4.940
NH_4Cl (mmol/hr)	0.54	1.13	1.63	2.41	3.48	4.47	5.60	6.49	8.42
Water (mmol/hr)	720	1551	2216	3214	4547	5825	7213	8325	10549
CO_2 (mmol/hr)	1.37	2.97	3.77	4.72	4.90	3.80	4.14	2.81	1.15
H_2 (mmol/hr)	1.68	3.92	4.91	6.42	6.52	4.03	4.90	2.62	0.40
Ethanol (mmol/hr)	0.53	1.01	1.32	1.51	1.64	1.79	1.69	1.50	0.95
Acetate (mmol/hr)	0.36	0.73	1.00	1.22	1.31	1.37	1.30	1.20	0.76
Lactate (mmol/hr)	0.29	0.48	0.32	0.06	0.08	0.11	0.00	0.00	0.00
Cells (mmol/hr)	0.07	0.17	0.24	0.30	0.35	0.44	0.47	0.52	0.45
HCl (mmol/hr)	0.07	0.17	0.24	0.30	0.35	0.44	0.47	0.52	0.45

Table A.4: Molar flow mass balance for the reconciled mass balance on the continuous reactor using *Clostridium Thermolacticum*

References

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Appendix B

Derivation of Carnot Temperature

Patel, Hildebrandt, and Glasser (2010) showed that for a chemical process there is a temperature called the Carnot temperature (T_c). This appendix shows the derivation of the Carnot temperature. A typical chemical process, as shown in Figure B.1 has material feeds into the system and product feeds out of the system and a energy requirement (Q). The heat into the system (Q) is assumed to be that the temperature of the process at the point of entry. The energy and entropy balances over the system are:

$$H_{in} + Q(T) = H_{out} \tag{B.1}$$

$$S_{in} + \frac{Q(T)}{T} = S_{out} + S_{lost} \tag{B.2}$$

(B.1) - T_0 (B.2)

$$H_{in} + Q(T) - T_0 S_{in} - Q(T)\frac{T_0}{T} = H_{out} - T_0 S_{out} - T_0 S_{lost} \tag{B.3}$$

$$[H_{in} - T_0 S_{in}] + Q(T) - Q(T)\frac{T_0}{T} = [H_{out} - T_0 S_{out}] - T_0 S_{lost} \tag{B.4}$$

Given $G = H - TS$ (B.4) becomes:

$$G_{in} + Q(T) = G_{out} + Q(T)\frac{T_0}{T} - T_0 S_{lost} \tag{B.5}$$

$$-\Delta G + Q(T) = Q(T)\frac{T_0}{T} - T_0 S_{lost} \tag{B.6}$$

Where $\Delta G = G_{out} - G_{in}$

From (B.1) where $\Delta H = H_{out} - H_{in}$:

$$\Delta H = Q(T) \tag{B.7}$$

Substituting (B.7) into (B.6) yields:

$$\Delta H = \Delta G + \Delta H \frac{T_0}{T} - T_0 S_{lost} \tag{B.8}$$

$$\Delta G = \Delta H - \Delta H \frac{T_0}{T} - T_0 S_{lost} \tag{B.9}$$

$$= \Delta H(1 - \frac{T_0}{T}) - T_0 S_{lost} \tag{B.10}$$

$T = T_c$ when $S_{lost} = 0$

$$\Delta G = \Delta H(1 - \frac{T_0}{T_c}) \tag{B.11}$$

$$T_c = \frac{T_0}{(1 - \frac{\Delta G}{\Delta H})} \tag{B.12}$$

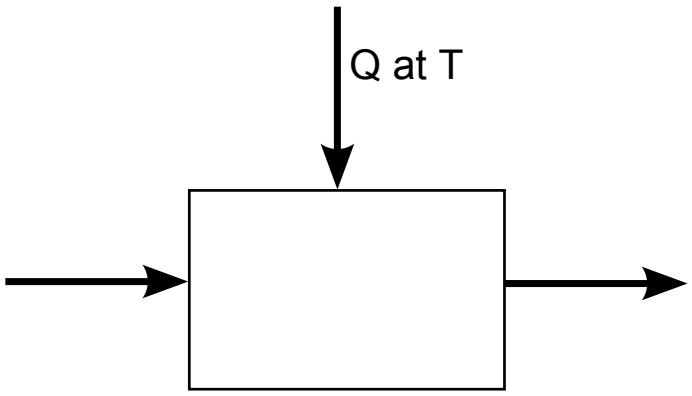

Figure B.1: Representation of a typical chemical process with a heat input at temperature T

References

Patel, B., D. Hildebrandt, and D. Glasser (2010). An overall thermodynamic view of processes: Comparison of fuel producing processes. *Chemical Engineering Research and Design 88*, 844–860.

Appendix C

Thermodynamic Data For Photosynthesis/Combustion Reactions

Chapter 4 uses thermodynamic data to show the relationship between the combustion reaction and photosynthesis. Table C.1 lists the chemical formula and the data as used in Chapter 4. The data is calculated using the properties of formation ($H_{f,i}$ and $G_{f,i}$) obtained from Sandler (1999). Figure C.1 shows the same data as Figure 4.2. The entropy of reaction is calculated using Equation (C.1) and is shown in Figure C.2 which reflects the same data as Figure 4.3. Figure C.3 and Figure C.4 correspond to Figure 4.4 A & B respectively.

$$\Delta S = \frac{\Delta H - \Delta G}{T_0} \tag{C.1}$$

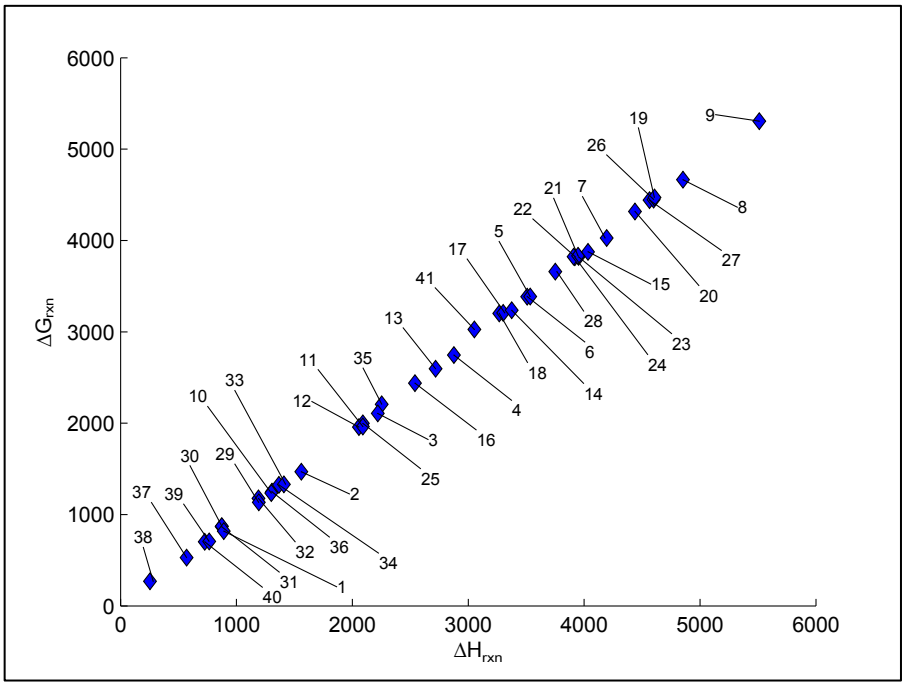

Figure C.1: Annotated ΔG-ΔH plot as shown in Figure 4.2

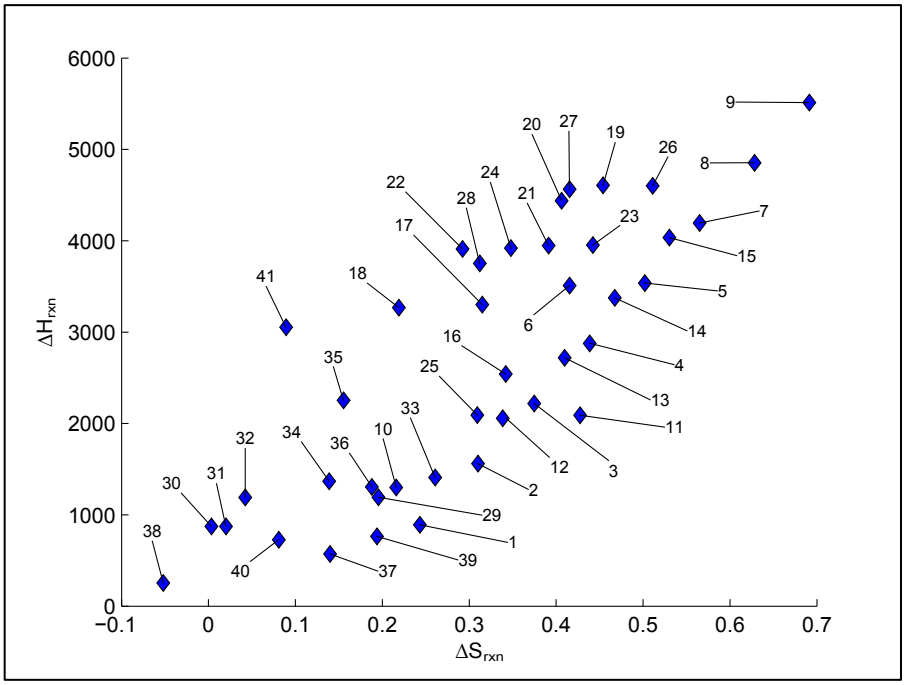

Figure C.2: Annotated ΔH-ΔS plot as shown in Figure 4.3

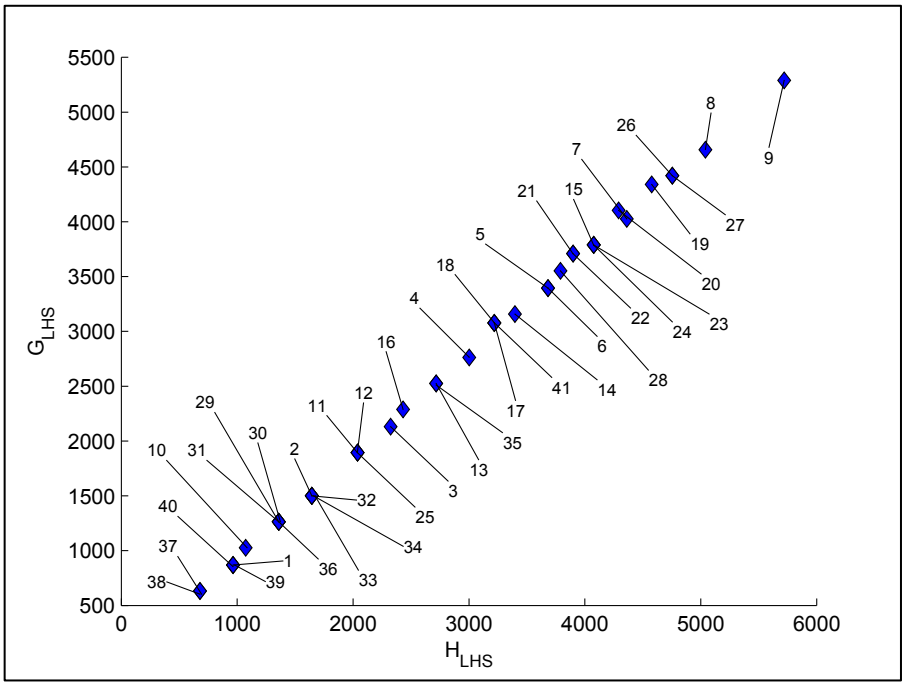

Figure C.3: Annotated ΔG_{LHS}-ΔH_{LHS} plot as shown in Figure 4.4A

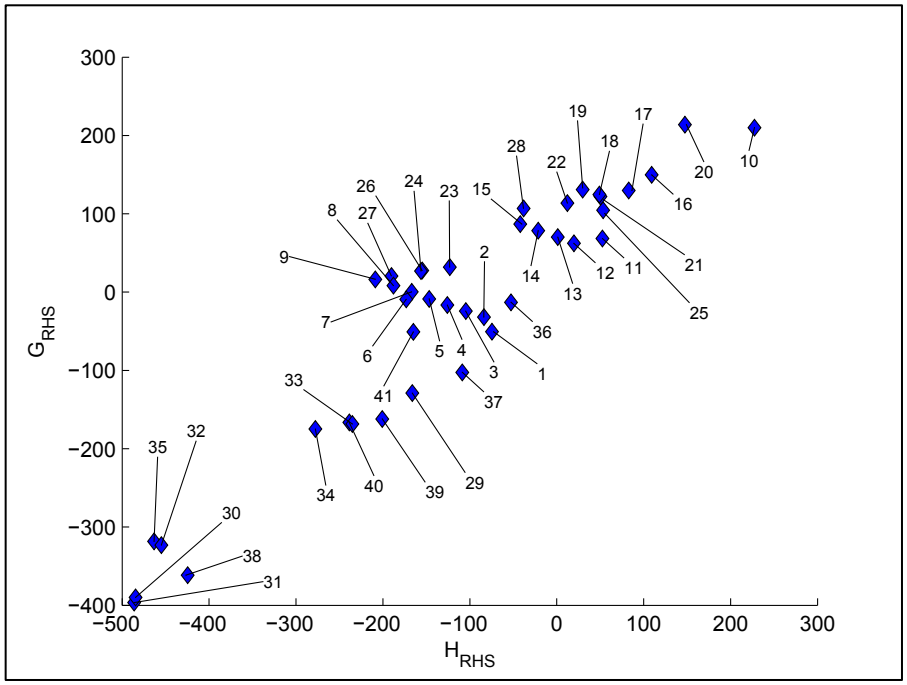

Figure C.4: Annotated ΔG_{RHS}-ΔH_{RHS} plot as shown in Figure 4.4B

Table C.1: Thermodynamic data for the photosynthesis and combustion reaction for various compounds

No.	Chemical	ΔH_{rxn}	ΔG_{rxn}	ΔS_{rxn}	H_{LHS}	H_{RHS}	G_{LHS}	G_{RHS}
1	CH_4	890.6	818.1	0.243	965.1	-74.5	868.6	-50.5
2	C_2H_6	1560.6	1468.2	0.31	1644.4	-83.8	1500.1	-31.9
3	C_3H_8	2219	2107.3	0.375	2323.7	-104.7	2131.6	-24.3
4	C_4H_{10}	2877.2	2746.5	0.439	3003	-125.8	2763.1	-16.6
5	C_5H_{12}	3535.5	3385.9	0.502	3682.3	-146.8	3394.6	-8.7
6	C_5H_{12}	3509.2	3385.4	0.415	3682.3	-173.1	3394.6	-9.2
7	C_6H_{14}	4194.7	4026.3	0.565	4361.6	-166.9	4026.1	0.2
8	C_7H_{16}	4853.1	4665.9	0.628	5040.9	-187.8	4657.6	8.3
9	C_8H_{18}	5511.4	5305.4	0.691	5720.2	-208.8	5289.1	16.3
10	C_2H_2	1300.3	1235.9	0.216	1072.8	227.5	1025.9	210
11	C_3H_6	2090.4	1963	0.428	2037.9	52.5	1894.5	68.5
12	C_3H_6	2057.6	1956.7	0.339	2037.9	19.7	1894.5	62.2

Table C.1 – continued from previous page

No.	Chemical	ΔH_{rxn}	ΔG_{rxn}	ΔS_{rxn}	H_{LHS}	H_{RHS}	G_{LHS}	G_{RHS}
13	C_4H_8	2718.4	2596.3	0.41	2717.2	1.2	2526	70.3
14	C_5H_{10}	3375.2	3235.9	0.467	3396.5	-21.3	3157.5	78.4
15	C_6H_{12}	4033.8	3875.8	0.53	4075.8	-42	3789	86.8
16	C_4H_6	2540.6	2438.7	0.342	2431.4	109.2	2288.9	149.8
17	C_6H_6	3301.3	3207.4	0.315	3218.4	82.9	3077.7	129.7
18	C_6H_6	3267.5	3202.2	0.219	3218.4	49.1	3077.7	124.5
19	C_8H_{10}	4606.9	4471.6	0.454	4577	29.9	4340.7	130.9
20	C_8H_8	4438.6	4317.5	0.406	4291.2	147.4	4103.6	213.9
21	C_7H_8	3947.9	3831.3	0.391	3897.7	50.2	3709.2	122.1
22	C_7H_8	3909.9	3822.8	0.292	3897.7	12.2	3709.2	113.6
23	C_6H_{12}	3952.7	3820.9	0.442	4075.8	-123.1	3789	31.9
24	C_6H_{12}	3919.6	3815.9	0.348	4075.8	-156.2	3789	26.9
25	C_3H_6	2091.2	1999	0.309	2037.9	53.3	1894.5	104.5
26	C_7H_{14}	4600.3	4448	0.511	4755.1	-154.8	4420.5	27.5
27	C_7H_{14}	4564.9	4441.1	0.415	4755.1	-190.2	4420.5	20.6
28	C_6H_{10}	3751.8	3658.8	0.312	3790	-38.2	3551.9	106.9
29	C_2H_4O	1192.4	1134.1	0.196	1358.6	-166.2	1263	-128.9
30	CH_3COOH	874.1	873.1	0.003	1358.6	-484.5	1263	-389.9
31	CH_3COOH	872.5	866.5	0.02	1358.6	-486.1	1263	-396.5
32	$C_2H_6O_2$	1189.6	1177	0.042	1644.4	-454.8	1500.1	-323.1
33	C_2H_6O	1409.3	1331.6	0.261	1644.4	-235.1	1500.1	-168.5
34	C_2H_6O	1366.7	1325.3	0.139	1644.4	-277.7	1500.1	-174.8
35	$CH_3COOC_2H_5$	2253.9	2207.6	0.155	2717.2	-463.3	2526	-318.4
36	C_2H_4O	1306	1250	0.188	1358.6	-52.6	1263	-13
37	CH_2O	570.7	529	0.14	679.3	-108.6	631.5	-102.5
38	HCOOH	254.6	270.1	-0.052	679.3	-424.7	631.5	-361.4

Table C.1 – continued from previous page

No.	Chemical	ΔH_{rxn}	ΔG_{rxn}	ΔS_{rxn}	H_{LHS}	H_{RHS}	G_{LHS}	G_{RHS}
39	CH_4O	764.4	706.6	0.194	965.1	-200.7	868.6	-162
40	CH_4O	726.4	702.3	0.081	965.1	-238.7	868.6	-166.3
41	C_6H_5OH	3053.4	3026.8	0.089	3218.4	-165	3077.7	-50.9

References

Sandler, S. I. (1999). *Chemical and Engineering Thermodynamics* (Third ed.). New York: Wiley.