THEORETICAL AND EXPERIMENTAL STUDY OF BIOBASED SUCCINIC ACID PRODUCTION

A Thesis

by

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ABSTRACT

Biomass based succinic acid is gaining increasing interest as a potential platform chemical for replacing a large petroleum-based bulk chemical market. Biomass as a renewable resource has proved the economic and sustainable potential to produce succinic acid by fermentation method. Biobased succinic acid has yet faced with the challenge of becoming competitive with petrochemical method because of its higher production cost.

To lower the production cost, extensive research efforts have been undertaken in upstream technology that involves strain development via metabolic engineering, and downstream technology that aims to improve efficiency of purification method. Many research studies have focused on either one of two technological areas, with little interest on interaction between them.

This present work integrates the processing steps from upstream and downstream technologies using a systematic approach and presents an optimal production pathway from a large number of possible process configurations. The development of such a process pathway involves selection of bioproducts, feedstock, pre-treatment technology, microorganism and product separation method. Performance criteria such as titre, rate, yield and minimum production cost, express the optimality of production pathway.

Optimization study indicates that succinic acid seems to be the most promising bioproduct among all other bioproducts. Corn stover is the suitable feedstock to produce succinic acid.

Based on the findings from optimization study, experimental work was performed with an aim of achieving better performance criteria than it is reported in literature. This work selected corn stover as feedstock, and a bacterium called, *Basfia succiniciproducens* for converting corn stover-derived glucose into succinic acid. To date, no deliberate experiment has been done on this bacterium to improve succinic acid production, despite its promising features. Highest succinic acid yield of 18 g/100g total

sugar (glucose plus xylose) was observed in this experiment. Genetically modified strain of the bacterium reported a much higher yield of 71 gm succinic acid/ 100gm of glucose.

DEDICATION

To:

My parents

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All work for the thesis was completed independently by the student.

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NOMENCLATURE

AA	Acetic Acid
ACE	ACEtate
AcCoA	Acetyl Co-enzyme A
ATP	Adenosine-Tri-Phosphate
ADA	ADipic Acid
ABXU	Acid Birchwood Xylanase Units
Bio	Biomass
BDO	1-4, ButaneDiOl
C	Concentration (g/L)
CO_2	Carbon dioxide
CRF	Capital Recovery Factor
DOE	Department Of Energy
DHAP	DiHydroxyAcetone Phosphate
EDP	Entner–Doudoroff Pathway
EIA	Energy Information Administration
E4P	d-Erythrose-4-Phosphate
ETH	ETHanol
EU	European Union
equ.	equation
FA	Formic Acid
F6P	Fructose-6-Phosphate
F1,6P	d-Fructose-1,6-isPhosphate
FUM	FUMarate
FOR	FORmate
FDCA	2,5-Furan DiCarboxylic Acid
FCI	Fixed Capital Investment
GLC	GLUcose

G6P	Glucose-6-Phosphate,
GHG	GreenHouse Gas
GA3P	GlycerAldehyde 3-Phosphate
HMF	Hydroxy Methyl Furfural
HPLC	High Performance Liquid Chromatography
3 HP	3-HydroxyPropionic acid
ISCC	International Sustainability & Carbon Certification
LA	Lactic Acid
LCA	Life Cycle Analysis
MAL	MALate
MJ	Mega Joule
MM	million
mM	milliMolar
NREL	National Renewable Energy Laboratory
na	not available
NADH	reduced-Nicotinamide Adenine Dinucleotide
NADPH	reduced-Nicotinamide Adenine Dinucleotide Phosphate
NLP	Non-Linear Programming
NREL	National Renewable Energy Laboratory
NPV	Net Profit Value
OAA	OxAloAcetate
OD	Optical Density
1,3PG	3-Phospho-d-Glyceroyl phosphate
3PG	3-Phospho-d-Glycerate
2PG	2-Phospho-d-Glycerate
PEP	PhosphoEnolPyruvate
PYR	PYRuvate
PE	Poly Ethylene
PEP	PhosphoEnolPyruvate

PPP	PhosPhate Pathway
PHB	Poly Hydroxy Butyrate
PLA	PolyLactic Acid
PHAs	Poly Hydroxy Alkanoates
PNNL	Pacific Northwest National Laboratory
PTFE	PolyTetraFluoroEthylene
RU5P	d-RibUlose-5-Phosphate
R5P	d-Ribose-5-Phosphate
RSB	Roundtable on Sustainable Biomaterials
RFS2	Renewable Fuels Standard program
SA	Succinic Acid
SUC	SUCcinate
S7P	d-Sedoheptulose-7-Phosphate
SLP	Sequential Linear Programming
TAC	Total Annualised Cost
TACC	Total Annualised Capital Cost
TAOC	Total Annualised Operating Cost
TRL	Technology Readiness Level
TRY	Titre, Rate, and Yield
TCA	TriCarboxylic Acid
TSB	Tryptone Soya Broth
THFA	TetraHydroFurfuryl Alcohol
THF	TetraHydroFuran
vvm	Volume of gas per Volume of liquid per Minute
XYL	XYLose
X5P	Xylulose-5-Phosphate

Parameters/ Variables

b	index for biomass feedstock
B_b^{Bio}	available total flow rate of biomass feedstock b
$E_s^{Prod'}$	cost of product s'
E_b^{Bio}	cost of biomass feedstock b
E_{bq}^{Cap}	capital cost for the conversion of biomass feedstock b
$E_{sq}^{Cap'}$	capital cost for the conversion of intermediate s
E_{bq}^{Opr}	operating cost for the conversion of biomass feedstock b
$E_{sq}^{Opr'}$	operating cost for the conversion of intermediate s
F^{II}_{sq}	Intermediate flowrate s of conversion pathway s
GP^{Total}	gross profit
R^{I}_{bqs}	Conversion rate of conversion technology q
$R_{II}^{sq'}$	Conversion rate of conversion technology q'
s	index for intermediate product
s'	index for product
$T_{s'}^{Prod}$	Total production rate of intermediate product s'
T_s^{Inte}	Total production rate of product s
V	total Volume
w	initial weight (g) of glucose or xylose
q	biomass conversion technology
q'	intermediate conversion pathway

Units

gal	gallon
GB	GigaByte
h	hour
kg	kilogram
ktpa	Kilo tons per annum
L or l	Litre

m	meter
MJ	MegaJoule
MMBtu	Metric Million British thermal unit
MMGPY	Metric Million Gallon Per Year
MW	MegaWatt
SCF	Standard Cubic Foot
s	seconds
Twa	Terra watt
t	tonne, equal to 1,000 kilograms.
y	year
kWh	kilo-Watt hour
TWa	Terra Watt

TABLE OF CONTENTS

Page

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGEMENTS v

CONTRIBUTORS AND FUNDING SOURCES vi

NOMENCLATURE vii

TABLE OF CONTENTS xii

LIST OF FIGURES xiv

LIST OF TABLES xvi

1. INTRODUCTION 1

    1.1 Sustainable potential of biomass for fuels and chemicals 2
    1.2 Why bioproduct, not biofuel? 3
    1.3 Market potential 5
    1.4 The limitation of traditional petrochemical processes 8
    1.5 New routes to succinic acid 8
    1.6 Succinic acid production by microbial fermentation 9
    1.7 Process schematic and description 9
    1.8 Problem statement 12
    1.9 Research objective 13
    1.10 Thesis outline 14

2. OPTIMAL DESIGN OF A BIOREFINERY 15

    2.1 Potential bioproducts 17
    2.2 Market analysis 18
    2.3 Mathematical formulation for biorefinery model 22
    2.4 Case studies 26

3. IDENTIFICATION OF A SUITABLE FEEDSTOCK FOR SUCCINIC ACID PRODUCTION 48

    3.1 Selection of a suitable feedstock 48

4. EXPERIMENTAL STUDY OF SUCCINIC ACID PRODUCTION 53
4.1 Materials and methods for pretreatment process 53
4.2 Materials and methods for fermentation process 55
4.3 Results and discussions 64
4.4 Future research directions 67
5. CONCLUSION 70
REFERENCES 72
APPENDIX A 79
APPENDIX B 81
APPENDIX C 85
APPENDIX D 86
APPENDIX E 91

LIST OF FIGURES

Page

Figure 1. Succinic acid derivatives 2
Figure 2. Biomass availability 3
Figure 3. Value in a barrel of crude oil 4
Figure 4. Proportion of biofuels & bio-products by industry's product offering 5
Figure 5. Forecasted global bio-succinic acid by application in the year 2020 6
Figure 6. Process flow scheme for 1,4-butanediol 6
Figure 7. Process flow scheme of biobased succinic acid production 11
Figure 8. Thesis work-flow chart 14
Figure 9. General representation of integrated biorefinery 16
Figure 10. Market price and market volume 19
Figure 11. Typical representation of biorefinery for mathematical formulation 22
Figure 12. Production pathway of case study 1 28
Figure 13. Commercialization status of priority based bioproducts 30
Figure 14. Case study 2: production pathway for a maximum yield, (a) when the feedstock is mixed food waste, (b) corn stover, (c) rapeseed. 32
Figure 15. Single feedstock scenario for maximum profit 35
Figure 16. Production pathway for a single feedstock scenario 36
Figure 17. Maximum profit for a mixture of two types feedstocks 37
Figure 18. Production pathway for two-feedstocks scenario 38
Figure 19. Maximum profit for a mixture of three types feedstocks 39
Figure 20. Percentage change in GHG emission 41
Figure 21. Percentage change in energy use 42
Figure 22. Problem statement for finding optimal pathway 44
Figure 23. Superstructure of succinic acid production 46
Figure 24. Optimal production pathway for succinic acid production 47
Figure 25. Metabolic network analysis of B.Succiniciproducens 57

Figure 26. Bio-fermenter 62
Figure 27. Time course of total sugar yield from corn stover 65
Figure 28. Succinic acid yield as a function of time 66

LIST OF TABLES

Page

Table 1. Estimated prices and market volumes of succinic acid in the year 2014 5
Table 2. List of commercial companies producing bio-based succinic acid..... 7
Table 3. List of potential products identified by US DOE and EU 17
Table 4. Biobased chemicals-this study 18
Table 5 Classification of potential products..... 20
Table 6. Market demand of biobased products 21
Table 7. Basis and assumptions of the financial parameters..... 25
Table 8. List of case studies 26
Table 9. Case study 1 (maximum profit) results 27
Table 10. Sub-categories of case study 1 29
Table 11. Succinic acid value per lb of succinic acid..... 31
Table 12. Case study 2 results - maximum yield of succinic acid..... 32
Table 13. Composition of feedstocks..... 34
Table 14. Results of case study 3 - single feedstock scenario..... 35
Table 15. Case study 3 - feedstock as a mixture of two types feedstocks 37
Table 16. Case study 3 - feedstock as a mixture of three types of biomass..... 39
Table 17. Summary of weightage assigned to each critical factor 48
Table 18. Cost, yield, and availability of biomass as reported in literature..... 50
Table 19. Ranking of feedstocks based on biomass availability in the world 51
Table 20. Ranking of feedstocks based on biomass availability in the U.S.A..... 52
Table 21. Chemical reactions of glycolysis 56
Table 22. Chemical reactions of TCA cycle 58
Table 23. Fermentation medium selected for this study 60
Table 24. Components of inoculation medium 61
Table 25. Composition of corn stover..... 63
Table 26. Results obtained in hydrolysis of corn stover..... 65

Table 27. Results obtained in fermentation of corn stover 66
Table 28. Cost of succinic acid production 68
Table 29. Composition of biomass feedstocks 79
Table 30. List of conversion technologies and its details 81
Table 31. Market price of bioproduct and feedstocks 85
Table 32. List of pretreatments and its yield for various agricultural residues 86
Table 33. Lignocellulosic biomass pretreatment technologies 90
Table 34. List of biobased succinic acid production methods 92

1. INTRODUCTION

Succinic acid has been identified by the U.S. Department of Energy (USDOE) as one of the top 12 biobased platform chemicals in the year 2004. Since then, extensive research has been carried out to produce succinic acid by fermentation [1,2]. Main driving forces behind this research interest are to replace the petrochemical products with their equivalent biobased products, and to achieve economic benefits of bioproducts. Succinic acid was ranked first among all other high prioritized chemicals based on some key deciding factors such as market data, building block of chemical and commercial deployment of technology [1].

Succinic acid ($C_4H_6O_4$, molecular weight =118.09 g/mol), a dicarboxylic acid, is a potential platform chemical to produce various high value-added products such as food, pharmaceutical products, surfactants, detergents, plastics, and ingredients to stimulate animal and plant growth.

Succinic acid can also be used as a precursor for many industrial chemicals as shown in Figure 1. Due to its versatile applications, succinic acid is rising to a bulk chemical in recent years. These applications could potentially lead to a market of several tons of succinic acid [3,5,8].

Green technology deals with producing biomass crops and converting them into products and energy. One of such technologies is the fermentation method that valorises biomass (green) and fixes CO_2 to produce the platform chemicals. In this way, fermentation also creates a carbon-negative cycle, reducing CO_2 emission. Green technology is therefore becoming a driving force in the chemical industry because of the declining fossil fuel reserve, and increasing concern on global climate change caused by pollutants from petrochemical technology. There is a necessity to replace the replenishing fossil-based hydrocarbon economy with renewable resource based economy.

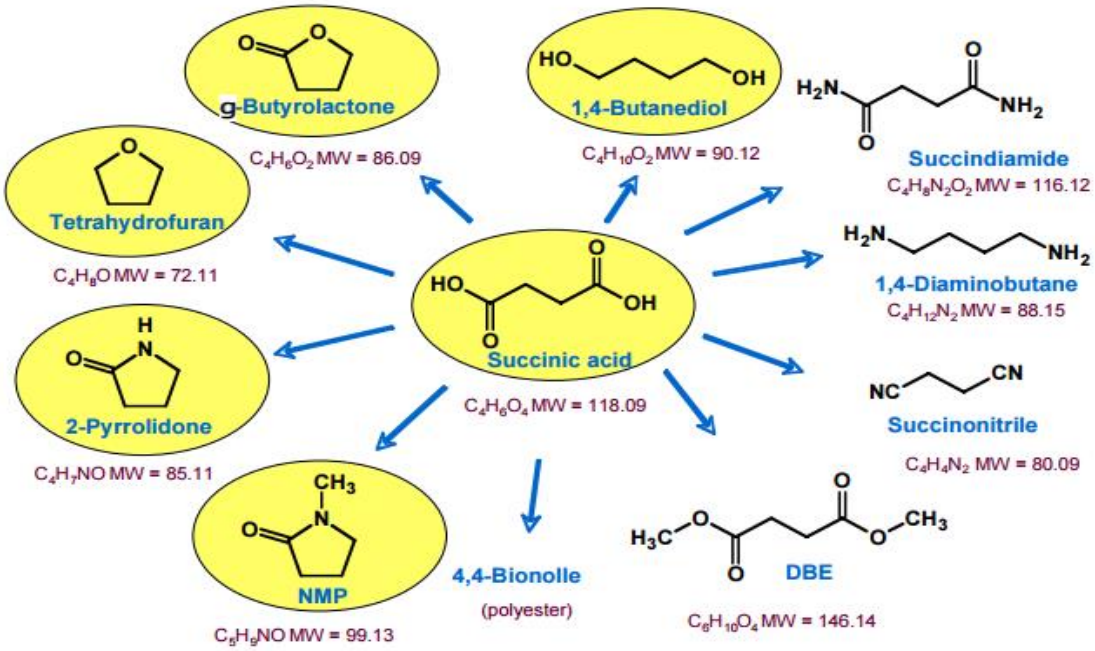


Figure 1. Succinic acid derivatives [3,4,5]

1.1 Sustainable potential of biomass for fuels and chemicals

The 2016 Billion Ton Report concluded that the United States has the potential to sustainably produce one billion tons of biomass, while meeting food needs. As shown in Figure 2, the report forecasts 1 to 1.2 billion tons of sustainable biomass by 2030, and 1.2 to 1.5 billion tons by 2040 [26]. Key biomass feedstocks include energy crops (e.g. miscanthus, switch grass), agricultural residue (e.g. corn stover, corn fibre, sugarcane bagasse) and various industrial waste and by-product streams (e.g. sugar cane molasses, cheese whey, crude glycerol from biodiesel production).

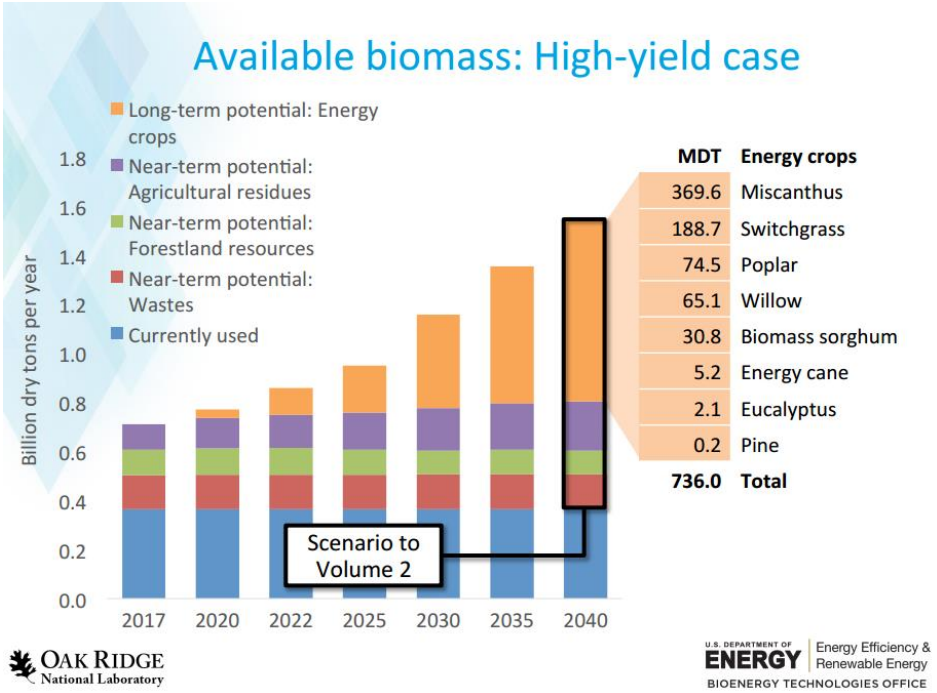


Figure 2. Biomass availability [26]

1.2 Why bioproduct, not biofuel?

Biomass has the potential to dramatically reduce dependence on foreign oil for fuels and chemicals, however it is not possible to replace the entire petroleum-based infrastructure with a biomass-based infrastructure. Enthalpy estimate of the annually produced total biomass is approximately 100 Twa [78]. This estimate considers the biomass derived from global photosynthetic activities.

However, sustainably grown biomass corresponds to a smaller amount of only about approximately 3 Twa [78]. The annual worldwide energy consumption today is about 16 Twa [78]. The world energy demand is much higher than energy potential of biomass. Thus, the biomass production is not likely to be a primary energy source that could replace entire fossil fuel.

The efficient use of biomass is therefore for a variety of specialty and commodity chemicals. Although 70.6 percent of a barrel of oil that is converted into fuels is worth

$385 billion annually, the 3.4 percent that is converted into petrochemicals is annually worth approximately $375 billion (Figure 3). Exploiting the enormous value of petrochemicals and specialty chemicals becomes the main goal of biomass utilization [27].

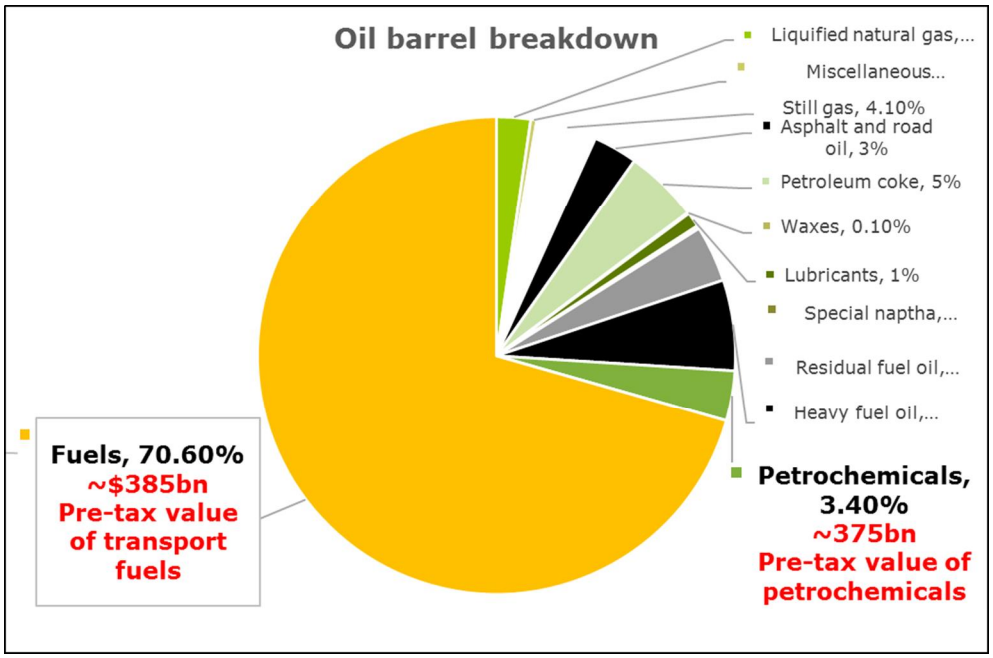


Figure 3. Value in a barrel of crude oil [27]

Because of the great recession between the year 2007 and 2009, the biofuels industry has been shifting their product offering from fuel related technology to specialty chemicals production. As of 2015, up to 54% of the biofuels industry has increased its product offering from biofuels production and relevant technology to a mix of bioproducts such as di-carboxylic acids and high value alcohols, when compared with less than 13% of such bioproducts offerings in 2012 (Figure 4).

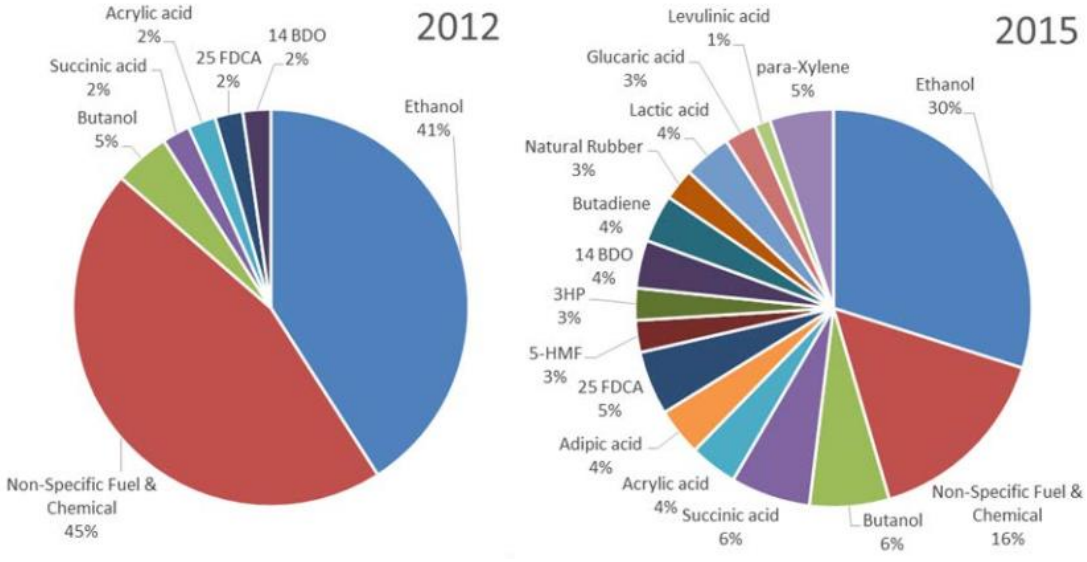


Figure 4. Proportion of biofuels & bio-products by industry's product offering [79]

1.3 Market potential

In the year 2013, total succinic acid production from petrochemical and biobased method was approximately 76 kilotons. Global production of bio-based succinic acid (SA) was 38 kilotons, constituting 49% of total market (Table 1). The current market price of biobased succinic acid is approximately $2940/ton, while the fossil-based equivalent is valued at around $2860/ton. The succinic acid market is expected to reach 600,000 tons by 2020 with a projected market size of $1 billion.

Table 1. Estimated prices and market volumes of succinic acid in the year 2014

Product	Bio-based market				Total market (bio+fossil)			Ref.
	Price ($/t)	Volume (ktpa)	Sales (m$/y)	% of total market	Price ($/t)	Volume (ktpa)	Sales (m$/y)	
Succinic acid	2,940	38	111	49%	2,860	76	191	[7 to 13]

By the year 2020, more than half of total succinic acid production finds applications in 1-4 butanediol (BDO) production (Figure 5).

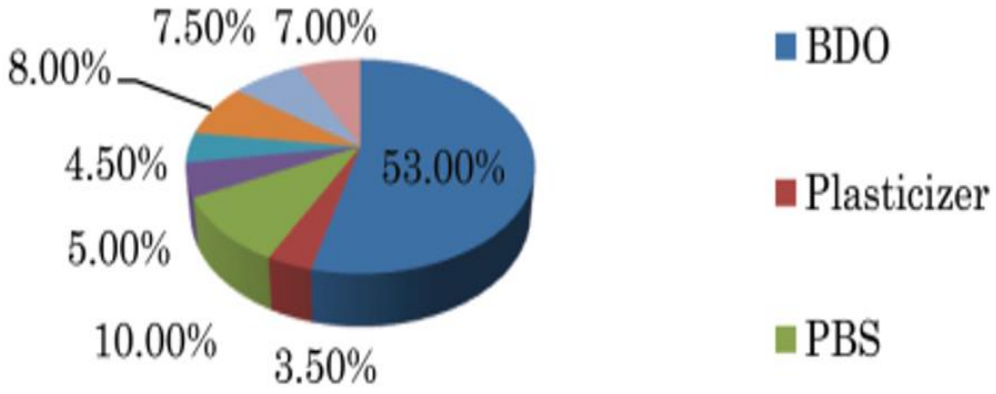


Figure 5. Forecasted global bio-succinic acid by application in the year 2020 [27]

1,4-butanediol (BDO) is comprised of a butane chain of four carbon groups with an alcohol(-OH) group bound to each terminal carbon of this chain. It is an odourless, thick, colourless, organic compound, generally used in the production of plastics, solvents, sportswear, and recreational drugs among others.

Petrochemical method produces BDO from butane, which is now replaced by two biological methods; 1. direct conversion of glucose into BDO using engineered E-coli, 2. conversion of glucose into BDO via succinic acid. Succinic acid on catalytic hydrogenation produces 1,4-butanediol (BDO) as shown in Figure 6.

Figure 6. Process flow scheme for 1,4-butanediol

The market potential and advantages presented by bioprocessing have led to investment by several companies (Table 2). The current Technology Readiness Level (TRL) of succinic acid production is TRL 8 that represents commercialised status of the technology. Manufacturing facilities were constructed in Europe and North America [9].

Table 2. List of commercial companies producing bio-based succinic acid

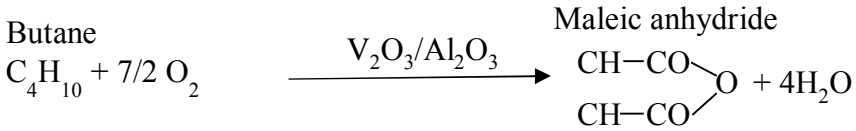
Company	Capacity t/y	Raw material	Fermentation/ Microorganism	Downstream recovery	Investment made in	Ref.
BioAmber (DNP/ard)	3000	Wheat glucose	E. coli	Electrodialysis	Europe, Pomacle, France	[18, 19]
BioAmber, Mitsui	30,000–50,000	Corn glucose	Low pH culture is targeted using Candida krusei	Direct succinic acid separation when low Ph conditions are used	Sarnia, Ontario, Canada	[9,18]
BioAmber, Mitsui	70,000–200,000	–	–	–	North America	[18]
Reverdia (Roquette & DSM)	10,000	Starch/ sugars	Low pH culture is targeted by *S.cerevisiae*	Direct separation of succinic acid	Cassano Spinola, Italy	[9,18]
Myriant, ThyssenKrupp	1000	Glucose	*E. coli*	Ammonia precipitation	Leuna, Germany	[18]
Myriant	14,000	Corn glucose	*E. coli*	Ammonia precipitation	Louisiana, USA	[9,18, 19]
Succinity (BASF & Corbion-Purac)	10,000	Glycerol/ sugars	*B. succiniciproducens*	Magnesium hydroxide as neutralizer followed by recycling	Montmelo, Spain	[9,18]

1.4 The limitation of traditional petrochemical processes

Succinic acid has been traditionally produced by petrochemical technology. Georgius Agricola first purified succinic acid from amber (Amber is a fossilized tree resin, known for its colour and beauty) in 1546. Since then, succinic acid has been produced by microbial fermentation [3]. In the early years of 1980, the industrial potential of microbial fermentation was discovered to produce succinic acid on a commercial scale [3].

Currently, petrochemical process provides fifty percentage of world's succinic acid production. In this process, a petroleum derivative, butane is used as a starting material to produce succinic acid in two steps; 1. partial oxidation of butane into maleic anhydride (equ. 1.1), 2. hydrogenation of the maleic anhydride into succinic acid (equ.1.2).

Partial oxidation of butane in fluidized bed catalytic reactor



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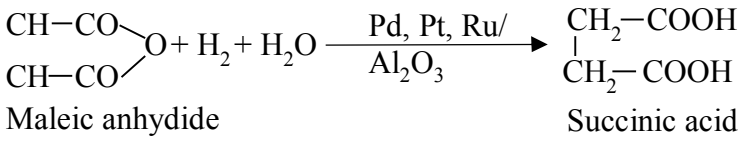


(1.2)

Hydrogenation of maleic hydride at 15 bar, 150°C, followed by intensive cooling

This petrochemical process is commercially well established, but the overall yield is less than 40% and purity of succinate is relatively low, which makes the purification of succinic acid expensive.

1.5 New routes to succinic acid

The economic potential of succinic acid drives research interest towards finding new conversion pathways to produce succinic acid. Among them, conversion of different feedstocks to succinic acid is gaining much attention towards future research studies.

The feedstocks include glucose (equ. 1.3), glycerol (equ. 1.4), ethanol (equ. 1.5), and a mixture of glucose and ethanol (equ. 1.6).

Glucose — Succinic acid

$$CH_2O + 1/7\ CO_2 \longrightarrow 8/7\ CH_{3/2}O + 1/7\ H_2O \quad (1.3)$$

Glycerol

$$CH_{8/3}O + 1/3\ CO_2 \longrightarrow 4/3\ CH_{3/2}O + 1/3\ H_2O \quad (1.4)$$

Ethanol

$$CH_3O_{1/2} + 5/7\ CO_2 \longrightarrow 12/7\ CH_{3/2}O + 3/14\ H_2O \quad (1.5)$$

Glucose Ethanol — Acetic acid

$$¾\ CH_2O + CH_3O_{1/2} + 3/4\ CO_2 \longrightarrow 2\ CH_{3/2}O + 1/2\ CH_2O + ¼\ H_2O \quad (1.6)$$

1.6 Succinic acid production by microbial fermentation

The fermentative production of succinic acid has been most extensively investigated with several bacteria that are capable of producing large amounts of succinic acid. The bacteria include *Basfia succiniciproducens, Actinobacillus succinogenes, Anaerobiospirillum succiniciproducens Mannheimia succiniciproducens*, and recombinant *Escherichia coli* [23,24,25]. The microbial conversion of biomass into succinic acid presents an environmentally friendly and energy-saving process.

1.7 Process schematic and description

As shown in Figure 7, Succinic acid is mainly produced in three steps: conversion feedstock into reduced sugar (C5, C6 sugar), fermentation of reduced sugar into crude succinic acid, purification of crude succinic acid.

First, the biomass feedstock is processed thorough sequential stages of pre-treatment; mechanical, chemical, and biochemical type of treatment. In the mechanical method, milling reduces size of feedstock to increase surface area which in turn improves efficiency of pre-treatment. After milling, the feedstock is treated by chemical method where smaller size feedstock is mixed with chemicals (e.g. H_2SO_4, NaOH). This prepares the biomass to be fermentable by enzymes in the downstream process. The

enzymatic hydrolysis converts the biomass into reduced sugar (e.g. glucose, xylose, arabinose).

Secondly, the reduced sugar is converted into succinic acid by an anaerobic or aerobic microorganism through fermentation process. Many naturally producing and genetically modified microbes have been extensively studied to ferment various agricultural biomass feedstocks (Appendix E). The crude succinic acid in fermentation broth contains many by-products and contaminants. In the final step, purification or separation stage separates pure succinic acid from the undesired components and contaminants.

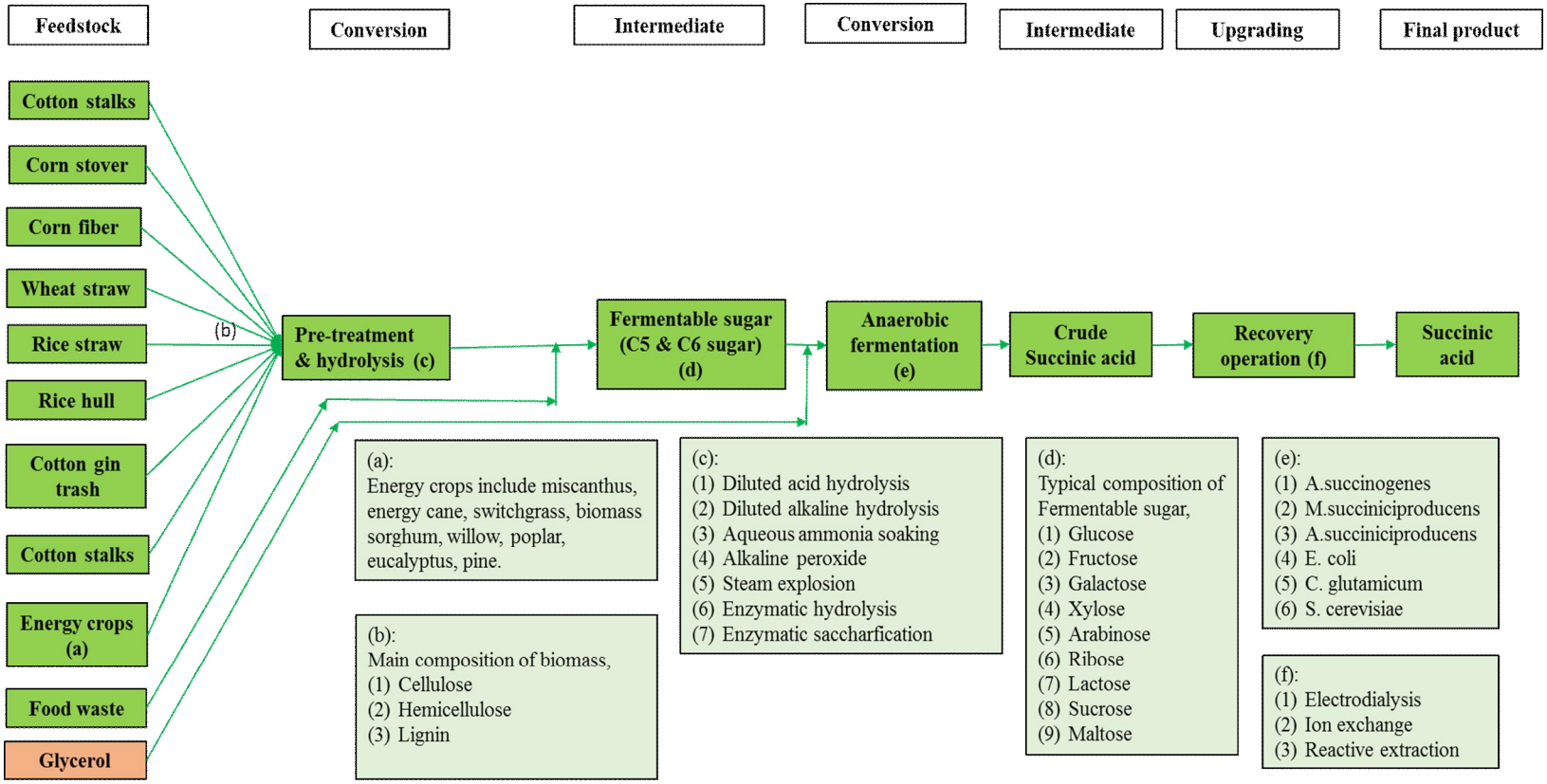


Figure 7. Process flow scheme of biobased succinic acid production

1.8 Problem statement

Although the cost of producing succinic acid by biological method is nearly competitive with petrochemical method, the biological method contributes almost half of world's succinic acid production (Table 1). Considering potential application of the succinic acid in bulk chemical market, the market demand is expected to increase at a rate of fifteen times the current market demand by the year 2020. The projected market demand considers an optimistic scenario, because production cost of $1000 per ton has been considered, though the current production cost is much higher, $2940 per ton [9]. The current biological process still needs improvement to produce succinic acid around $1000 per ton of succinic acid.

To decrease the production costs of biobased succinic acid, two different research areas were established [20]:

The first research area is the improvement of upstream technology which refers the engineering of bacterial strains to enhance product yield, and selection of a suitable feedstock.

The second research direction is the development of downstream technologies which deals with separation of succinic acid from the aqueous fermentation broth. The separation cost accounts for more than 50 % of the total cost of fermentative process. Several methods have been evaluated to identify cost-efficient purification method. No single method has yet proved to be simple and efficient. Improvement is still needed in key performance indicators notably, titre, yield, purity, and energy consumption. Careful consideration should be given to select a suitable purification method.

A systemic approach is needed to synthesize possible production pathways from both upstream and downstream technologies of succinic acid production, and present an optimal production pathway. The pathway identifies the desired products, suitable feedstocks, and conversion technologies.

1.9 Research objective

Objectives of this research work were established to address the problems identified in section 1.8. The objectives are summarised as follows,

1. Determine optimal design of a biorefinery: Given a large number of potential biomass feedstocks and multi bioproducts, identify an optimal production configuration that meets certain objectives such as maximum profit, maximum yield, multi-feedstocks, minimal GHG and energy use,
2. Identify a suitable biomass feedstock that can provide better TRY (titre, rate, yield) to produce succinic acid. It should also present a large, sustainable supply at an economical cost and release lesser Greenhouse gas emission (GHG) than fossil based technology,
3. Based on theoretical studies as listed above in objectives 1 and 2, perform an experiment to produce succinic acid with an aim of achieving maximum titre, rate, and yield.

1.10 Thesis outline

Overall framework as shown in Figure 8 presents the thesis work-flow.

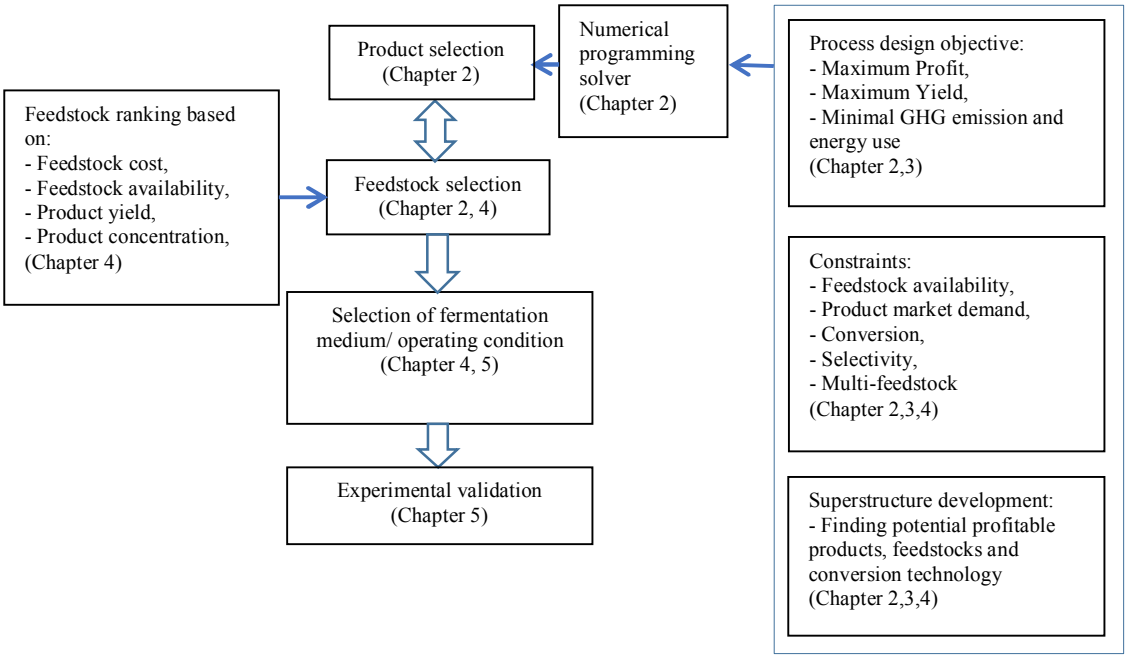


Figure 8. Thesis work-flow chart

The biorefinery development starts with a question of "what products should we make?". This question sums up overall objective of this thesis: identify the potential bioproduct that satisfies mainly two goals, 1. replacement of petroleum based product, 2. enabling biorefinery to achieve economic benefits. Though focus is towards product, conversion technology has been tailored depending on the structural features of feedstock. Therefore, feedstock selection is also an integral part of product identification.

Once potential products were identified, all possible production pathways will be integrated in a superstructure. The superstructure will be modelled and solved using numerical solver of an optimization software to generate an optimal pathway. Multiple optimal pathways can be generated depending on objective functions and constraints.

2. OPTIMAL DESIGN OF A BIOREFINERY

Many production pathways are possible to produce bioproducts using different types of feedstocks and conversion technologies. To identify the optimal design of a biorefinery, a systematic approach as proposed by Pham and El-Halwagi [30] was followed.

The first step in the approach is to develop a superstructure which will show all possible production pathways that contain the optimal one. Using the approach, a superstructure (Figure 9) was developed. In this step, potential bioproducts were selected based on market demand and price as explained in section 2.1. The biomass feedstocks were selected based on ranking criteria as described in section 3. Composition of feedstocks (Appendix A) is one of key factors in deciding the suitability of feedstock because percentage of cellulose and hemicellulose in biomass determines product yield. Various conversion technologies were identified from literature sources. Key reactions of the conversion technologies are listed in Appendix B.

After developing the biorefinery configuration as shown in Figure 9, a mathematical programming model was formulated to simulate the biorefinery. The model was optimized with certain objectives and constraints. As a result, an optimal design was found out, which typically consists of preferable products, a suitable feedstock, and optimal conversion technologies. The optimal design was then considered as design case for developing a flexible biorefinery model. The flexible design can process more than one type of biomass feedstock to generate different optimal pathways.

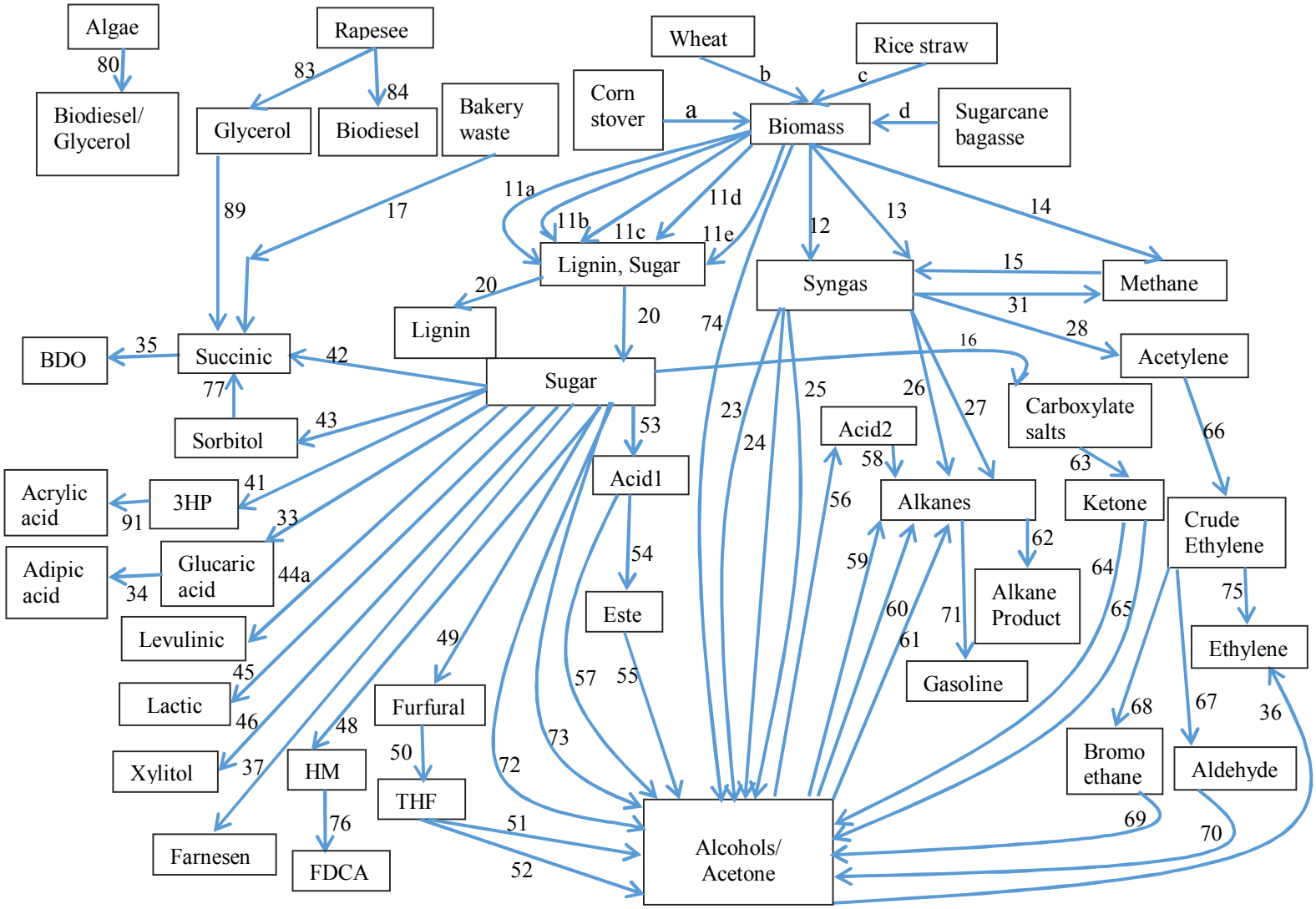


Figure 9. General representation of integrated biorefinery (refer Appendix B for description about conversion pathways)

2.1 Potential bioproducts

US DOE screened 300 potential candidates and selected 12 chemicals (Table 3) using an iterative review process that is based on the building blocks of petrochemical method, chemical data, market data, properties, and the relevant industry experience of the team at PNNL and NREL [9].

EU selected ten products (Table 3) that are at least Technology Readiness Level (TRL) 5, with at least one EU developer has significant potential for market expansion [9]. Each product is selected after a detailed review about the bioproduct production process, the plants and partnerships involved in its production in EU and rest of world, the value proposition (production economics, greenhouse gas savings), and the expected market growth rate.

Table 3. List of potential products identified by US DOE and EU

Biobased chemicals-US DOE	Biobased chemicals-EU
1,4 succinic, fumaric and malic acids	Acrylic acid
2,5 furan dicarboxylic acid	Adipic acid (ADA)
3 hydroxy propionic acid	1,4 –Butanediol (BDO)
aspartic acid	Farnesene
glucaric acid	2,5 furan dicarboxylic acid (FDCA)
glutamic acid	Isobutene
Itaconic acid	Poly hydroxy alkanoates (PHAs)
Levulinic acid	Poly ethylene (PE)
3-hydroxybutyrolactone	Polylactic acid (PLA)
glycerol	Succinic acid
sorbitol	
xylitol/arabinitol	

The superstructure (Figure 9) and Table 4 selected most of the bioproducts from the chemicals identified by US DOE and EU (Table 3). Few other bioproducts were referred from literature sources based on bioproduct market potential and commercial application status. Due to lack of information about production cost and conversion technology, a small number of potential chemicals were excluded from the study.

Table 4. Biobased chemicals-this study

Biobased products - this study	
1,4 succinic acid	xylitol
Adipic acid	Lactic acid
2,5 furan dicarboxylic acid	Methanol, Ethanol, Propanol, Butanol, pentanol, pentanediol
3 hydroxy propionic acid	Ethane, propane, butane, pentane, hexane, heptane, octane, nonane, decane
glucaric acid	Ethylene
levulinic acid	Acetone
glycerol	Gasoline
sorbitol	Biodiesel
THFA	

2.2 Market analysis

To assess market prices and volumes, a plot between price and market volume are presented in the Figure 10.

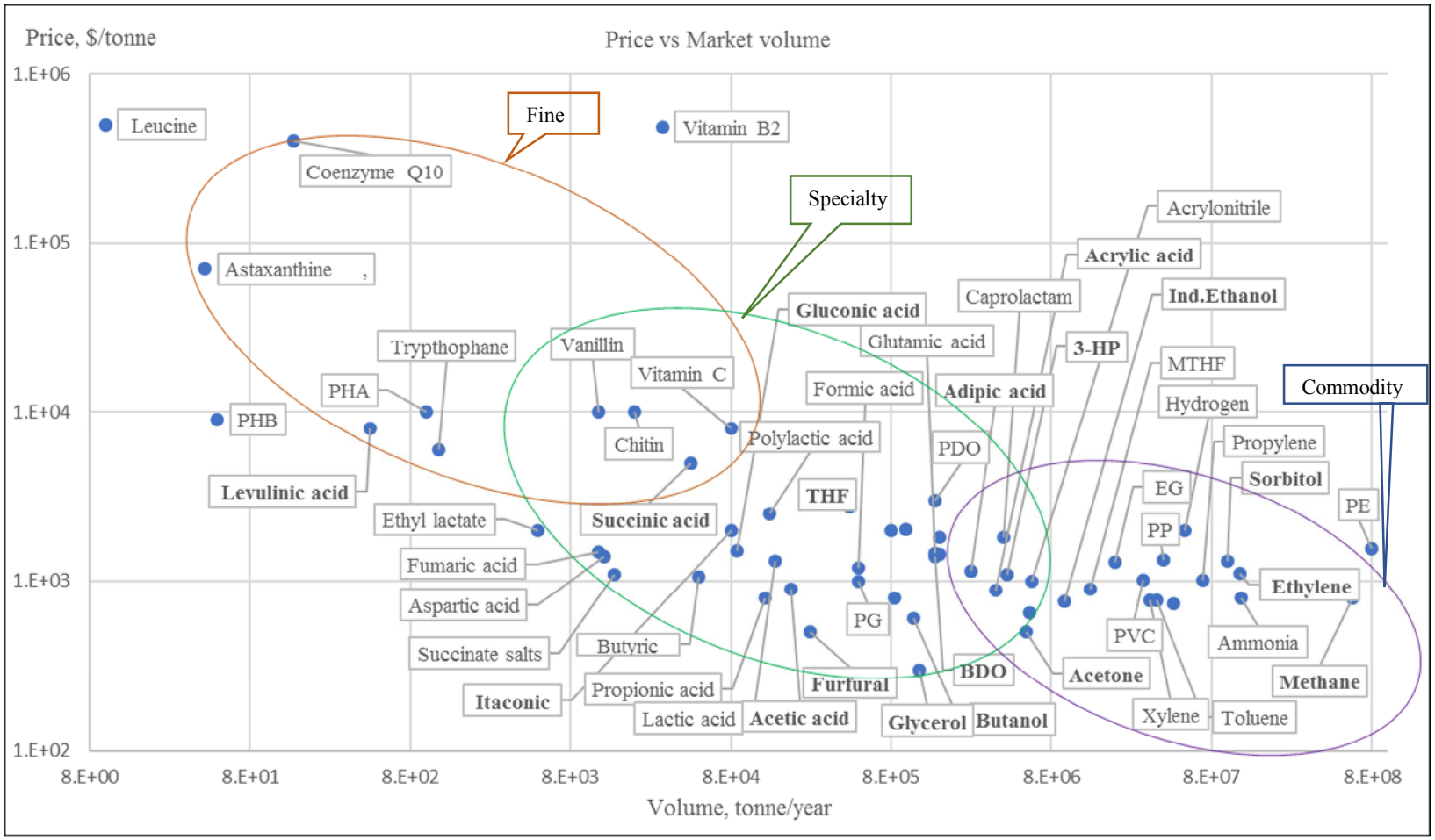


Figure 10. Market price and market volume

Figure 10 classifies the bioproducts as commodity, specialty and fine based on the market volume and price as shown in Table 5.

Table 5 Classification of potential products [62]

Chemicals	Price($/tonne)	Market size (Tonne/year)
Fine	>=10000	1 to 1000
Speciality	>=1000 and <=10000	>1000
Commodity	<=1000	>1 million

This study targets the bioproducts that has high value greater than $1000/ tonne and significant market demand of greater than 1000 tonnes/year. Though fine chemicals have high value, its low market demand makes it less attractive. Commodity chemicals have large market size however, it has low value and its demand is generally disrupted by volatile oil price. Speciality chemicals provide the benefits that fine and commodity chemicals lack; high value of products and significant market volume. Therefore, speciality chemicals were selected in this study. Table 6 presents the market volume and price of the selected bioproducts in the order from highest to lowest market potential of the bioproducts.

Table 6 indicates that high-demand bioproducts such as ethylene, p-xylene, adipic acid shows no production by biological method because bioprocessing technologies of the bioproducts have not yet attained commercial deployment status. Biobased succinic acid contributed almost 50% of world's succinic acid demand in 2013, yet it requires a cost-effective technology to replace the remaining quantity that is currently produced from petrochemical method. Few other bioproducts such as Levulinic acid, sorbitol, and xylitol have already reached full potential; it replaced 100% of petroleum counterpart.

Table 6. Market demand of biobased products [9]

Bio-products	Price	Current supply of Bioproduct Volume	Total market volume (Fossil + bio-based)	Percentage of bioproduct in total market volume	Potential demand of bioproduct in existing market
	($/t)	(ktpa)	(ktpa)	%	(ktpa)
Ethylene	1,300-2,000	200	127000	0.2	126800
p-xylene	1414	1.5	35,925	0.0	35,924
Ethylene glycol	1300	425	28,000	1.5	27,575
Iso-butene	1850	0.01	15,000	0.0	15,000
Acetic acid	617	1,357	13,570	10.0	12,213
Ethanol	815	71,310	76,677	93.0	5,367
Acetone	1400	174	5,500	3.2	5,326
Acrylic acid	2688	0.3	5,210	0.0	5,210
Adipic acid	2150	0.001	3,019	0.0	3,019
BDO	3000	3	2,500	0.1	2,497
n-butanol	1890	590	3,000	19.7	2,410
Isoprene	2000	0.02	850	0.0	850
Isobutanol	1721	105	500	21.0	395
Succinic acid	2940	38	76	50.0	38
5-HMF	2655	0.02	0.1	99.0	0
PDO	1760	128	128	98.4	0
Lactic acid	1450	472	472	20.0	0
3-HPA	1100	0.04	0.04	100.0	0
FDCA	NA high)	0.045	0.045	100.0	0
Levulinic acid	6500	3	3	100.0	0
Farnesene	5581	12	12.2	100.0	0
PHAs	6500	17	17	100.0	0
Itaconic acid	1900	41	41.4	100.0	0
Algal lipids	1000	122	122	100.0	0
Xylitol	3900	160	160	100.0	0
Sorbitol	650	164	164	100.0	0
Furfural	1000	700	700	100.0	0

2.3 Mathematical formulation for biorefinery model

To identify an optimal process route out of all possible options as shown in superstructure (Figure 9), a non-linear programming (NLP) model was developed. The optimal process route either generate a maximum annual after-tax net profit (AANP) or produce a maximum product yield.

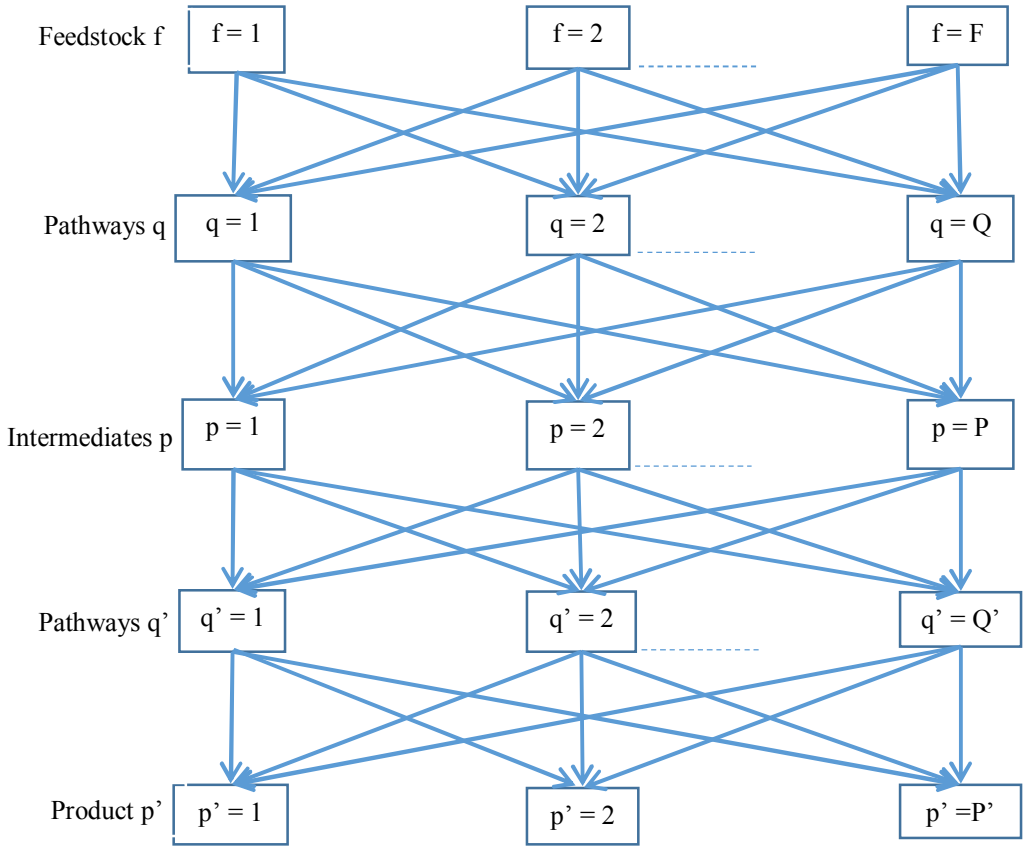


Figure 11. Typical representation of biorefinery for mathematical formulation [63]

Figure 11 exhibits that biomass feedstock f can be converted into intermediate product p via conversion pathway q with their respective flow rate, F^{bio}_{fq} .

$$F^{bio}_{f} = \sum_{q=1}^{Q} F^{I}_{fq} \quad \forall f \tag{2.1}$$

In Equation (2.1), F^{bio}_{fq} is the available total flow rate of biomass feedstock f. After the feedstock f is passing through the biomass conversion pathway q, intermediate product p is generated based on conversion rate of conversion pathway q, R^{I}_{fqp}. This results a total intermediate production rate of $T^{inter}p$, as shown in Equation (2.2).

$$T^{inter}_{p} = \sum_{f=1}^{F} \sum_{q=1}^{Q} (F^{I}_{fq} R^{I}_{fqp}) \quad \forall p \tag{2.2}$$

Subsequently, the intermediate product p is then further converted to product p' via conversion pathway q'. The total production rate of intermediate product T_s^{Inter} is split to all possible conversion pathway q' with flow rate $F^{II}_{pq'}$ can be represented by Equation (2.3).

$$T^{inter}_{p} = \sum_{q'=1}^{Q'} (F^{II}_{pq'}) \quad \forall f \tag{2.3}$$

The total production rate of final product p', $T^{Prod}_{p'}$ can be calculated based on given conversion rate of conversion pathway q', $R^{II}_{pq'p'}$ by equation (2.4).

$$T^{prod}_{p'} = \sum_{p=1}^{P} \sum_{q'=1}^{Q'} (F^{II}_{pq'} R^{II}_{pqp'}) \quad \forall p' \tag{2.4}$$

By following Equation (2.1) – (2.4), the material balance of the biomass, intermediates and final products will be performed. Objective function of maximizing product yield is represented by following equation,

Maximise $T^{Prod}_{p'}$ (2.5)

Other than maximising the product yield, maximizing annual after-tax net profit (AANP) can also be considered as one of objective functions in the biorefinery model.

Maximise AANP,

AANP is expressed as follows,

$$AANP = GP^{total} * (1\text{-Tax rate}) + \text{Depreciation}$$

$$GP^{total} = \sum_{p'=1}^{P'} T^{prod}_{p'} C^{prod}_{p'} - \sum_{f=1}^{F} F^{bio}_{f} C^{bio}_{f} - TAC$$

$$TAC = TACC + TAOC$$

$$TAOC = \sum_{q=1}^{Q} \sum_{f=1}^{F} F^{I}_{fq} C^{opt}_{fq} + \sum_{f=1}^{F} F^{II}_{pq'} C^{opt'}_{pq'}$$

$$TACC = \sum_{q=1}^{Q} \sum_{f=1}^{F} F^{I}_{fq} C^{cap}_{fq} CRF + \sum_{f=1}^{F} F^{II}_{pq'} C^{cap'}_{pq'} CRF$$

$$C^{cap'}_{pq'} = C^{cap}_{nq'} \left[\frac{F^{II}_{fq'}}{F_{nq'}} \right]^{0.7}$$

$$C^{cap}_{fq} = C^{cap}_{nq} \left[\frac{F^{I}_{fq}}{F_{nq}} \right]^{0.7}$$

Where AANP is the annualised after-tax net profit of biorefinery configuration, GPt^{otal} is the gross profit, TAC is the total annualised cost, TACC is the total annualised capital cost, TAOC is the total annualised operating cost, CRF is the capital recovery factor, $C^{prod}_{p'}$ is the cost of product p', C^{bio}_{f} is the cost of biomass feedstock f, C^{cap}_{fq} is the capital cost for the conversion of biomass feedstock b, $C^{Cap'}_{pq'}$ is the capital cost for the conversion of intermediate p, C^{opt}_{fq} is the operating cost for the conversion of biomass feedstock f, $C^{opt'}_{pq'}$ is the operating cost for the conversion of intermediate product p, $C^{cap}_{nq,}$ $C^{cap}_{nq'}$ are the capital cost for nominal capacity of conversion

technology q and q' respectively, F_{nq}, $F_{nq'}$ are the nominal flowrate of conversion technology q, q' respectively.

Annual capital cost (AOC) and annual operating cost (AOC) for each conversion pathway are presented in Appendix B. Capital cost means fixed capital investment (FCI) for nominal capacity of each conversion technology. Sixth-tenth rule was applied to calculate capital cost for the predicted flow rate as determined by the simulation model.

Following equation expresses the sixth-tenths factor,

$FCI_B = FCI_A*(Capacity_B/ Capacity_A)^x$,

where exponent x is 0.7, FCI_B is fixed capital investment for the predicted capacity, $Capacity_B$, FCI_A is fixed capital investment for the nominal capacity, $Capacity_A$ of similar product. Market price of bioproducts and biomass feedstocks are presented in Appendix C. Values are based on current market condition.

The simulation model uses financial parameters (Table 7) to determine annual after-tax net profit (AANP). The financial parameters were referred from Modified Accelerated Cost Recovery System (MACRS) of NREL [64].

Table 7. Basis and assumptions of the financial parameters

Parameters	Values
Plant life	20 years
General plant depreciation	10-year linear depreciation
Salvage value	no
After-tax discount rate	10%
Income tax rate	39%
Subsidy	No
Operating period	8,000 hours per year

2.4 Case studies

The simulation model was run for different case studies to obtain an optimal design. Three case studies were considered to analyze biorefinery model from different objective functions (Table 8).

Table 8. List of case studies

Case #	Objective function	Case description
1	Maximum profit	This case determines the production pathway that can generate a maximum profit, given the constraints; feedstock availability, product demand.
2	Maximum succinic acid yield	Case study 2 screens all possible feedstocks and selects the feedstock that can produce a maximum quantity of succinic acid.
3	Maximum profit from processing multi-feedstocks	This case selects the best combination of feedstocks that can generate a maximum profit.

2.4.1 Case study 1: maximum profit for biorefinery production

Case study 1 aims to maximize profit of a biorefinery. Current market demand of bioproduct and current worldwide availability of feedstock are the main constraints to achieve the objective. This scenario assesses the maximum revenue potential of bioproducts market. Based on the ranking of biomass feedstocks as described in section 3, corn stover was placed at highest ranking, therefore corn stover was considered as best feedstock. Simulation model was run with corn stover as feedstock.

Solution of the model generates an annual net (after-tax) profit of $5.84 billion and favours the production of high value chemicals (succinic acid, acrylic acid, adipic acid, BDO) and commodity chemicals (ethanol, ethylene) as presented in Table 9.

Table 9. Case study 1 (maximum profit) results

Objective function: Annual (after-tax) net profit, Feedstock: Corn stover, Feed flowrate= feed availability, Product flowrate=Market demand of product			
Bioproducts	Annual (after-tax) net profit Billion$/ annum	NPV MM$	TAC Billion $/ annum
High-value products: succinic, acrylic, adipic, glucaric, BDO, Commodity products: ethylene, ethanol	5.84	785	10

The resulting biorefinery configuration along with its net profit and conversion technology are shown in Figure 12.

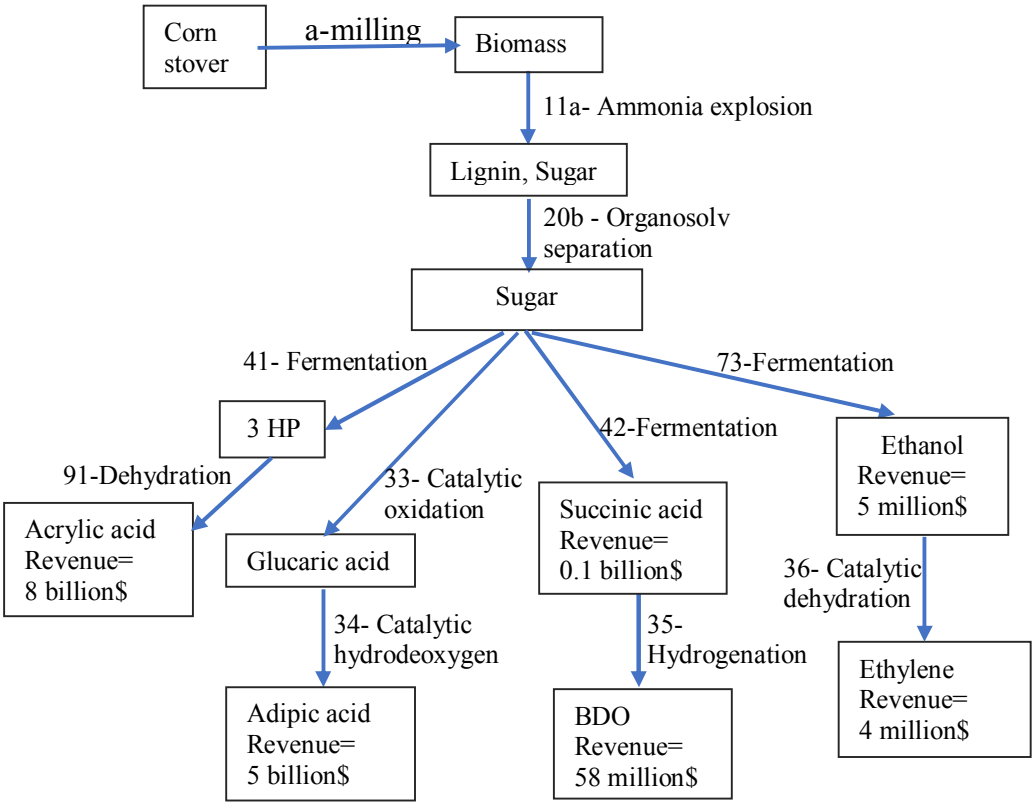


Figure 12. Production pathway of case study 1

The synthesis problem was formulated as a Non-Linear Program (NLP) and solved by using Sequential Linear Programming (SLP) solver option in optimization software LINGO (version 16). Once the program generated an optimal solution, an integer constant was added into the program to exclude the first optimal solution and to generate new solutions. The procedure was continued till obtaining all possible optimal solutions. The optimal solutions were then ranked from highest to lowest annual (after-tax) net profit and Net present value (NPV) as shown in Table 10.

Table 10. Sub-categories of case study 1

Objective function: Annual (after-tax) net profit, Feedstock: Corn stover Feed flowrate= feed availability, Product flowrate=Market demand of product					
Rank #	Product	Annual (after-tax) net profit	NPV MM$	TAC billion$/ annum	Production million tonne/ annum
1	acrylic, adipic, succinic, BDO	4.6 billion$/ annum	734	5.6	5.2 (acrylic), 3 (adipic), 0.03 BDO), 0.03 (succinic)
2	Acrylic acid	2.9 billion$/ annum	380	3	5.2
3	Adipic acid	1.6 billion$/ annum	340	2.5	3
4	succinic, BDO ethanol	1 billion$/ annum	64.5	0.05	0.03 (succinic), 0.03 (BDO), 5.3 (ethanol)
5	Ethanol, Ethylene	1.25 billion$/ annum	59	4.5	5.3 (ethanol), 5.3 (ethylene)
6	Ethanol	1 billion$/ annum	46	0.05	5.3 (ethanol)
7	BDO, succinic	49 MM$/annum	18.5	0.07	0.03 (succinic), 0.03 (BDO)
8	Succinic acid	23 MM$/annum	13	0.05	0.04

2.4.1.1 Assessment of technology development status

The ranked bioproducts (Table 10) were further analysed from the view of commercial readiness of conversion technology. A key metric used to assess status of technology development is the TRL (Technology Readiness Level). TRL is a relative measure, introduced by NASA, to rank the maturity of developing technologies on a scale of 1 to 9. TRL 1 represents the basic research on an invention or concept, TRL 5 to pilot scale testing, TRL 7 to at pre-commercial scale testing, while TRL 8 corresponds to full commercial application of technology [9]. TRL status of various biobased products are shown in Figure 13.

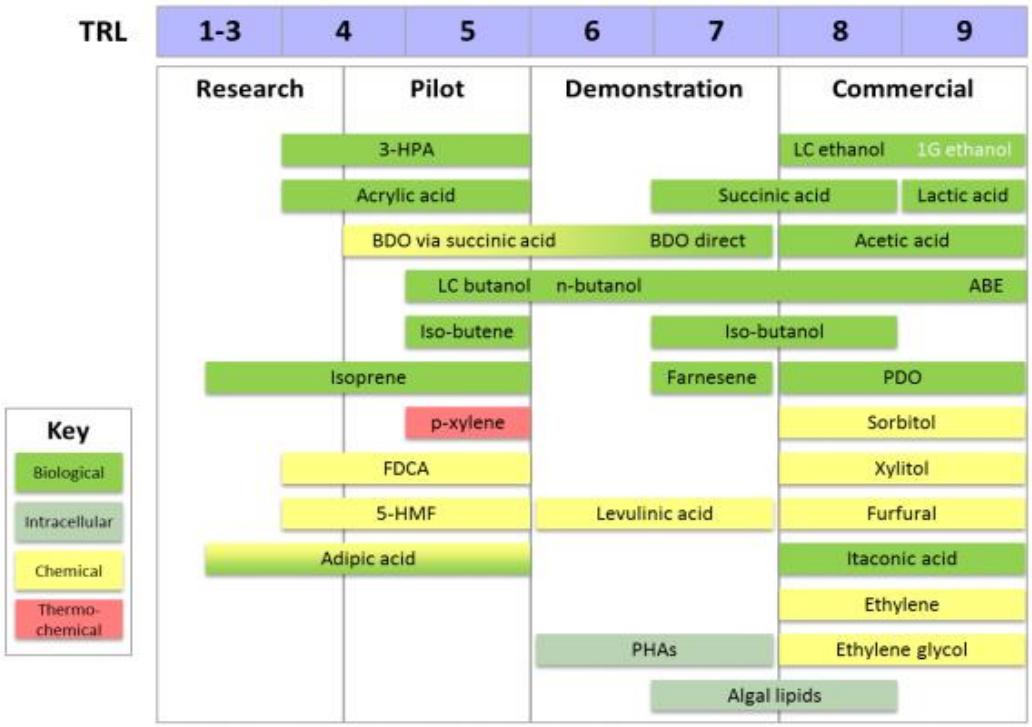


Figure 13. Commercialization status of priority based bioproducts [9]

Out of all bioproducts considered in this study, succinic acid technology has reached the TRL of 8; commercially proven technology. Acrylic acid, adipic acid and BDO have yet to develop into a commercially deployable technology. Though the high value chemicals such as sorbitol, xylitol, Itaconic acid have achieved TRL 9, no additional production is required because the bio based products have already replaced petrochemical equivalents.

2.4.1.2 Results and discussions

Because of less total annualised cost (TAC) and higher net profit, high value chemicals are favourable than commodity products. Within production economics of high value products, succinic acid is most attractive product because of commercial readiness of its conversion technology. Production of BDO via succinic acid expands the current demand of succinic acid. Significant market demand in existing as well as future

market condition necessitate finding a cost-effective production method in competitive with fossil based method. Cost of succinic acid production by different feedstocks is listed in Table 11. Currently, biological method ($2940/ton succinic acid) using corn based sugar as feedstock is nearly competitive with petrochemical method ($2860/ ton). Based on conceptual study, lignocellulosic produces succinic acid at a much lesser cost than starch (corn) based or fossil based feedstock.

Table 11. Succinic acid value per lb of succinic acid

Feedstock	Technology	Cost, $/ lb SA	Cost, $/ ton SA
Butane	Petrochemical	1.3	2860
Corn	Biological	1.3	2940
Corn stover	Biological	0.8	1780

2.4.2 Case study 2: maximum yield for production of succinic acid

Objective of this case study is to select a technological pathway that will maximize succinic acid yield. Feedstocks are shown in superstructure (Figure 9). The feed rate was taken from worldwide availability of feedstock. Appendix B lists the key reactions involved in the conversion technology.

The synthesis problem was formulated as a Non-Linear Program(NLP) and solved by using Sequential Linear Programming solver (SLP) option in the optimization software LINGO (version 16). Once a maximum yield for one type of biomass was obtained, an integer cut was added to exclude the first optimal solution and to generate another one. This process was repeated for each type of feedstock to produce succinic acid.

Top three optimal pathways (Figure 14) were selected from all possible options generated by the simulation model. The top-three feedstocks are corn stover, rapeseed

and bakery waste. Among them, bakery waste produced highest succinic acid yield as shown in Table 12.

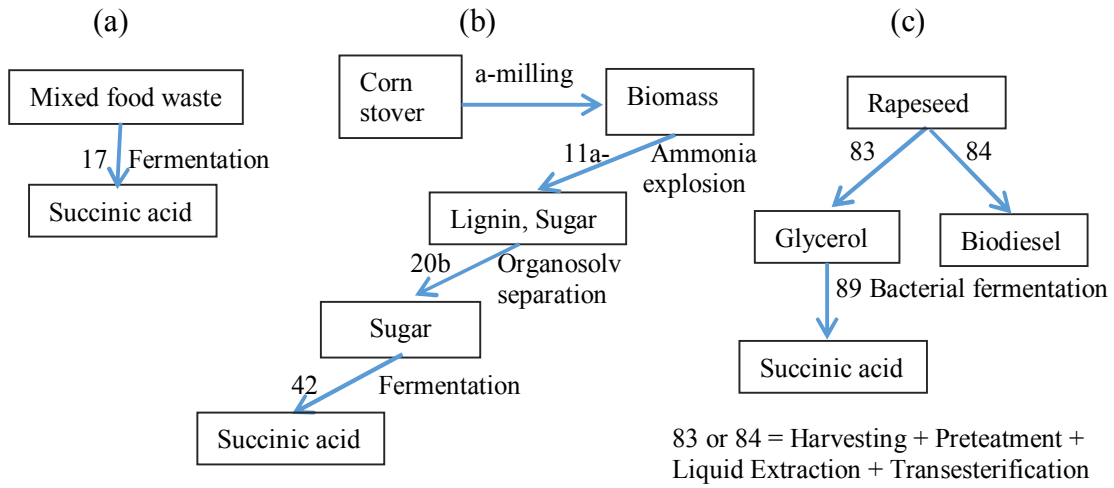


Figure 14. Case study 2: production pathway for a maximum yield, (a) when the feedstock is mixed food waste, (b) corn stover, (c) rapeseed.

Table 12. Case study 2 results - maximum yield of succinic acid

Objective function: Maximum yield of succinic acid, Feed flowrate= feed availability			
Rank #	Feed	Product	Production (million tonne/annum)
1	Bakery waste	Succinic acid	880
2	Corn stover	Succinic acid	391
3	Rapeseed	Succinic acid	0.6

2.4.2.1 Results and discussions

Bakery waste or food waste showed highest availability among all other possible feedstocks such as corn stover, rapeseed, wheat straw, rice straw, sugarcane bagasse. Because of enormous availability, food waste can produce a maximum quantity of succinic acid. The technology of converting food waste into succinic acid was tested on a lab scale [65]. Collection and segregation of food waste are major drawbacks, preventing the technology to reach commercial status. Research studies should focus on improving supply chain of food waste and performance of conversion technology.

2.4.3 Development of a flexible biorefinery

To maximize usefulness of a biorefinery and minimize the total annualised cost, the process design should include all possible sources of uncertainty such as variation in availability, cost, price, composition, and environmental impact of the feedstock. A flexible plant should be capable to process a variety of feedstocks in a way that promotes sustainability and profitability. To accomplish this, case study 3 was considered. Case study 3 can answer the following question,

Should feedstocks be processed as a single feedstock or multi-feedstocks?

Case study 3 can also be explained as follows,

Given a design case which can handle only a single feedstock (corn stover), design a flexible case to process different combinations of feedstocks without compromising production rate and profit.

Table 13 shows composition of the feedstocks, considered in this case. Cellulose, hemicellulose, and lignin are main components in the feedstock.

Table 13. Composition of feedstocks

Component	Formula	MW (g/gmol)	Composition (%)			
			Corn stover	Wheat straw	Rice straw	Sugarcane bagasse
Cellulose	$C_6H_{10}O_5$	162.14	37.73	36	32	38
Hemicellulose	$C_5H_8O_4$	132.12	26	25	24	27
Lignin	$C_8H_8O_3$	152.15	19.24	15	18	18
Ash	-	-	3.79	-	-	-
Other solids	$C_8H_8O_3$	152.15	13.24	-	-	-

2.4.3.1 Case study 3: maximum profit from processing a single feedstock

Firstly, the problem was formulated using optimization software with the objective of maximizing the profit for a single feedstock. This problem takes into one type of biomass feedstock and determines the annual after-tax net profit.

Based on simulation results, feedstocks were ranked from highest to lowest net profit (Table 14 and Figure 15). Corn stover generated a maximum profit of $ 5.8 billion as compared to other feedstocks. The single feedstock scenario selects succinic acid and BDO as optimal products as shown in Figure 16.

Ammonia explosion converts cellulose and hemicellulose in biomass into reducing sugar which is then converted into succinic acid by anaerobic fermentation. Catalytic hydrogenation of succinic acid produces BDO.

Table 14. Results of case study 3 - single feedstock scenario

Objective function: maximum Annual (after-tax) net profit Feed flowrate = 10000 tonnes/ annum, Product flowrate = market demand of product					
Rank #	Feed	Product	Annual (After-tax) net profit, MM $	NPV MM$	TAC Billion $/ annum
1	Corn stover	succinic, BDO	17.6	2.5	17.7
2	Sugarcane bagasse	succinic, BDO	11	1.7	11
3	rice straw	succinic, BDO	9.5	1.6	9.5
4	wheat straw	succinic, BDO	9.3	1.5	9.4

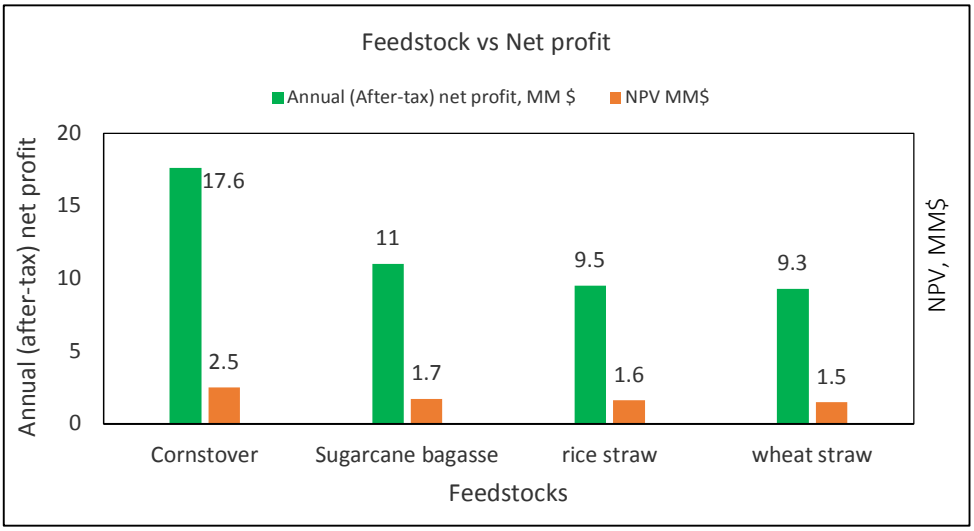
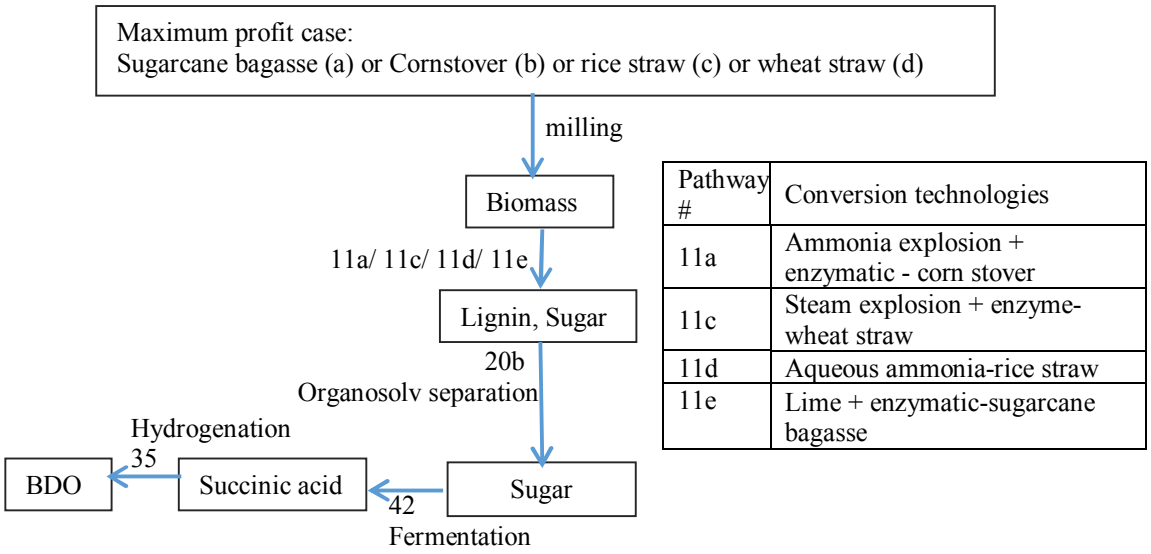


Figure 15. Single feedstock scenario for maximum profit



Pathway #	Conversion technologies
11a	Ammonia explosion + enzymatic - corn stover
11c	Steam explosion + enzyme- wheat straw
11d	Aqueous ammonia-rice straw
11e	Lime + enzymatic-sugarcane bagasse

Figure 16. Production pathway for a single feedstock scenario

The scope of optimization study was extended to all possible combinations: a mixture of two and three types of biomass feedstocks.

2.4.3.2 Case study 3: maximum profit from processing a mixture of two feedstocks

Optimal two-feedstock mixture was found to be a mixture of corn stover and sugarcane bagasse (Table 15 and Figure 17). The optimal mixture showed a maximum profit of 38 million$/ annum among other possible options. BDO, succinic acid, ethanol and ethylene were the optimal products.

Production pathway of two-feedstock mixture is shown in Figure 18. The pathway is different from a single feedstock scenario. Ammonia explosion converts cellulose and hemicellulose in biomass into reducing sugar which is then converted into succinic acid and ethanol. Anaerobic fermentation converts reduced sugar into succinic acid. Catalytic hydrogenation of succinic acid produces BDO.

Mixed culture fermentation is applied to convert the reduced sugar into ethanol via acid fermentation and ketonisation. Catalytic hydrogenation of ethanol produces

ethylene. In case of less feed rate, say 30,000 tons/ annum, mixed culture fermentation is the optimal process for ethanol production, whereas feed rate of much higher than 30,000 tons/ annum, yeast fermentation is the optimal conversion technology.

Table 15. Case study 3 - feedstock as a mixture of two types feedstocks

Objective function: maximum Annual (after-tax) net profit Feed flowrate for each type of biomass = 10000 tonnes/annum, Product flowrate as determined by simulation					
Rank #	Feed	Product	Annual (After-tax) net profit, MM $	NPV MM $	TAC MM $/ annum
1	C + S	BDO, succinic acid, ethanol, ethylene	29	5.85	46.2
2	C + R	BDO, succinic acid, ethanol, ethylene	27	5.6	43.7
3	C + W	BDO, succinic acid, ethanol, ethylene	27	5.6	43.4
4	R + S	BDO, succinic acid, ethanol, ethylene	20	4.6	33
5	W + R	BDO, succinic acid, ethanol, ethylene	19	4.3	30
C- Corn stover, W- wheat straw, R- rice straw, S- Sugarcane bagasse					

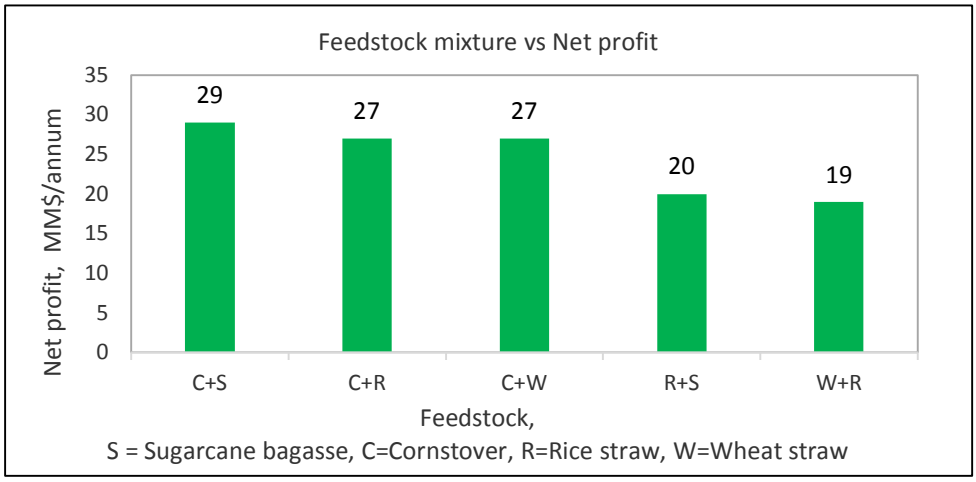


Figure 17. Maximum profit for a mixture of two types feedstocks

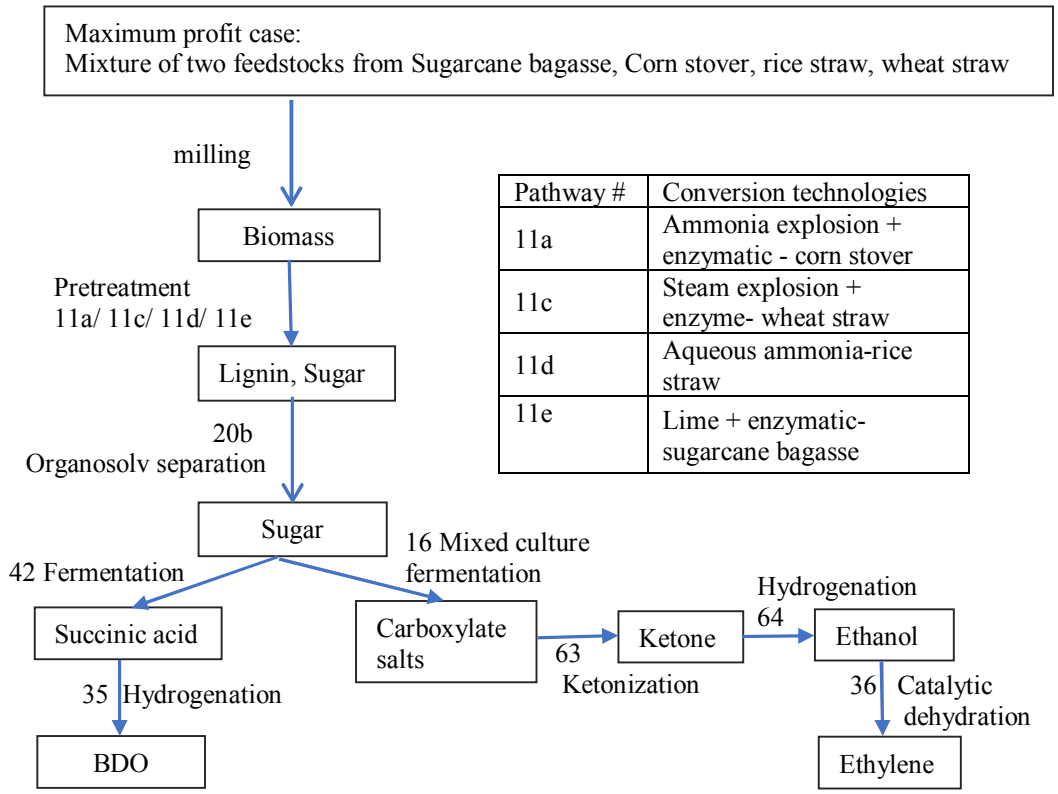


Figure 18. Production pathway for two-feedstocks scenario

2.4.3.3 Case study 3: maximum profit from processing a mixture of three feedstocks

In case of three-feedstocks mixture, a combination of corn stover, rice straw and sugarcane bagasse was found to be the optimal mixture, generating a maximum profit of 38 million$/ annum (Table 16 and Figure 19). BDO, succinic acid, ethanol and ethylene were the optimal products. Production pathway is same as that of two-type feedstocks as shown in Figure 18.

Table 16. Case study 3 - feedstock as a mixture of three types of biomass

Objective function: maximum Annual (after-tax) net profit Feed flowrate for each type of biomass = 10000 tonnes/annum, Product flowrate as determined by simulation					
Rank #	Feed	Product	Annual (After-tax) net profit, MM $	NPV MM $	TAC MM $/ annum
1	C + R + S	BDO, succinic acid, ethanol, ethylene	38	4.3	38.2
2	C + W + S	BDO, succinic acid, ethanol, ethylene	38	4.3	38.1
3	C + W + R	BDO, succinic acid, ethanol, ethylene	36	4.2	36.5
4	R + W + S	BDO, succinic acid, ethanol, ethylene	30	3.5	30
C- corn stover, W- wheat straw, R- rice straw, S- sugarcane bagasse					

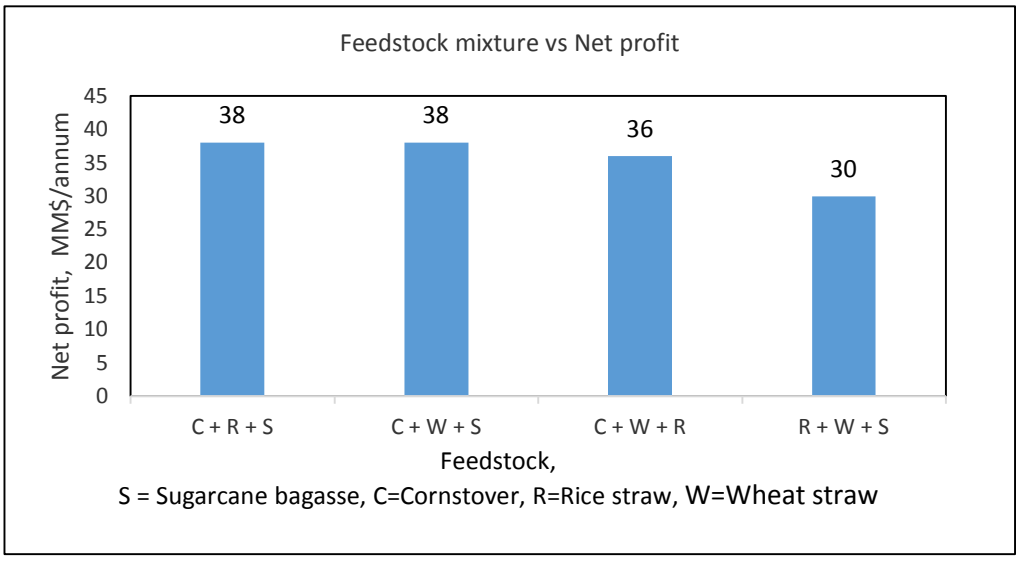


Figure 19. Maximum profit for a mixture of three types feedstocks

2.4.3.4 Results and discussions

Based on the simulation results of multi-feedstock studies, feedstocks can be prioritised in an order from highly preferable to less favourable feedstock as follows,

- Corn stover,
- Sugarcane bagasse
- Rice straw
- Wheat straw

This sort of prioritisation would allow us to select suitable feedstock for succinic acid production.

Bioproducts can also be prioritised within the list of most selective products. While performing optimization, simulation solver first selects succinic acid and BDO as optimal products to generate a maximum profit. If excess feedstocks are available, other high value bioproducts are selected. Based on simulation results, the bioproducts can be ordered from highly favourable to less promising bioproducts as follows, succinic acid, BDO, acrylic acid, ethanol, ethylene, adipic acid.

2.4.4 Analysis of Greenhouse Gas (GHG) emissions and energy use

In addition to economic benefit, biobased chemicals offer environmental advantage by means of reducing greenhouse gas (GHG) emissions and fossil energy use as compared to petrochemicals. To assess the environmental benefits, this analysis studied the GHG emission and energy use of the biobased chemicals as selected in this study (Table 4).

2.4.4.1 Method

Matthew et al [66] summarised the values of GHG and energy use of petrochemicals from a detailed review on LCAs of biobased products [66, 67], which forms the basis for this study. Most of LCA studies estimated the values from different literature sources: Renewable Fuels Standard (RFS2) program, Roundtable on

Sustainable Biomaterials (RSB) and the International Sustainability & Carbon Certification (ISCC) [66, 67].

Generally, energy use and GHG emission values are estimated per 1 lb of target product. Energy use is typically expressed in following metrics: non-renewable energy use (NREU), fossil fuel input and cumulative energy demand (CED). Values of GHG emissions and net energy use were taken from research studies [66,67]. All results are presented as the relative difference between biobased chemical and petrochemical equivalents rather than as absolute value.

2.4.4.2 GHG emission

GHG emission of the biobased products were plotted as percentage change in GHG in relative to petrochemical method. As shown in Figure 20, the relative GHG emissions varies from a 371% increase for p-xylene production to a 177% decrease in emissions for poly hydroxy butyrate (PHB) production, when compared with their petroleum based equivalents. PHB shows highest GHG emission reduction and the second highest GHG emission reduction occurs in succinic acid production (87% decrease).

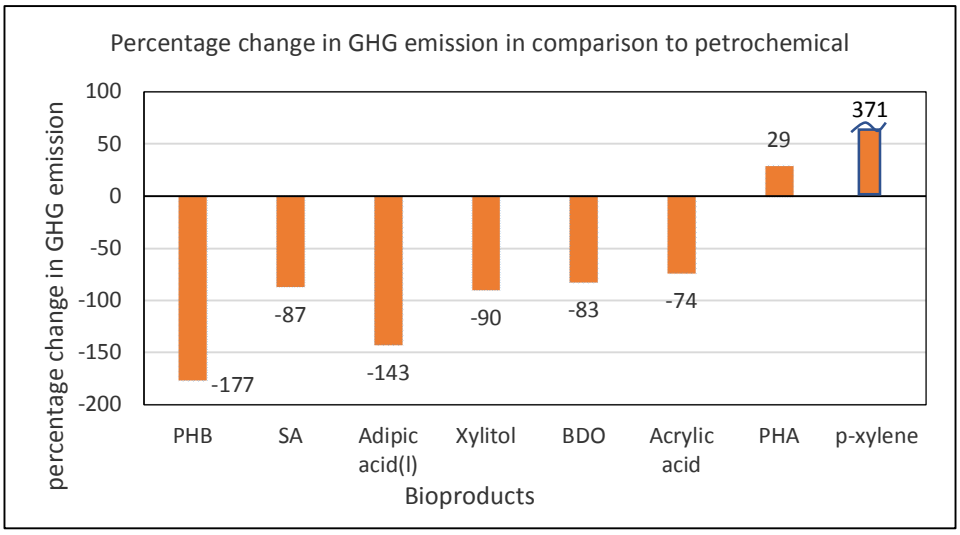


Figure 20. Percentage change in GHG emission

2.4.4.3 Energy use

Relative difference between energy-use values of biobased and fossil-based chemicals were plotted in Figure 21. Energy use was measured in terms of amount of energy in MJ consumed to produce 1 lb of target product. As shown in Figure 21, PHB, xylitol and styrene have the highest reduction in energy consumption (>85%). Succinic acid shows significant (>50%) reduction in energy use.

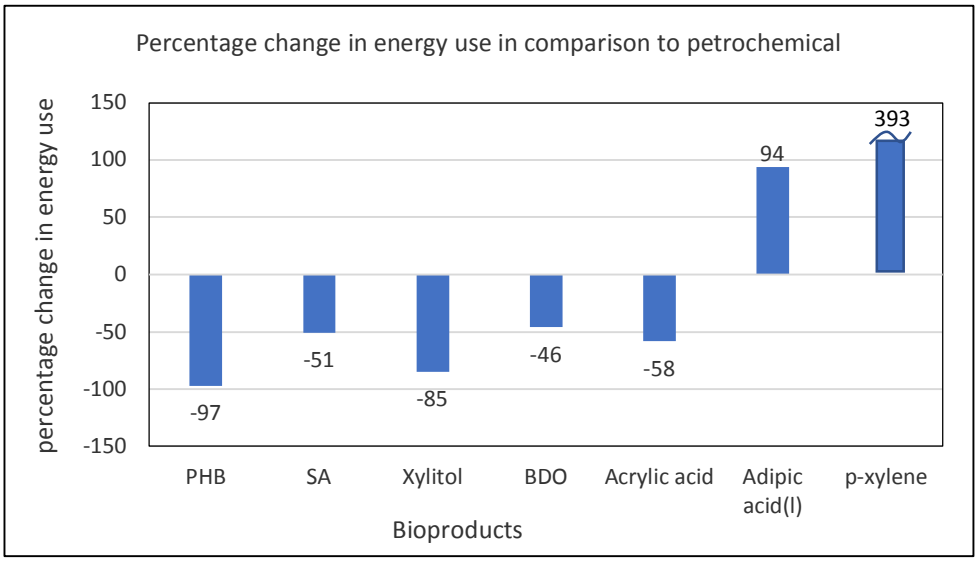


Figure 21. Percentage change in energy use

2.4.4.4 Results and discussions

Succinic acid shows higher percentage reduction in GHG and energy use as compared to petrochemical equivalent.

2.4.5 Evaluation of optimal design of succinic acid production

After a detailed literature study on succinic acid production, an optimal pathway was selected. The pathway was then given as input to the simulation model of biorefinery.

A systematic approach was followed to find the optimal pathway. Problem in this case is explained as follows: Given several potential biomass feedstocks and a desired product as succinic acid, find an optimal process that meets the objective functions: maximum yield, higher production concentration, maximum production rate, minimal cost of production. This task requires to find out the efficient pretretment technology, productive microorganism and product separation method as shown in Figure 22.

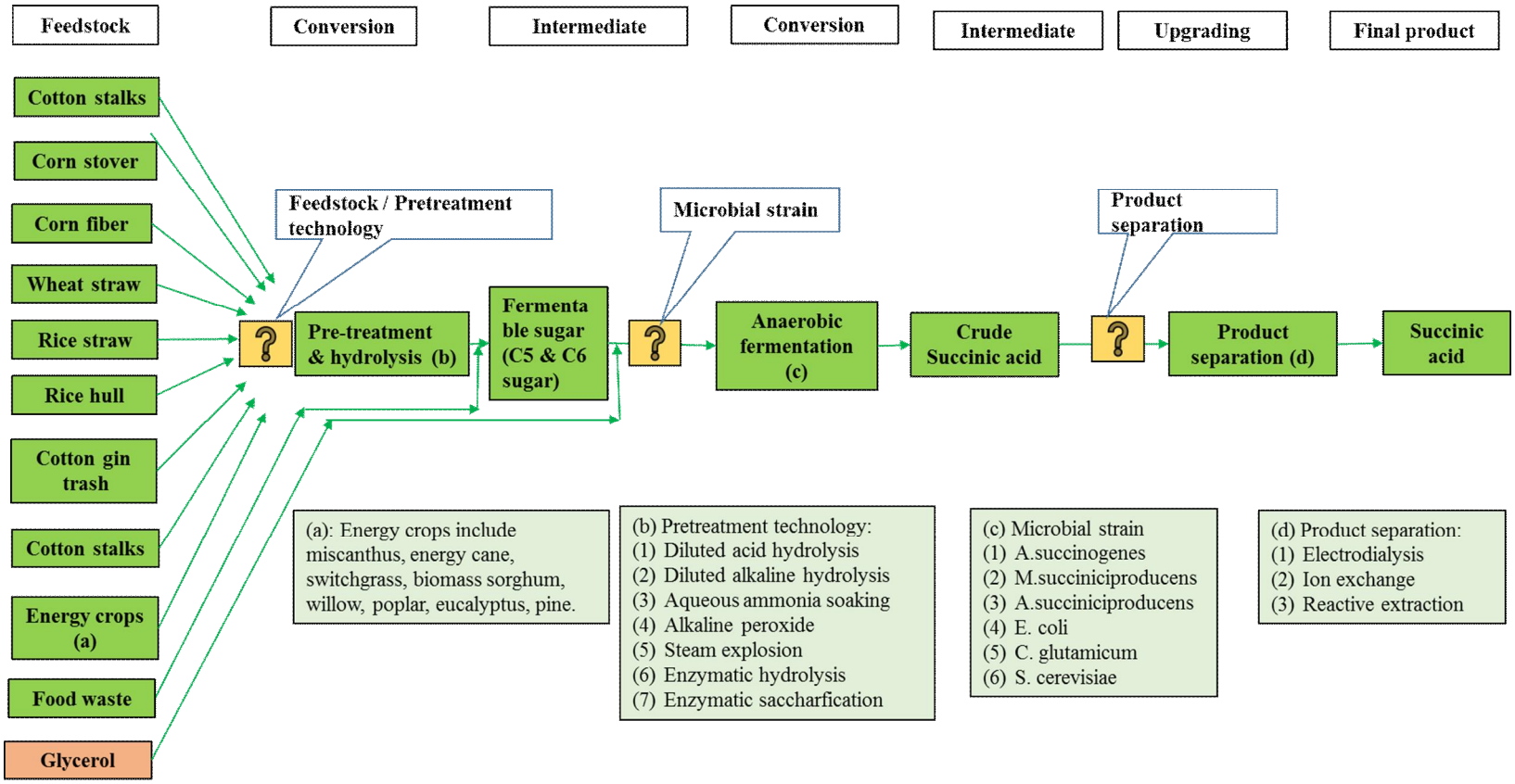


Figure 22. Problem statement for finding optimal pathway

2.4.5.1 Methodology and results

All possible production pathways were integrated in a superstructure (Figure 23). With reference to literature studies, the production pathways were screened to eliminate the pathways that reported low product yield, higher cost of production, less production rate, and less availability of feedstocks.

Main purpose of the screening is to select the suitable feedstock, pretreatment technology, microbe, fermentation medium and product separation method from all available alternatives.

Biomass feedstock and its sugar yield for various pretreatment technologies were tabulated in Appendix D, Table 29. Advantages and disadvantages of each pretereatment technology were presented in Appendix D, Table 30.

Various bacteria and its performance characteristics (yield, product concentration, production rate) on different feedstocks were shown in Appendix E.

Based on the data collection, reaction path that reported higher yield on multiple experiments was selected as best production pathway. The optimal pathway was found out for each biomass feedstock as displayed in Figure 24.

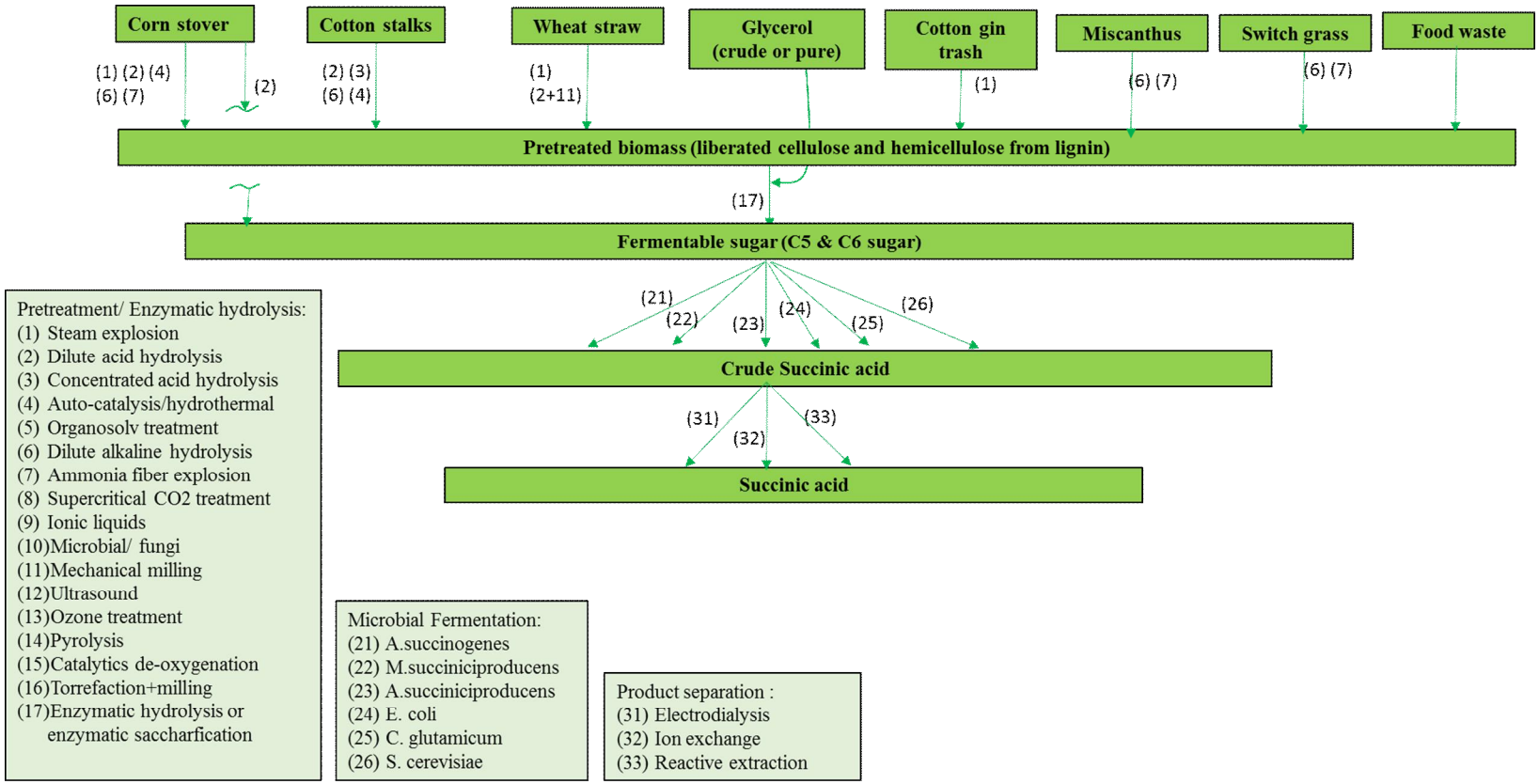


Figure 23. Superstructure of succinic acid production

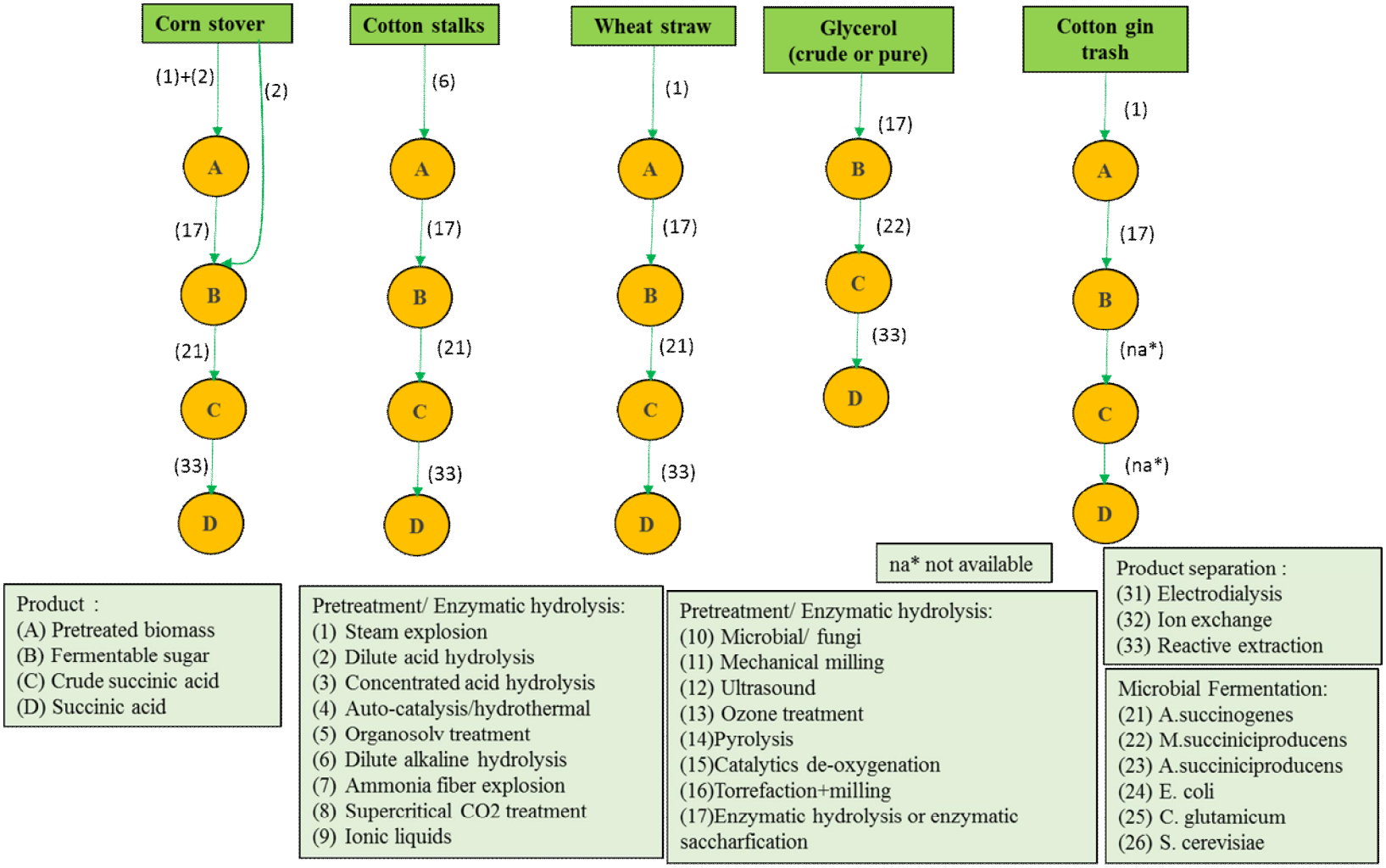


Figure 24. Optimal production pathway for succinic acid production

3. IDENTIFICATION OF A SUITABLE FEEDSTOCK FOR SUCCINIC ACID PRODUCTION

3.1 Selection of a suitable feedstock

Findings from biorefinery modelling indicates that corn stover is the suitable feedstock to produce succinic acid. Along with the modelling, an evaluation matrix was developed to identify best feedstock to produce succinic acid.

The evaluation matrix assigned weightage to key performance parameters of feedstock. The key performance parameters were identified as feedstock availability, feedstock cost, product yield and product concentration [26].

Feedstocks were compared in terms of the key performance factors. Each factor was assigned with a percentage weight (Table 17), so that each weight adds up to 100%. The feedstock cost and succinic acid yield directly influence the production cost of succinic acid, therefore they were given high weightage of forty and thirty percentage respectively whereas, feedstock availability and succinic acid concentration were considered as secondary criteria and assigned with twenty and ten percentage respectively. The total weightage expresses the level of feasibility that the biomass offers for industrial production of succinic acid.

Table 17. Summary of weightage assigned to each critical factor

	Critical factors				
	Cost of feedstock	yield	concentration	availability	total weightage
weightage of Factor	40	30	10	20	100

The key performance parameters are defined as follows,

$$\text{Cost} = \frac{(\text{highest cost of biomass - cost of biomass per ton}) \times 40}{\text{highest cost of biomass - lowest cost of biomass}} \quad (3.1)$$

$$\text{Yield} = \frac{\text{yield of biomass} \times 40}{\text{highest yield of biomass}} \quad (3.2)$$

$$\text{Availability} = \frac{\text{availability of biomass} \times 20}{\text{highest availability of biomass}} \quad (3.3)$$

$$\text{Concentration} = \frac{\text{concentration of product} \times 10}{\text{highest concentration achieved}} \quad (3.4)$$

Information about the key performance parameters were collected from various literature sources (Table 18). Based on the data collection, each key parameter was quantified using the weightage as shown in Table 17 and the parameter equations (3.1), (3.2), (3.3), (3.4). The total weightage was calculated for each feedstock as shown in last column of Table 19 and Table 20. Feedstock that has maximum total weightage is the most preferable feedstock. Feedstocks were positioned from highest to lowest rank based on the total weightage. Ranking of feedstock was done for two scenarios: worldwide availability of feedstock (Table 19) and feedstock availability in the USA (Table 20).

Table 18. Cost, yield, and availability of biomass as reported in literature [24]

biomass	cost of raw material ($ tonne^{-1})	ref	yield (g g^{-1})	ref	U.S./World availability (million tonnes y^{-1})	ref
corn stover/ straw	40	26	0.81	23	**24**/ 1015	26
corn core/cob	40	31	0.89	23	0.8/ 84	26
Glycerol*	**219.5**	55	**1.33**	34	0.6/ 600	35
wheat straw	60	39	0.74	23	8/915	38,40
cotton straw	38	41	1.23	42	3.7/ 107	43
Rice straw	40	26	0.63	23	4.3/ 800	26,44
rapeseed meal	324		**0.12** (SSF)		4.9 /30.8	39
waste bread	60		1.16		10.7/na	52
sugar cane bagasse	**23**		0.89		4/73.6	26
sugar cane molasses	140		0.96		1/56	26
soybean meal	345		0.64		45/ 151.6	80
wood (oak) hydrolysate	65		0.88		**0.04**/na	26
sake lees	na		**0.59**		na	
food waste	0	52	0.22	51	63/ 1600	52
switch grass	40		na		83.5/ na	53
willow or hybrid poplar	40		na		61.32/ na	53

na: not available. Number in bold indicates the highest or lowest value.

*U.S. Biodiesel annual production = 1.2 billion gallons. Biodiesel production will generate about 10% (w/w) glycerol as the main by-product. Thus, every gallon of biodiesel produced generates approximately 1.05 pounds (0.5 kg) of glycerol. This indicates a 1.2 billion of biodiesel will generate 0.6 million ton of glycerine per year.

Table 19. Ranking of feedstocks based on biomass availability in the world

Ranking	biomass	Scores for cost (per 40)	Scores for yield (per 30)	Scores for concentration (per 10)	Scores for availability (per20)	Total
1	corn stover/ straw	35.4	12.2	9.6	20.0	77.1
2	Glycerol	17.3	40	0.9	11.8	70
3	wheat straw	33.0	11.1	3.6	18.0	65.8
4	Rice straw	35.4	9.5	3.4	15.8	64.0
5	sugar cane bagasse	37.3	13.4	7.5	1.5	59.7
6	waste bread	33.0	17.4	9.0	0.0	59.5
7	cotton straw	35.6	18.5	3.0	2.1	59.2
8	corn core/cob	35.4	13.4	6.1	1.7	56.5
9	wood (oak) hydrolysate	32.5	13.2	4.6	na	50.3
10	food waste	40.0	3.3	5.7	na	49.0
11	sugar cane molasses	23.8	14.4	8.9	1.1	48.2
12	Glycerol	14.6	20.0	0.9	11.8	47.3
13	switch grass	35.4	na	na	na	35.4
14	willow or hybrid poplar	35.4	na	na	na	35.4
15	sake lees	na	8.9	10.0	na	18.9
16	soybean meal	0.0	9.6	2.1	3.0	14.8
17	rapeseed meal	2.4	1.8	3.0	0.6	7.8

As shown in Table 19, corn stover was placed at highest ranking as compared to all other feedstocks available in the world. This indicates that corn stover is best feedstock because of its less expensive cost, abundant availability, and higher yield. Corn stover was therefore selected for experiments on succinic acid production.

Glycerol was ranked next to corn stover, although glycerol is expensive feedstock ($219.5/ton). Glycerol does not require any pre-treatment technology and is highly available biomass. In addition, research experiments reported highest yield (1.33 g/ g) of

succinic acid using glycerol as feedstock. Other promising biomass feedstocks are cotton stalk (rank 3), wheat straw (rank 4), rice straw (rank 5).

Table 20. Ranking of feedstocks based on biomass availability in the U.S.A

Ranking	biomass	Scores for cost (per 40)	Scores for yield (per 30)	Scores for concentration (per 10)	Scores for availability (per20)	Total
1	waste bread	33.0	26.2	9.0	3.4	71.6
2	food waste	40.0	5.0	5.7	20.0	70.7
3	cotton straw	35.6	27.7	3.0	1.2	67.5
4	sugar cane bagasse	37.3	20.1	7.5	1.3	66.2
5	corn stover/ straw	35.4	12.2	9.6	7.6	64.8
6	switch grass	35.4	na	na	26.5	61.9
7	corn core/cob	35.4	20.1	6.1	0.3	61.8
8	wood (oak) hydrolysate	32.5	19.8	4.6	0.0	56.9
9	wheat straw	33.0	16.7	3.6	2.5	55.9
10	willow or hybrid poplar	35.4	na	na	19.5	54.8
11	sugar cane molasses	23.8	21.7	8.9	0.3	54.6
12	Rice straw	35.4	14.2	3.4	1.4	54.3
13	Glycerol	14.6	30.0	0.9	0.2	45.7
14	soybean meal	0.0	14.4	2.1	14.3	30.9
15	sake lees	na	13.3	10.0	na	23.3
16	rapeseed meal	2.4	2.7	3.0	1.6	9.7

The ranking is different in case of biomass availability in the USA (Table 20); bakery waste was ranked first and food waste was the second preferable feedstock.

4. EXPERIMENTAL STUDY OF SUCCINIC ACID PRODUCTION

Based on simulation findings, an experimental set up was designed. As shown in Figure 22, the experimental design identifies the following: suitable feedstock, pretreatment technology, bacterium, fermentation medium, operating condition, and product separation method.

4.1 Materials and methods for pretreatment process

4.1.1 Raw material

Samples of corn stover were obtained from Texas A&M AgriLife, the agriculture college at Texas A&M University. The corn stover was then washed with water to remove dirt particles. The washed corn stover was then dried with air and convection oven till the moisture content remains constant. It was stored in air-tight container to maintain constant moisture, and to reduce air contact. The samples were grounded in a Wiley mill to obtain an average particle size of 20 mesh screen. By ensuring uniform particle size, performance of pretreatment was maintained.

4.1.2 Selection of pretreatment method

Key factors that guide the selection of pretreatment method include feedstock composition, sugar yield, inhibitor formation, technology cost, commercial readiness level, environmental impact of pretreatment technology. Based on literature survey as tabulated in Appendix D Table 29, dilute acid treatment, ammonia explosion and steam explosion seem to be the efficient and cost-effective methods. As listed in Appendix D, Table 30, steam explosion has many advantages: low cost, maximum glucose yield, low environmental impact, commercial application status. Considering equipment availability in the lab, this experimental work selected ultrasonic and dilute sulphuric acid(H_2SO_4) treatment. Biomass will first be treated with ultrasonic method, followed by dilute acid (H_2SO_4) treatment.

4.1.3 Enzymes

Once mechanical pretreatment is complete with ultrasonic and dilute acid method, the hydrolysate will then be fermented with hydrolytic enzymes. The enzymes were provided by Genencor International. This enzyme consists of two types of commercial enzymes; Accellerase 1500 a cellulases complex and Accellerase XY a hemicellulases enzyme complex, both produced from genetically modified strains of Trichoderma reesei. As indicated by enzyme supplier, Accellerase 1500 provides endoglucanase Activity and Beta-Glucosidase Activity, while Accellerase XY presents xylanase activity.

4.1.4 Hydrolysate preparation

In this experiment, three different pretreatments (ultrasonication, dilute acid and enzymatic hydrolysis) were used to convert biomass to reduced sugar, mainly glucose and xylose. Corn stover solids were added in to a 2% dilute sulphuric acid at a loading rate of 10 gm corn stover per 100 gm of solution. The mixture was subject to ultrasonication using an ultrasonicator at 100 kHz for 30 minutes. Once the ultrasonic process was done, the treated biomass was heated in a hot air convection oven at 120°C for 45 minutes. After the acid treatment was complete, the biomass was washed with water until pH of filtrate reached above 4.5. As per enzyme-provider recommendation, the successive, enzymatic treatment generally exhibits better performance at a pH between 4.5 and 7.

The acid-treated biomass was then reacted with hydrolytic enzymes (Accellerase 1500, and Accellerase XY). These enzymatic reactions took place in 250 mL Erlenmeyer flasks with ACCELLERASE® 1500 dosage rate of 0.3 mL per gram cellulose + ACCELLERASE® XY enzyme dosage rate of 0.10 mL per gram cellulose. 10 mL solution of 50 mM sodium acetate buffer was added to maintain pH of 5. The mixture was heated in a digital hot plate stirrer at 50°C. Stirrer speed, 100 rpm was maintained to ensure thorough mixing.

During enzymatic hydrolysis, samples were taken on a regular time interval. The samples were centrifuged at 10,000 rpm for 5 min. Using 0.5 μm hydrophilic PTFE syringe filters, the supernatant was filtered. This filtrate was analysed using high performance liquid chromatography for sugar content such as glucose, xylose, mannose, arabinose, galactose and cellobiose.

The efficiency of pretereatment was measured by comparing total sugar yield before and after enzymatic hydrolysis. The total sugar yield represents mainly glucose and xylose formation. To perform the calculation, following equation [71] was used,

% total sugar (xylose + glucose) yield = $[(c \times V)/ (w \times C_f]$ 100%, where,

c is the concentration (g/L) of total sugars (glucose+xylose) in the hydrolysed sample, as determined by HPLC,

V is the total volume (L) of hydrolysate,

w is the initial weight (g) of glucose and xylose, determined by NREL protocol [64]

C_f is the correction factor to account for addition of water molecules to the anhydroglucose residues in cellulose or hemicellulose (C_f is 1.11 for glucan and 1.14 for xylan and arabinan).

After the pretreatment step was done, the corn stover hydrolysate was used as substrate for successive fermentation to produce succinic acid.

4.2 Materials and methods for fermentation process

4.2.1 Selection of microorganism

Several succinic-producing microorganisms were screened to identify the most efficient one. *Actinobacillus succinogenes* has many salient features, including the following; fermentation capability to process a broad range of biomass based sugars such as arabinose, cellobiose, fructose. Also, the bacterium has good tolerance to a high concentration of glucose, high acid concentration. The bacterium reported a higher yield of 80 gm succinic acid/100gm of glucose [55, 56, 57].

Many researchers have experimented with several other native and engineered bacteria using biomass hydrolysates (Appendix E). However, less number of research studies have reported for a bacterium, *Basfia succiniciproducens*, despite its promising features.

Of the natural SA producing bacteria, *Basfia succiniciproducens* was isolated in 2008 from bovine rumen [56]. *B*.succiniciproducens is a member of the Pasteurellaceae family and is characterized as non-pathogenic, gram-negative, facultative anaerobic, and capnophilic. For this experimental work, *B*.succiniciproducens was selected to ferment biomass hydrolysate with a future goal of engineering the strain to enhance SA production.

4.2.1.1 Metabolic network of native B. succiniciproducens

Metabolic pathway of *B*.succiniciproducens is comprised glycolysis, pentose phosphate pathway (PPP), tricarboxylic acid (TCA), Entner–Doudoroff pathway (EDP), cycle, anaplerotic carboxylation and decarboxylation, fermentation pathways, and anabolic pathway. Most natural producing bacteria synthesis succinic acid using a partial Tri-Carboxylic Acid (TCP) cycle. TCP carboxylates phosphoenolpyruvate (PEP) into succinic acid routes as shown in Figure 25.

Glycolysis [61]:

As listed in Table 21, Glucose is phosphorylated into glucose-6-phospate (G6P) with the action of a permease enzyme. G6P is further catabolized to phosphoenolpyruvate (PEP) through the glycolytic pathway. Phosphoenolpyruvate (PEP) produces succinate and other by-products through TCA cycle.

Table 21. Chemical reactions of glycolysis

GLC+ATP → G6P	1,3PG↔3PG + ATP
F6P↔G6P	3PG↔2PG
F6P+ATP→F1,6P	2PG↔PEP
F1,6P↔DHAP+GA3P	PEP↔PYR +ATP
DHAP↔GA3P	
GA3P↔1,3PG+NADH +ATP	

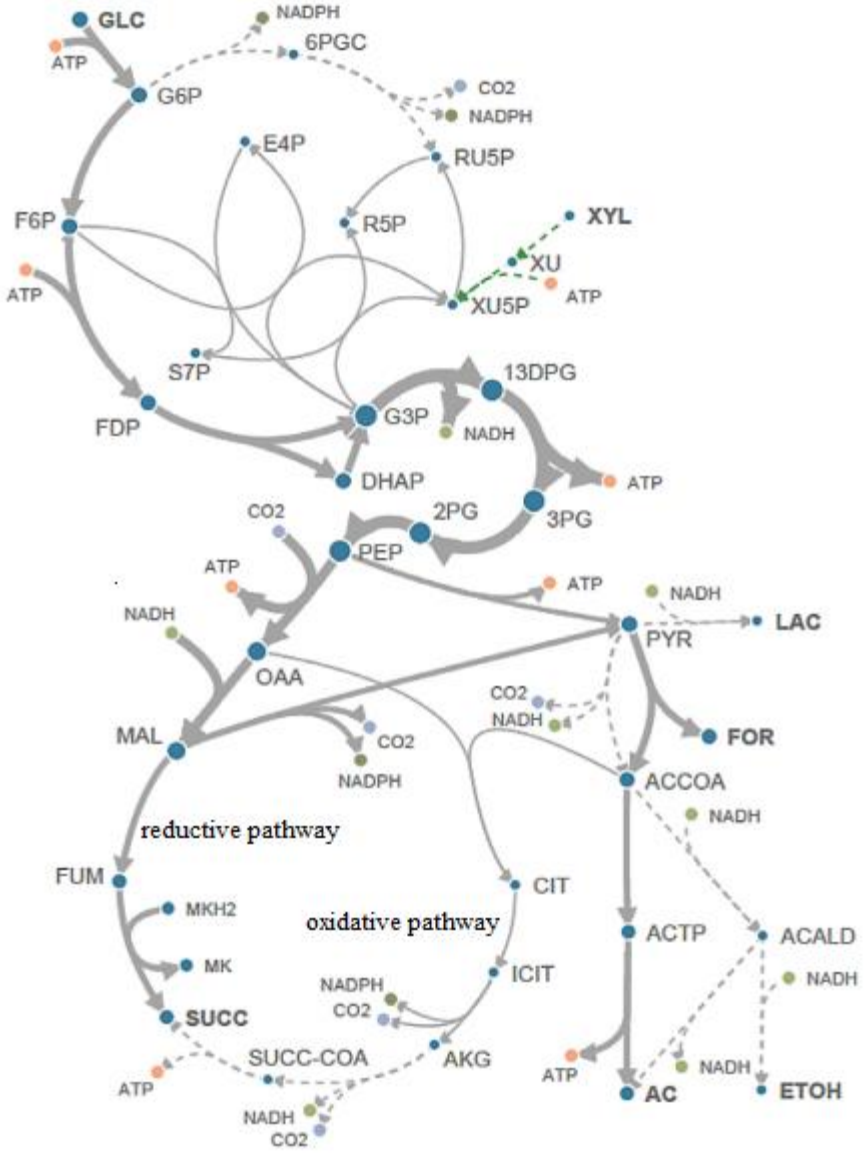


Figure 25. Metabolic network analysis of *B.Succiniciproducens* [61] (Thicker arrows indicate higher flux, while dashed arrows indicate less or zero flux)

TCA cycle [61]:

PEP can take either C4 pathway to produce succinate via oxyloacetate, malate, fumarate or C3 pathway to produce formate, acetate and ethanol depending CO_2 level (Table 22). In case of ample quantity of CO_2, PEP takes C4 pathway; PEP consumes CO_2 to produce succinate and ATP with action of enzyme, called PEP carboxykinase.

Gibbs energy of the reaction is 5.6 kJ mol-1. If there is not enough CO2, PEP prefers C3 pathway.

Table 22. Chemical reactions of TCA cycle

TCA Cycle	
C4 pathway to Succinate (Reductive pathway)	C3 Pathway to byproducts (Oxidative pathway)
PEP+CO2↔OAA+ATP OAA→PYR+CO2 OAA+NADH↔MAL MAL→PYR+NADPH+CO2 MAL↔FUM FUM+NADH+2/3 ATP↔SUC	PEP↔PYR +ATP PYR→AcCoA+ NADH +FOR AcCoA→ACE +ATP AcCoA+2NADH→ETH ADP + GLC → 1.0 ATP + 2.0 LAC 3.0 ADP + GLC + 2.0 NAD →2.0 AC + 3.0 ATP + 2.0 FOR + 2.0 NADH 3.0 ADP + GLC + 4.0 NAD → 2.0 AC + 3.0 ATP + 2.0 CO2 + 4.0 NADH
Xylose Catabolism: Xyl+ATP→X5P X5P↔RU5P	
Biomass formation: 13.49NADPH + 0.00041G6P + 0.000126F6P + 0.000686R5P + 0.000099G3P + 0.0013703PG + 0.000528pep + 0.002764Pyr + 0.003006AcCoA' + 0.001502OAA + 0.046930ATP → Bio + 0.002727NADH	

Redox reaction:

Theoretically, 1.71 mol of succinic acid can be produced from the fixation of 1 mol of CO_2 and 1 mol of glucose or 1 mol of glycerol. 2 moles of NADH are oxidised through the reductive pathway of the TCA cycle during conversion of OAA to succinate via malate and fumarate. One mole of NADH is produced within the reductive pathway, but remaining one should be supplied by other parts of the metabolism such as glycolysis, C3 pathway. Because C4 pathway requires one mole of nicotinamide adenine dinucleotide (NADH), the pathway is referred as reducing branch. An oxidative branch in C3 pathway (pyruvate to acetyl CoA) supplies additional one mole of NADH to C4 pathway to maintain cell's redox.

In addition to succinic acid, TCA cycle produces by-products: lactic acid (LA), formic acid (FA), and acetic acid (AA). The theoretical yield cannot be achieved due to biomass and by-product formation.

Theoretically, ~1.71 mol (1.12 gm/ gm glucose) succinate can be produced per mol glucose (plus CO2), based on stochiometric balance (equation 4.1):

$$C_6H_{12}O_6 + 0.86\ HCO_3^- \rightarrow 1.71\ (CH_2)_2(CO_2)_2^{2-} + 1.74\ H_2O + 2.58\ H^+ \quad (4.1)$$

(Glucose) (succinate) $\Delta GH^{o'} = -173$ kJ/mol

$$CO2 + H20 \rightarrow HCO_3^- + H^+ \quad (4.2)$$

Carbonic anhydrase, which is found within bacterium, converts CO_2 and water into carbonic acid. Carbonic acid is dissociated into protons, and bicarbonate ions (equation 4.2).

In the presence of CO2 and additional reducing power (e.g. glycerol, sorbitol, H2), the theoretical yield increases to 2 mol succinate per mol glucose (equation 4.3):

$$C_6H_{12}O_6 + 2\ HCO_3^- + 2H_2 \rightarrow 2\ (CH_2)_2(CO_2)_2^{2-} + 2H_2O + 2H^+ \quad (4.3)$$

(Glucose) (succinate) $\Delta GH^{o'} = -317$kJ/mol

Two research works relevant to this organism demonstrated an improved succinic acid production via fermentation [69] and metabolic engineering [70]. In the former,

Basfia succiniciproducens (*B*.succiniciproducens) was cultivated in continuous fermentation using glycerol as a substrate.

Succinic acid productivities and titres resulted low for commercial application. In the latter one, authors reported improved succinic acid yields from 0.48 to 0.71 g/g using genetically modified strain of *B*.succiniciproducens. Genetic modification deals with deletion of by-product producing mutants.

4.2.2 Fermentation medium

Fermentation media from several experiments were referred from literature sources. The media components were screened to prepare an optimal fermentation medium. Components of the selected fermentation medium are shown in Table 23. This fermentation medium is used in this experiment.

Table 23. Fermentation medium selected for this study

	Compound	Concentration, $g.l^{-1}$
Organic	Glucose (25-80)	40
	Yeast extract (5-10)	6
	Corn steep liquor (5-10)	10
Mineral salt	K_2HPO_4	3
	NaCl	1
	$MgCl_2.6H_2O$	0.2
	$CaCl_2.2H_2O$	0.2
	$MgCO_3$	10
Working volume, mL	450	

4.2.3 Fermentation condition

CO_2 fixation is required to enhance succinic acid production. Therefore, CO_2 (g) was supplied to the fermentation broth. Excessive amount of $MgCO_3$ up to 10 g/L was added as a buffer medium to maintain pH between 6 and 7. $MgCO_3$ also acts as a carbon source. Fermentation temperature of 37°C and agitator speed of 300 rpm were maintained throughout the incubation period using automatic control system of bio-fermenter.

4.2.4 Microorganism

Native *Basfia succiniciproducens*, DSM 22022 in pellet form was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany. This bacterium is also named as CCUG 57335 and JF4016.

As per bacterium supplier recommendation, inoculation medium was prepared by thoroughly mixing 1.5 gm of Tryptone Soya Broth (TSB) in 100 ml of distilled water. Composition of TSB is shown in Table 24.

The bacterium was revived in 100 mL of inoculation medium. Cells were incubated at 37°C and 180 rpm for 16 hr using a hot plate stirrer. The hot plate stirrer was placed inside hot air convection heater. During the microbial growth, 10 mL sample was withdrawn from the culture medium at regular time intervals to determine optical density using spectrophotometer. Cells were incubated until optical density of cell reached 0.1 at 600 nm. Flasks were sterilized before using it for bacterial growth.

Table 24. Components of inoculation medium

Components	Concentration (gm/L)
Peptone from casein	17
Peptone from soymeal	3
D (+) Glucose	2.5
NaCl	5
K2HPO4	2.5

4.2.5 Experimental design for batch fermentation

Fermentation experiments were performed on a 2 L capacity of Biostat CT bioreactor (2 L total volume) as shown in Figure 26, with 400 mL working volume.

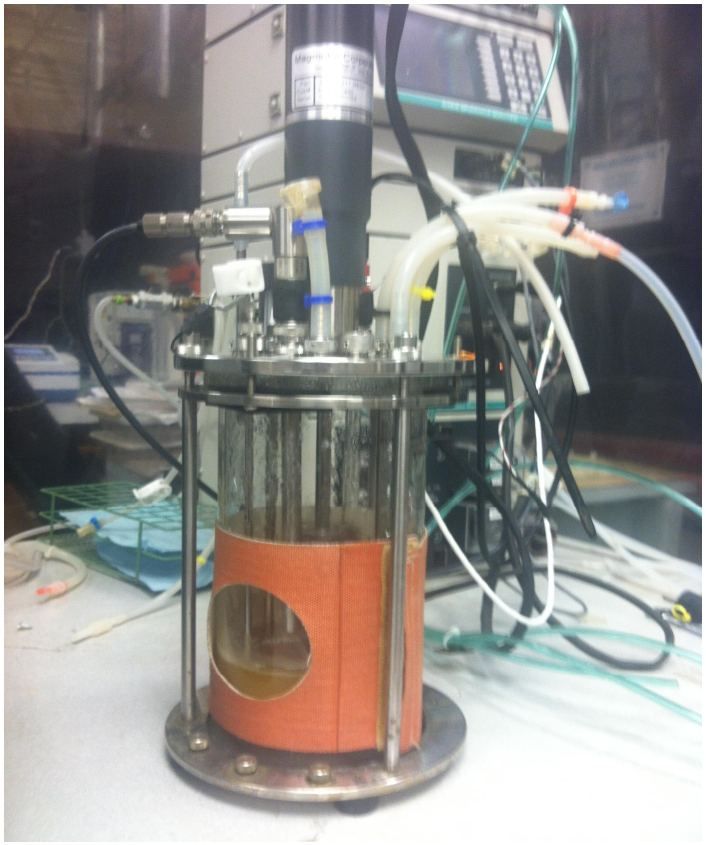


Figure 26. Bio-fermenter

Fermentation medium of 250 mL was prepared by mixing carbon source, nitrogen source and mineral salts as listed in Table 23. Pretreated-corn stover was added into the fermentation medium. Additional glucose was charged into the fermentation medium at a concentration of 40gm glucose/ litre of fermentation medium. This provides high substrate concentration which in turn improves microbial growth.

The fermentation medium was fed with 50 ml inoculation medium. The culture was sparged with CO_2 at 0.1 vvm to maintain anaerobic condition. Temperature, 37°C and agitator speed 300 rpm were set using automatic controllers in bio-fermenter. pH between 6.5 and 7 was maintained via addition of $MgCO_3$.

During the fermentation, 10 mL samples were withdrawn from the reactors at regular time intervals. The samples were centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through 0.5 μm hydrophilic PTFE syringe filters and analysed using HPLC for measuring succinic acid concentration along with other acid concentration such as formic acid, lactic acid, acetic acid.

4.2.6 Analytical methods

Texas A&M AgriLife provided samples of corn stover along with its structural composition as shown in Table 25 [72]. Texas A&M AgriLife applied the analytical protocols developed by NREL of US DOE to quantify the structural composition. The composition of corn stover was used to perform calculations in this experiment.

Table 25. Composition of corn stover

Component	Formula	MW (g/gmol)	Composition (%)
Cellulose	$C_6H_{10}O_5$	162.14	37.73
Hemicellulose	$C_5H_8O_4$	132.12	26
Lignin	$C_8H_8O_3$	152.15	19.24
Ash	-	-	3.79
Other solids	$C_8H_8O_3$	152.15	13.24

To determine sugar concentration mainly, glucose, xylose, arabinose, mannose, galactose, the samples were injected in a high performance liquid chromatography (HPLC) (Waters 2690, Separations Module). HPLC is equipped with auto sampler, a

Shodex SP 810 packed column and a Refractive Index (RI) detector. 5 mL of fermentation broth was centrifuged (10,000 rpm, 3 min). The supernatant was then filtered through a 0.5µm syringe filter. The filtrate was then analysed in high pressure liquid chromatography (HPLC).

Bacterial growth was analysed using spectrophotometer. Optical density was measured at 600 nm (OD600). The OD expresses quantity of suspended cells in the fermentation broth.

Concentration of succinic acid and other organic acids were measured by injecting 5 µL of filtered sample onto the high performance liquid chromatography (HPLC). The specification of HPLC is listed as follows,

Column: Acclaim HPLC Organic Acid Analysis Column, 5µm,

Column dimension: 4 x 150 mm,

Column temperature: 30°C,

Mobile phase: 0.01 M sodium sulfate (Na_2SO_4),

Flowrate of mobile phase: 0.6 mL/min

Detector: Ultra Violet (UV),

Detection wavelength: 210 nm,

Retention time: 2.7 min – 2.9 min.

4.3 Results and discussions

4.3.1 Evaluation of pretreatment method for sugar production

A combination of pretreatment methods (hydrolytic enzyme + ultrasonic + dilute acid) converted corn stover biomass into sugar. Table 26 and Figure 27 show the time course taken for the total sugar (glucose + xylose) production. The enzymes rapidly hydrolysed corn stover-substrates within approximately 60 hours, and produced a maximum glucose concentration of 2.8 gm/ litre of solution and xylose concentration of 1 gm/L. Beyond the duration, sugar concentration began to drop. This might be due to decomposition of sugar molecules. Highest glucose yield up to 35 gm per 100 gm of

biomass, and xylose yield of 17 gm per 100 gm of biomass were calculated from the glucose and xylose concentration before and after pretreatment. The total sugar yield of 25 gm per 100 gm of biomass was determined from the maximum values of glucose and xylose yield.

Table 26. Results obtained in hydrolysis of corn stover

Time, hr	Glucose concentration, g/L	Glucose yield, gm/100 gm of corn stover	Xylose concentration, g/ L	Xylose yield, gm/100 gm of corn stover	Total Sugar yield, gm/100 gm of corn stover	Rate, gm/ L/ hr
24	0.9	11	0.5	8	10	0.04
32	1.6	19	0.6	10	15	0.05
48	2.2	26	0.6	10	18	0.05
56	2.6	31	0.8	13	22	0.05
64	2.8	34	0.9	15	24	0.04
72	2.8	34	1	17	25	0.04
82	2.6	31	0.8	13	22	0.03

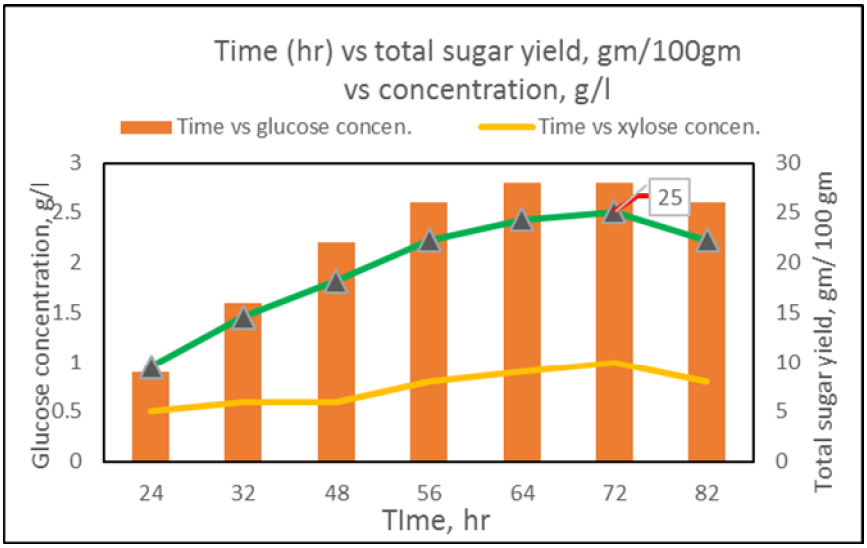


Figure 27. Time course of total sugar yield from corn stover

4.3.2 Evaluation of fermentation method for succinic acid production

B.succiniciproducen fermented corn stover hydrolysate to produce succinic acid. Table 27 and Figure 28 show concentration of succinic acid, produced at different process duration. After 48 hours of fermentation, the highest titre value of 1.6 gm succinic acid /litre of fermentation broth was observed. Extending the fermentation duration did not improve succinic acid concentration. Maximum yield of 18 gm succinic acid per 100 gm of total sugar, and the corresponding production rate of 0.02 g/L/hr were calculated from the highest succinic acid concentration.

Table 27. Results obtained in fermentation of corn stover

Time, hr	Succinic acid concentration, gm/L	Succinic acid yield, gm/100 gm	Rate, g/ L/ hr
18	0.3	3	0.02
24	0.5	6	0.02
42	0.8	9	0.02
54	1.2	13	0.02
72	1.6	18	0.02
80	1.6	17	0.02

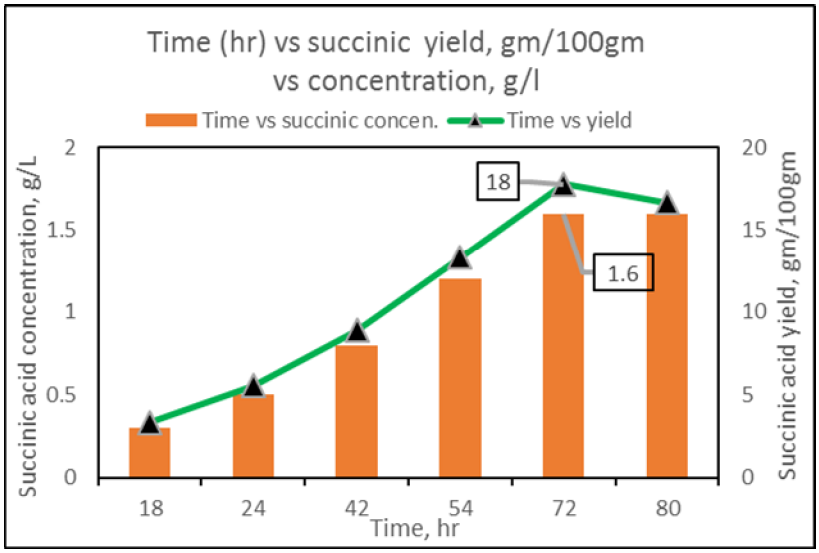


Figure 28. Succinic acid yield as a function of time

4.4 Future research directions

Highest yield of 1.08 gm succinic acid /gm glucose was reported in case of a fermentation that used glucose as a substrate, and a genetically modified strain of *B.succiniciproducens* to ferment the substrate. The current experiment observed a maximum yield 0.17 gm of succinic acid/ gm of total sugar using a wild type of the same bacterium. Major factors that can lower the succinic acid yield are discussed as follows,

Fermentation requires pH between 6.0 and 7.2 with an optimal pH of 6.8 [25]. Though higher pH will accelerate cell growth, but will increase by-product formation. [4]. Below a pH of 6.0, little cell growth occurs due to increased energy demand for cell maintenance [4]. This current experiment was not able to maintain the optimal pH throughout the fermentation course. Metabolic engineering of the bacterium would provide a good tolerance to high acidic environment, which in turn would increase succinic acid yield.

During the fermentation process, succinic acid was produced along with the by-products such as formic, lactic, and acetic acid. The organic acids caused pH to decrease. Because of this, base was continuously added to the fermentation medium to keep the pH at or near its optimal. This was done through addition of magnesium carbonate ($mgCO_3$) and CO_2. If too much base is added, the osmolarity of the fermentation medium changes and cells will begin to flocculate; their productivity decreases as they spend energy on cell maintenance [31]. An optimal proportion of $mgCO_3$ and CO_2 should be known prior to addition. The present work did not run many experiments to find out the optimal proportion.

The by-product formation can reduce product yield, because the bacterium takes away from the carbon source which is otherwise used by the bacteria to produce main product [74]. Among other by-products, formic acid is the strongest inhibitor to succinic acid production [7]. Metabolic engineering could help to knock out the by-product pathway in metabolic network of bacterium. Deletion of by-product pathways would result a better TRY (titre, rate, yield) of succinic acid [70].

Optimum concentration of substrate or carbon source is also a concern. Initial glucose concentrations above a 100g/L hindered cell growth and succinate production, while below a 20g/L produced very less product that it was impractical to perform successive product separation [75]. Additional studies indicated the optimal initial glucose concentration between 50g/L and 60g/L [31]. In this experiment, initial glucose concertation of 40 g/L was used. The fermentation medium should have been fed with the optimal glucose concentration to achieve maximum product yield.

4.4.1 "Starch versus lignocellulosic" feedstock

As reported by US DOE in 2004, biobased succinic acid requires a minimum productivity of 2.5 g/L/hr of succinic acid to compete economically with fossil based succinic acid [1]. Recent studies proved the potential of bacterium to produce a much higher rate of 11.8 g/L/hr [77], which is now promising the economic potential of fermentation technology.

Lignocellulosic biomass presents a cost-advantage to "1st generation", food-based sugars. Based on conceptual and industrial data, cost of succinic acid production using lignocellulosic as feedstock is 28% less than fossil based, or biological method that uses starch based sugar as feedstock (Table 28).

Table 28. Cost of succinic acid production

Feedstock	Technology	Cost, $/ lb SA	Cost, $/ kg SA
Butane	Petrochemical	1.3	2.86
Sugar	Biological	1.3	2.94
Corn stover	Biological	0.94	2.00

Though the lignocellulosic biomass is competitive, purification step poses the cost intensive process for succinic acid production, and is identified as major area for

improvement [5]. Purification can account for up to 60% to 70% of production cost [74]. Starch based sugar generally provides a higher yield of 0.8 ton product per ton feedstock, whereas lignocellulosic based sugar gives a lower yield of 0.7 ton/ton. This is because of the highly pure, starch based feedstock that produces crude succinic acid with fewer impurities. The crude product with less contaminant requires less dilution water for washing and leads to lower product losses from purification.

To date, starch-based sugar has been used as feedstock to produce succinic acid on a commercial scale. Several companies (Table 2), notably BioAmber have begun commercial operation of succinic acid using glucose derived from corn (starch based sugar). The plant is in Sarnia, Ontario, with a nameplate capacity of 30,000 tons per year. Technology of the company will be cost-competitive with fossil based method even if oil price drops down as low as $35/barrel [76].

Historically, the market size for succinic acid is small, with immediate use in a narrow range of applications such as pharmaceuticals and food ingredients. This is because of higher cost of producing succinic acid from petroleum feedstocks. As a result, the current market for petroleum-based succinic acid is only approximately 40,000 tons/year, representing a small market size of $300 million. Industrial reports forecast succinic acid demand of approximately 600,000 tons by the year 2020 with a value of $1 billion. This significant demand makes the future price of succinic acid largely unknown. Simulation results, however, suggest that $2000/ton is the minimum acceptable price assuming current process technology.

For such a limited applications and unpredictable price, starch based feedstock seems suitable than lignocellulosic because of its advantages: sufficient availability of feedstock, proven commercial technology and efficient separation method. Only 6% of the sugar produced in existing corn wet mills in the USA can produce $2 billion worth of succinic acid and BDO. Though Lignocellulosic is cost-advantageous, it would become competitive with starch based feedstock, provided that the market size of succinic acid is large enough to utilize abundant source of biomass.

5. CONCLUSION

This thesis has aimed to identify the potential bioproducts by developing a biorefinery model that meet the objective function: maximizing profit while minimizing GHG emission and energy use. An evaluation matrix was constructed to find a suitable feedstock. Based on findings from modelling of superstructure, an experiment was designed with aim of producing succinic acid at higher titre, rate, and yield than it is reported in literature source.

Optimization results indicate that speciality chemicals are highly favourable than commodity chemicals in case of commercialising a biorefinery. Optimization studies resulted with succinic acid as the preferable product, corn stover as the suitable feedstock and anaerobic fermentation as the optimal conversion technology.

Though many high value chemicals such as BDO, acrylic acid, adipic acid are attractive, conversion technologies of those chemicals have not yet reached commercial deployment status. Few other bioproducts such as Levulinic acid, xylitol, sorbitol, do not require any additional production, because supply of those bioproducts have already met the total market demand; it replaced 100 percentage of petrochemical equivalents.

Succinic acid shows many advantages as compared to other bioproducts; proven commercial application of technology, high product demand in existing as well as future market condition, significant GHG (-87%) and energy-use (-51%) reduction in relative to petrochemical technology. Currently, biological method contributes almost 50% of worldwide production of succinic acid. Fossil based method produces remaining quantity. Thus, there is a realistic scenario to replace the remaining quantity by biobased method.

Based on optimization findings, an experiment was conducted to produce succinic acid by utilizing suitable pretreatment and fermentation methods. The experiment used corn stover as feedstock, and selected a bacterium, *Basfia succiniciproducens* to ferment the feedstock. A maximum glucose yield of 0.35 gm / gm of corn stover was observed. Literature sources recorded a highest glucose yield of 0.98gm / gm of corn stover [60].

Highest succinic yield of 0.18 g /g total sugar(glucose+xylose) was observed after 72 hours of anaerobic fermentation. The corresponding titre value was 1.6 gm/L.

Native bacterium, *Basfia succiniciproducens* can produce succinic acid at a high titre of 30.9 gm/L [61]. Genetically modified strain of this bacterium produced a much higher yield of 0.71 gm succinic acid/ gm of glucose [70]. When compared to past research works, this experiment shows a much lower concentration and yield. The maximum values of TRY (Titre, Rate, Yield) can be achieved by increasing number of trials in the experiment.

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APPENDIX A

COMPOSITION OF VARIOUS LIGNOCELLULOSIC BIOMASS

Table 29. Composition of biomass feedstocks

Lignocellulosic material	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Bamboo	21 - 31	15 - 26	26 - 43
Banana waste	14	14.8	13.2
Barley straw	14 - 15	24 - 29	31 - 34
Bast fibre jute	21 - 26	18 - 21	45 - 53
Bast fibre kenaf	15 - 19	22 - 23	31 - 39
Black gram residue	23.14	32.48	26.80
Coastal Bermuda grass	6.4 19.4	35.7 13.3	25 47.8
Coconut coir	18	26	48
Corn cob	15 4.5 -6.6	35 38 - 42	45 35
Corn stalks	17	24	43
Corn stover	18 18	22 20.5	40 41.7
Cotton gin waste	-	16	78
Elephant grass	24	24	22
Esparto grass	17 - 19	27 - 32	33 - 38
Flax straw	22	27	29
Grasses (average)	10 - 30	25 - 50	25 - 40
Hardwood stem	18 - 25	24 - 40	40 - 55
Leaves	0	80 - 85	15 - 20
Millet husk	14	27	33
Newspaper	18 - 30	25 - 40	40 - 55
Nut shells	30 - 40	25 - 30	25 - 30
Oat straw	16 - 19	27 - 38	31 - 37
Orchard grass	4.7	40	32
Pinewood	20	24	39
Poplar wood	26	17	35
Rice husk	14	24	31
Rice straw	18	24	32.1
Rye straw	16 - 19	27 - 30	33 - 35
Sabai grass	20.88	23.72	49.90
Softwood stem	25 - 35	25 - 35	45 - 50

Table 29 continued.

Lignocellulosic material	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Sugarcane bagasse	19 - 24 18.4 24	27 - 32 26.9 25	32 - 44 38.1 43
Sugarcane tops	36.1	24.2	33.3
Sunhemp residue	17.4 - 18.4	11.9 - 13	43.4 - 48
Sweet sorghum bagasse	18	25	45
Switchgrass	12 18	31 22	45 31
Timothy grass	18	30	34
Waste papers from chemical pulps	5 - 10	10 - 20	60 - 70
Wheat bran	3 – 10 16 - 21 7.7	22 – 25 26 - 32 30.8	7 – 11 29 - 35 41.3
Wheat straw	15 17 14.5	50 23 24.8	30 33 36

APPENDIX B

LIST OF CONVERSION TECHNOLOGIES

Table 30. List of conversion technologies and its details

Pathway #	Conversion technologies	Product	Conversion %	Selectivity %	Annual capital cost/ tonne (U.S.$)	Annual operating cost/ tonne (U.S.$)
11a	Ammonia explosion + enzymatic corn stover	Sugars, Lignin	98	-	19.64	11.30
11b	Acid impregnation + Steam explosion + enzyme (corn stover)	Sugars, Lignin	85	-	28	18
11c	Steam explosion + enzyme (wheat straw)	Sugars, Lignin	85	-	13.90	7.97
11d	Aqueous ammonia (rice straw)	Sugars, Lignin	89	-	22.3	7.97
11e	Lime +enzymatic (sugarcane bagasse)	Sugars, Lignin	92	-	28	7.97
12	Pyrolysis	Syngas	94	-	62.86	36
13	Gasification	Syngas	90	-	86.43	55
14	Anaerobic digestion	Methane	40	-	26.23	15
15	Water gas shift reaction	Syngas	100	-	15.11	8.66
16	Mixed culture fermentation	Carboxylate salts	80	65	60	94
17	Fermentation	Succinic acid	55	-	4868	2984
20a	Organosolv separation	Lignin	79	-	40.68	23.30
20b	Organosolv separation	Sugars	97	-	40.68	23.30
23	Conversion of syngas 1	Methanol	25.1	2.6	38.56	22.10
		Ethanol		61.4		
24	Conversion of syngas 2	Methanol	24.6	3.9	41.10	23.60
		Ethanol		56.1		
25	Hydrogenation of CO	Methanol	28.8	20.7	40.19	23.00
		Ethanol		23.8		
		Propanol		14.1		
		Butanol		7.5		

Table 30 continued.

Pathway #	Conversion technologies	Product	Conversion %	Selectivity %	Annual capital cost/ tonne (U.S.$)	Annual operating cost/ tonne (U.S.$)
26	Fischer-Tropsch process 1	Hydrocarbon C2-C4	40	16	193.41	111
		Hydrocarbon C5-C9		27		
		HydrocarbonC10		26		
27	Fischer-Tropsch process 2	Hydrocarbon C2-C4	75	23	181.93	104
		Hydrocarbon C5-C9		19		
		Hydrocarbon C10		9.7		
28	Pyrolysis	Acetylene	80	80	96	121
31	Methanation	Methane	97	92	193	267
32	Bacterial fermentation	Adipic acid	74	-	4000	2150
33	Catalytic oxidation (Pt)	Glucaric acid	66	-	370	253
34	Catalytic hydrodeoxygenation	Adipic acid	77	-	290	192
35	Hydrogenation (Pt)	1,4-Butanediol (BDO)	99.7	85	350	397
		Tetrahydrofuran (THF)		8.28		
		Gamma-Butyrolactone (GBL)		2		
		n-Butanol		2.87		
36	Catalytic dehydration	Ethylene	99	-	24	730
37	Bacetrial fermentation	Farnesene	29	-	1480	980
41	Bacterial fermentation(blank)	3 Hydroxy propionic acid (HP)	72	-	na	na
42	Bacterial fermentation	Succinic acid	84	-	675	1100
43	Hydrogenation of glucose (blank)	sorbitol	87.5	-	0.1	407.7
44a	convert cellulose/glucose to Levulinic acid with H2SO4 as catalyst	Levulinic acid	60	-	117.3	259.7
45	Bacterial fermentation	Lactic acid	98	-	504	2174.4
46	Bacterial fermentation	Xylitol	93	-	573	87

Table 30 continued.

Pathway #	Conversion technologies	Product	Conversion %	Selectivity %	Annual capital cost/ tonne (U.S.$)	Annual operating cost/ tonne (U.S.$)
47	selective oxidation of cyclopentane (blank)	Glutaric acid	92.3	-	na	na
48	Autohydrolysis	HMF	90.9	-	63.46	36.40
49	Dehydration of sugars	Furfural	40.9	-	27.62	15.80
50	Hydrogenation of furfural	THFA	98.2	-	30.22	17.30
51	Hydrogenation of THFA 1	Pentanediol	99	-	43.52	24.90
		Pentanol		-		
52	Hydrogenation of THFA 2	Pentanediol	60	-	45.45	26.00
		Pentanol		-		
53	Acid fermentation	Acetic Acid (Acid 1)	70	-	5	43
54	Esterification	Ethyl acetate (Ester)	90	-	5	43
55	Hydrogenolysis	Ethanol	73	-	5	82
56	Monsanto process	Acetic or Ethanoic Acid (Acid -2)	99	-	40.68	23.30
57	Hydrogenation	Ethanol	61.7	-	169	132
58	Decarboxylation of acids	Hydrocarbon C2	62	21.3	45.94	26.30
59	Dehydration of alcohols 1	Hydrocarbon C2	67	-	40.5	23.20
60	Dehydration of alcohols 2	Hydrocarbon C3	59	28.8	37.47	21.50
		Hydrocarbon C4		37.3		
61	Dehydration of alcohols 3	Hydrocarbon C5	64	15.2	34.45	19.70
		Hydrocarbon C6		5.5		
		Hydrocarbon C7		5.6		
		Hydrocarbon C8		4.2		
62	Fractional distillation of alkanes	Hydrocarbon C8	99	-	169.48	98.2
		Hydrocarbon C2-C7, C9-C10	99	-		
63	Ketonization	ketone	99.5	-	120	187
64	Hydrogenation	Ethanol	98.4	-	60	94

Table 30 continued.

Pathway #	Conversion technologies	Product	Conversion %	Selectivity %	Annual capital cost/ tonne (U.S.$)	Annual operating cost/ tonne (U.S.$)
65	Grignard synthesis	alcohol	88	-	60	94
66	Hydrogeneation	Ethylene	85.9	56.4	104	164
67	Hydroformylation	Acetaldehyde	95		104	164
68	Hydrobromination	Bromoethane	25	90	104	164
69	Hydrolysis	Ethanol	45	64	104	164
70	Hydrogenation	Ethanol	48	84	104	164
71	Oligomerization	Gasoline	99	1.017	104	13.3
72	Bacterial fermentation	Ethanol	41	-	31.43	18.00
73	Yeast fermentation	Ethanol	61.9	-	40.62	22
74	ABE fermentation	butanol	42	23	137.3	590
		acetone		12		
		ethanol		4		
75	Purification of crude ethylene	Pure ethylene	99	-	5	60
76	Oxidation	2,5-Furan dicarboxylic acid (FDCA)	98	58	476	329
80	Harvesting+Preteatment+Liquid Extraction+Transesterification	Biodiesel and Glycerol	36	-	1300	1970
83	Harvesting+Preteatment+Liquid Extraction+Transesterification	Glycerol	3	-	1000	10000
84	Harvesting+Preteatment+Liquid Extraction+Transesterification	Biodiesel	40	-	54	1030
89	Bacterial fermentation of glycerol	Succinic acid	75	-	1334	11013
91	Dehydration	Acrylic acid	79	-	611	413

na-not available

APPENDIX C

MARKET PRICE OF BIOPRODUCTS NAD BIOMASS FEEDSTOCKS

Table 31. Market price of bioproduct and feedstocks

Feedstocks	$/ ton	Bioproducts	$/ ton
Agricultural residues (corn stover, wheat straw, rice straw, sugarcane bagasse)	40	Glucaric	1000
Algae	790	Glutaric acid	1000
Bakery/ food waste	0	Heptane	470
Rapeseed	335	Hexane	480
		HMF	2655
Bioproducts	$/ ton	HP	1100
Acetone	715	Lactic acid	1600
Acrylic acid	1540	Levulinic acid	6500
Adipic	1800	Methanol	314
Butane	593	Nonane	480
Butanediol (BDO)	1800	Octane	1000
Butanol	1250	Pentane	1000
Decane	2750	Pentanediol	2000
Ethane	200	Pentanol	1200
Ethanol	500	Propane	573
Ethylene	800	Propanol	950
Farnescene	5500	Sorbitol	650
FDCA	1100	Succinic acid	3200
Gasoline	830	Xylitol	3900

APPENDIX D

LIST OF PRETREATMENT METHODS

Table 32. List of pretreatments and its yield for various agricultural residues

Substrate	Pre-treatment	Hydrolysis	Yield of sugars*
Corn stover	Dilute acid: 140 °C, 1.0 wt% H_2SO_4, 40 min	15FPU/g for cellulose from Celluclast 1.5L and 26.25CBU/g for β-glucosidase from Novozyme 188.	82.3 in 72 h
	Liquid hot water: 190 °C, 15 min	15 FPU/g for ellulose from Spezyme CP and 65 IU/g for β-glucosidase from Novozyme 188.	69.6 in 72 h
	NaOH or Ca(OH)2: 55°C, 7.3 wt% Ca(OH)2, 4 weeks	15FPU/g for cellulose from Spezyme CP and 40CBU/g for β-glucosidase from Novozyme 188.	98 in 96 h
	AFEX(Ammonia fiber expansion): 90°C, 1:1 ammonia to biomass loading, 60%moisturecontent, 5 min	15FPU/g for cellulose from Celluclast 1.5L and 26.25CBU/g for β-glucosidase from Novozyme 188	76.6 in 72 h
	AFEX	Accellerase 1000	50.6 Glucose release(%), 30.0 Xylose release (%)
		Spezyme CP	44 Glucose release(%), 34.4 Xylose release (%)
	0.25% NaOH	Accellerase 1000	54 Glucose release(%), 23 Xylose release (%)
		Spezyme CP	40.7 Glucose release (%), 35.4 Xylose release (%)
	Alkaline peroxide	Accellerase 1000	68.6 Glucose release (%), 38.0 Xylose release (%)
		Spezyme CP	58.4 Glucose release (%), 49.6 Xylose release (%)
	0.2-0.98% H_2SO_4, 140-200°C,0-80min		Maximum xylose yield of 71-85%

Table 32 continued.

Substrate	Pre-treatment	Hydrolysis	Yield of sugars*
	AFEX**: Anhydrous ammonia , 1g/g of biomass, 90°C, 5min	Enzymatic hydrolysis	94.4 % (glucose+xylose) release
	SO_2:	Enzymatic hydrolysis	93.9 % (glucose+xylose) release
	0.49% H_2SO_4,160°C,20min	Enzymatic hydrolysis	92.4 % (glucose+xylose) release
	ARP :15% aqueous ammonia	Enzymatic hydrolysis	89.4 % (glucose+xylose) release
	170°C,27.5min Liquid Hot Water (LHW), 190°C,15min	Enzymatic hydrolysis	87.2 % (glucose+xylose) release
	Lime : 0.08 g/g biomass, 55°C, 4 weeks	Enzymatic hydrolysis	86.8%(glucose+xylose) release
Corn straw	Diluted acid		84.4(cellulose),14 (hemicellulose), 90 (lignin)
	Diluted alkaline		70.0(cellulose),62.7 (hemicellulose), 24.6 (lignin)
	dilute-alkali	enzymatic hydrolysis	Total sugar(215.7 g L-1)
	Aqueous-ammonia soaking		85.4(cellulose),85.2 (hemicellulose), 71 (lignin)
	Alkaline peroxide		81.9(cellulose),87 hemicellulose), 61 (lignin)
	2% NaOH, 80 C, 1 h	Enzymatic hydrolysis by ellulose of Trichoderma reesei ZU-02 and cellobiose of Aspergillus niger ZU-07	Xylose 23.6 g/L, glucose 56.7 g/L, arabinose 5.7 g/L
Corn-cob	$NH_3 \cdot H_2O$		86.47(cellulose),42(hemicellulose),10 (lignin) in %
	NaOH		86.46(cellulose),47(hemicellulose),7(lignin) in %
	dilute-alkali	enzymatic hydrolysis	Total sugar(223.8 g L-1)
	H_2SO_4		91.39(cellulose),17(hemicellulose),76 (lignin) in %
	H_2SO_4-$NH_3 \cdot H_2O$		85.40(cellulose),79(hemicellulose),83(lignin) in %
Cotton stalks	2% H_2SO_4, 60 min, 121 C/15 psi	Enzymatic hydrolysis	23.85Glucanconversion(%),0.0Xylan conversion (%)
	2% NaOH, 60 min, 121 C/15 psi	Enzymatic hydrolysis	60.79 Glucan conversion (%), 62.57 Xylan conversion (%)

Table 32 continued.

Substrate	Pre-treatment	Hydrolysis	Yield of sugars*
	2% H_2O_2, 60 min, 121 C/15 psi	Enzymatic hydrolysis	49.82 Glucan conversion (%), 7.78 Xylan conversion(%),61(cellulose),5(hemicellulose), 26 (lignin)
	Autohydrolysis, 90 min Ozone treatment for 30 min		17(cellulose), 17(hemicellulose), 12 (lignin) % reduction from untreated cotton stalks
Cotton waste	5% Dilute acid hydrolysis 5% Dilute alkaline hydrolysis	Enzymatic hydrolysis Enzymatic hydrolysis	90 mg/ml sugar released, 63 mg/ml sugar released
Cotton gin trash	Steam explosion, 185-238°C, 20-265sec	Enzymatic hydrolysis	77-104.5% fiber recovery (low temperature, low residence time-higher fiber recovey)
Sugarcane bagasse	Ball milling (4 h)	Enzymatic (Acremonium ellulose at 5 FPU/g substrate of ellulose and 20 U/g substrate of xylanase from Optimash BG at 45 C, pH 5.0 for 72 h	89.2 0.7% (glucose), 77.2 0.9% (xylose)
	1% sulfuric acid (v/v) at 60 C, 24 h (SLR 1:6)	In an autoclave at 121 C for 40 min after removing the excess acid (1% (v/v) sulfuric acid)	Total sugar concentration of approximately 68.0 g/L
Wheat Straw	Knife milling with 0.7e1.0 mm rejection screen, washed with water and dried	At 90 C with 1.85% (w:v) sulfuric acid for 18 h; liquid to solid ratio of 20:1. Suspension centrifuged and the residue is washed with hot water	D-xylose: 12.80 0.25 g/L, D-glucose: 1.70 0.30 g/L
	dilute-alkali pretreatment	enzymatic hydrolysis	Total sugar of 235.5 g L-1
	Steam explosion : 190°C, 10 min	15 FPU/g for ellulose from Celluclast 1.5 L 12.6 IU/g for β-glucosidase from Novozyme 188	85 in 72 h
Rice straw	Chopped to 5e6 mm size range	4.4% sulfuric acid at 1:10 solid to liquid ratio in boiling water bath, 1 h, filtered and pH adjusted to 5.5	Total sugar (20 g/L)
	Soaked in water at 170 C and 7.6 kg/cm2, 30 min, finally cooled and pH adjusted to 5.5		Total sugar (23 g/L)
	Chopped, steam exploded (3.5 Mpa, 275 C, 2 min)	Enzymatic saccharification (cytolase, novozyme) (50 C,	Xylose yield (10e5 g/L)

Table 32 continued.

Substrate	Pre-treatment	Hydrolysis	Yield of sugars*
		120 h)	
	dilute-alkali	enzymatic hydrolysis	Total sugar(176.5 g L-1)
	2% NaOH, 80 C, 1 h	Enzymatic hydrolysis by cellulose of Trichoderma reesei ZU-02 and cellobiose of Aspergillus niger ZU-07	Xylose 23.6 g/L, glucose 56.7 g/L, arabinose 5.7 g/L
Olive tree	Conc acid H_2SO_4 160°C, 30-60 min		56% sugar yield
* as percentage based on dry weight of raw material, ** About 97% of ammonia can recycled back to pretreatment.			

Table 33. Lignocellulosic biomass pretreatment technologies [9]

Technology	TRL	Opportunities	Barriers	Mitigations
Steam explosion	6 - 8	Cost-effective, High glucose yields Lignin and hemicelluloses removal Low environmental impact	Often catalyst needed to optimise pre-treatment, Formation of inhibitors and toxic, compounds	Development of new catalysts Developing Microorganisms more tolerant to inhibitors
Dilute acid pretreatment	5 - 7	Good removal of hemicelluloses	Degradation by-products (salts) and inhibitors Corrosion	Developing Microorganisms more tolerant to inhibitors, Reducing intensity of pretreatment , New enzyme developments
Concentrated acid hydrolysis	4 - 5	No enzymes needed Good removal of hemicelluloses	High chemical use and capex Corrosion and toxic hazard Degradation by-products (salts) and inhibitors	Recovery and reuse of chemicals Developing new catalysts More tolerant microorganisms
Auto-catalysis/ hydrothermal	4 - 6	No chemical use or residues High glucose yields	Higher operating temperature Inhibitor formation	Develop methods to add value to lignin
Organosolv treatment	4 - 6	Causes lignin and hemicellulose hydrolysis	High capital and operating costs Solvent may inhibit cell growth	Develop methods to add value to lignin Recovery and reuse of chemicals
Alkaline pre-treatment (e.g. dilute ammonia, NaOH, lime)	5 - 7	Low capital costs Low inhibitor formation High glucose yields	Residue formation Need to recycle chemicals Enzyme adjustment needed	New enzyme development Recovery and reuse of chemicals
Ammonia Fibre Explosion (AFEX)	3 - 5	No need for small particles Low inhibitor formation High accessible surface area	High cost due to solvent	Recovery and reuse of chemicals
Supercritical (CO_2) pre-treatment	2 - 4	Increases accessible surface area Low inhibitors or residues	Does not affect lignin and hemicelluloses V. high pressure, high capex	Develop methods to add value to lignin Improve process technology
Ionic liquids	2 - 3	Effective dissolution of all lignocellulose components Low degradation products	Expensive technology and recovery required	Develop methods to add value to lignin Recovery and reuse of chemicals Develop process technology
Microbial/fungi	3 - 4	Low energy requirement, No corrosion, Suitable for lignin and hemicelluloses removal	Time consuming Some saccharide losses	Development of robust microorganisms
Mechanical milling	5 - 6	Reduces cellulose crystallinity No inhibitors or residues	High energy consumption Poor sugar yields	Process integration, combine with mild chemical treatments

APPENDIX E

LIST OF BIOBASED SUCCINIC ACID PRODUCTION METHODS

Table 34. List of biobased succinic acid production methods

Carbon source	Microbial strain		Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA: FA:AA, (mol/mol)[a]
Succinic acid production from pure carbon sources by A. succinogenes								
Glucose	A.succinogenes	130Z	YE (6)/CSL (10)	CO_2 sparging, continuous, 0.158 L	48.5	nk	0.84	1:0:0:0.38
Glucose	*A.succinogenes*	CGMCC 1593	YE (10)/CSL (6)/Vit	CO_2, sparging, fed-batch, bioreactor, 3L	60.2	1.3	0.75	1:0:0.13:0.31
Glycerol	*A.succinogenes*	130Z	YE (5–10)/Vit	CO_2 sparging, batch, bottle reactors, 0.07 L	26.7	0.23	0.96	1:0:0.15:0.14
Glycerol	*A.succinogenes*	130 Z	YE (10)	CO_2, sparging, fed-batch, bioreactor, 1.5L	49.6	0.62	0.92	1:0:0.39:0.16
Sucrose	*A.succinogenes*	NJ113	YE (10)/CSL (5)	CO_2, sparging, fed-batch, bioreactor, 1.5L	60.4	2.16	0.72	1:0:0.55:0.29
Cellobiose	*A.succinogenes*	NJ113	YE (10)/CSL (5)	CO_2, sparging, fed-batch, bioreactor, 0.03L	38.9	1.08	0.66	1:0:0:0.69
Succinic acid production from crude renewable resources by A. succinogenes								
Corn fiber	*A.succinogenes*	FZ6 (mutant)	YE (10), Biotin (10 g)	CO_2 sparging,batch,vials,0.01L	70.6	0.7	0.88	1:0:0.01:0.08: f
	A.succinogenes	NJ113	YE (10)/CSL (5)	CO_2 sparging, batch, bioreactor, 4.5 L	35.4	0.98	0.72	nk
Corncob	*A.succinogenes*	CICC 11014	YE (11)	CO_2 sparging, batch anaerobic bottles,0.025L	23.6	0.49	0.58	nk
Corncore	*A.succinogenes*	CGMCC 1593	YE(5)GLU(10)CSL(5)	CO_2 sparging, batch, bioreactor, 5 L	32		89.1	nk

Table 34 continued.

Carbon source	Microbial strain		Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA:FA:AA, (mol/mol)[a]
Corn stover	*A.succinogenes*	CGMCC 1593	CSL (20)	CO_2 sparging, batch, SSF[b],bioreactor, 2 L	47.4	0.99	0.72[c]	1:0.06:0.06:0.44
Corn straw	*A.succinogenes*	CGMCC 1593	YE(5)GLU(10)CSL(5)	CO_2 sparging, batch, bioreactor, 5 L	33.7		81	nk
Corn straw	*A.succinogenes*	CGMCC 1593	YE (15)	CO_2, sparging, fed-batch, bioreactor, nk	53.2	1.21	0.82	1:0:0:0.22
Corn stalk	*A.succinogenes*	CGMCC 2650 or BE-1	YE (30)/Urea (2)	CO_2 sparging, batch, nk	17.8	0.56	0.66	nk
Wheat milling by-products	*A.succinogenes*	130Z	YE (2.5)	CO_2 sparging, batch, bioreactor, 0.5 L	62.1	0.91	1.02	nk
Wheat straw	*A.succinogenes*	CGMCC1593	YE(5)GLU(10)CSL(5)	CO_2 sparging, batch, bioreactor, 5 L	18.96		74.1	
Rice straw	*A.succinogenes*	CGMCC1593	YE(5)GLU(10)CSL(5)	CO_2 sparging, batch, bioreactor, 5 L	17.64		62.8	
Waste bread	*A.succinogenes*	130Z	Bread hydrolysate ,(200 mg/L free amino nitrogen)	CO_2 sparging, batch, bioreactor, nk	47.3	1.12	nk	nk
Cotton stalk	*A.succinogenes*	130Z	YE (30)/Urea (2)	CO_2 sparging, batch, SSF[b],flask, nk	63	1.17	0.64	nk
Cane molasses	*A.succinogenes*	CGMCC 1593	YE (10)	CO_2, sparging, fed-batch, bioreactor, nk	55.2	1.15	nk	1:0:0.16:0.32
Cane molasses	*A.succinogenes*	GXAS137	YE (8.8)	CO_2, sparging, fed-batch, bioreactor, 0.8L	64.3	1.07	0.76	1:0:0:0.39
Sugarcane bagasse cellulose	*A.succinogenes*	NJ113	YE (10)/CSL (5)	CO_2 sparging, batch, bioreactor, 1.5L	20	0.61	0.65	1:0:0:1.28
Sugar cane bagasse	*A.succinogenes*	NJ113	YE (10)/CSL (5)	CO_2 sparging, batch, bioreactor, 1.5L	23.7	0.99	0.79	1:0:0:0.37

Table 34 continued.

Carbon source	Microbial strain		Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA:FA:AA, (mol/mol)[a]
Sugarcane bagasse	*A.succinogenes*	CIP 106512	YE (2)	CO_2 sparging, batch, bioreactor, 1.5L	22.5	1.01	0.43	nk
Corn stover hydrolysate	*A.succinogenes*				56	1.08	0.73	
Macroalgal hydrolysate	*A.succinogenes*	130Z	YE (16.7)	CO_2 sparging, batch, bioreactor, 1.5L	33	1.27	0.75	1:0.18:0.28:0.54:g
Rapeseed meal	*A.succinogenes*	130 Z	YE (15)	CO_2 sparging, batch, SSF[b],bioreactor, 1.2L	23.4	0.33	0.115[d]	1:0:0:0.71
Whey	*A.succinogenes*	130 Z	YE (5)/Pep (10)	CO_2 sparging, batch, SSF[b],bioreactor, 1.2L	21.3	0.43[c]	0.44	1:0.02:0.68:0.78:h
Sake lees hydrolysate	*A.succinogenes*	130 Z	SLH/YE/biotin	CO_2 sparging, batch, SSF[b],bioreactor, 1.5L	52.3	1.74	0.85	1:0:0:0.30
Sucrose + sugarcane molasses	*A.succinogenes*				22	1.01	0.43	
Sugarcane molasses	*A.succinogenes*				51	0.84	0.79	
Glucose	*A.succinogenes*	130 Z			69-80	1.2-1.7	0.68-0.87	
Glucose	*A.succinogenes*	130 Z			4.1	0.3	0.5	
Glucose	*A.succinogenes*	130 Z			94-106	2-2.8	0.78-0.82	
Whey	*A.succinogenes*	130 Z			70.6	0.7	0.88	
Sake lees hydrolysate	*A.succinogenes*	130 Z		batch	48	0.94	0.75	
Glucose	*A.succinogenes*	FZ53		batch	105.8	1.36	0.83	
Cotton stalk	*A.succinogenes*	CGMCC 2650 or BE-1	YE (30)/Urea (2)	CO_2 sparging, batch, nk	15.8	0.62	1.23	

Table 34 continued.

Carbon source	Microbial strain		Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA: FA:AA, (mol/mol)[a]
Succinic acid production from pure carbon sources by various strains								
Glucose	A.succinogenes	ATCC 53488	YE (5)/Pep (10)/$(NH_4)_2SO_4$ (5)	CO_2 sparging, batch, bioreactor, nk	32.2	1.19	0.9	1:0:0:0.52
Galactose	A.succinogenes	ATCC 29305	YE (2.5)/Pep (2.5)/$(NH_4)_2SO_4$ (5)	CO_2 sparging, batch, bioreactor, 1L	15.3	1.46	0.9	1:0:0:0.60
Glucose	E coli	AFP184	CSL (33)/$(NH_4)_2SO_4$ (3)	dual phase, batch, bioreactor, 8L	45.4	2.84	0.92	1:0:0:0.24
Xylose					29.2	1.79	0.69	1:0:0:0.45
Fructose					27.7	1.54	0.46	1:0:0:0.34
Glucose	E.coli	AFP111	(NH4)2HPO4(8)/NH4Cl (0.2)/(NH4)2SO4 (0.8)/Vit	dual phase, fed-batch, bioreactor, 3L	101	1.18	0.78	1:0:0:0.07
Sugarcane bagasse hydrolysate	*E.coli*			Two-stage[k]	19	0.79	0.96	
Soybean meal hydrolysate	*E.coli*				37	0.77		
Beechwood xylan	*E.coli*			Anaerobic	14	0.12	0.37	
Sucrose + sugarcane molasses	*E.coli*			Microaerobic	56	0.78	0.96	
Pure glycerol	*E.coli*			Dual phase aeration, batch	14	0.19	0.69	
Softwood dilute acid hydrolysate	*E.coli*	AFP184		Dual phase aeration, batch	42.2	0.78	0.72	
Sucrose	*E.coli*	W3110		Dual phase aeration, batch	24	0.81	1.2	
Sugar cane Molasses	*E.coli*	W3110		Dual phase aeration, batch	26	0.87	0.52	
Bio-oil	*E.coli*	MG-PYC			11.5			

Table 34 continued.

Carbon source	Microbial strain		Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA:FA:AA, (mol/mol)[a]
Representative succinic acid production from crude renewable resources by various strains								
Corn stalk	E.coli	SD121	YE (10)/Tryp (20)/$(NH_4)_2SO_4 \cdot 7H2O$ (3)	dual phase, batch, bioreactor, 1L	57.8	0.87	0.96	1:0:0:0.29:i
Whey	*A.succiniciproducens*	ATCC 29305	CSL (20)/Tryptophane (0.02)	CO_2 sparging, fed-batch, bioreactor, nk	34.7	1.02	0.91	nk
	A.succiniciproducens	ATCC 29305	CSL (20)/Tryptophane (0.02)	CO_2 sparging, fed-batch, bioreactor, nk	19.8	3	0.64	nk
Whey	*M. succiniciproducens*	MBEL55E	CSL (7.5)	CO_2 sparging, fed-batch, bioreactor, 1L	13.4	1.18	0.71	1:0.06:1.10:0.73
	M. succiniciproducens	MBEL55E	YE (2.5)	CO_2 sparging, fed-batch, bioreactor, 1L	13.5	1.21	0.72	1:0.05:1.11:0.74
	M. succiniciproducens	MBEL55E	CSL (5)	CO_2 sparging, continuous, bioreactor, 0.5L	10[e]	3.9[e]	0.69[e]	1:0:0.80:0.79
Glycerol	*M. succiniciproducens*	ATCC29305		Batch	19	0.15	1.6	
Glycerol	*E.coli MLB*			Two-stage fermentation	360.2mM		0.93	
Wood hydrolysate	*M. succiniciproducens*	ATCC29305		Batch	24	0.74	0.88	
Galactose	*M. succiniciproducens*	ATCC29305		Batch	15.3	1.46	0.87	
Wood hydrolysate	*M. succiniciproducens*	ATCC29305		Continuous	8.2	3.19	0.55	
Cane molasses	*E. coli*	AFP111/pTrc C-cscA	$(NH_4)_2HPO_4$ (8)/NH_4Cl (0.2)/(NH4)2SO4 (0.7)/Vit	Dual phase, fed-batch, bioreactor, 1.5L	37.3	1.04	0.79	1:0:0:0.17:j
Cane molasses	*E. coli*	KJ122-pKJSUC-24T	$(NH_4)_2HPO_4$ (19.9)/$NH_4H_2PO_4$ (7.5)/Vit	CO_2 sparging, batch, bioreactor, 7.5 L	55.8	0.77	0.96	1:0:0:0.18

Table 34 continued.

Carbon source	Microbial strain		Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA: FA:AA, (mol/mol)[a]
Softwood hydrolysate	*E. coli*	AFP184	YE (15)/CSL (15)/$(NH_4)_2SO_4$ (3.3)	Dual phase, batch, bioreactor, 0.7 L	42.2	1	0.72	nk
Pre-treated wood hydrolysate	*M. succiniciproducens*	MBEL55E	YE (5)	CO_2 sparging, batch, bioreactor, 1 L	11.73	1.17	0.56	1:0.23:0.45:0.59
Pre-treated wood hydrolysate	*M. succiniciproducens*	MBEL55E	YE (5)	CO2 sparging, continuous, bioreactor, 0.5 L	7.98	3.19	0.55	nk
Glucose	*E. coli*	NZN111			28	0.7	0.74	
	E. coli	AFP111/ pTrc99A-pyc			99	1.3	1.17	
Glucose	*C. glutamicum*	ΔldhApCRA717			146	3.2	0.92	
Glucose	C. glutamicum	ΔldhApCRA717			83	11.8	0.9	
D-glucose or sucrose	Basfia succiniciproducens				5.8	1.5	0.6	
crude glycerol	B.succiniciproducens				8.4	0.9	1.2	
Arundo donax (energy crop)	*B.succiniciproducens*				17	0.2	0.75	
Corn stover	*B.succiniciproducens*				30	0.43	0.69	
Glucose	Genetically modified *B.succiniciproducens*						1.08	

Nitrogen Source: YE: Yeast extract, CSL: Corn steep liquor, Tryp: Tryptone, Pep: Peptone, Vit: Vitamin supplementation.

nk: not known.

[a] mol/mol ratio of fermentation by-products SA: Succinic acid, LA: Lactic acid, FA: Formic acid, AA: Acetic acid.

[b] Simultaneous saccharification and fermentation.

Table 34 continued.

Carbon source	Microbial strain	Nitrogen–nutrient source (g/L)	Type of fermentation, working volume	SA concentration, (g/L)	SA productivity, (g/L/h)	Yield, $g_{sa}/g_{totalsugars}$	SA:LA: FA:AA, (mol/mol)[a]

[c] Yield: $g_{succinic\ acid}/g_{substrate}$

[d] Yield: $g_{succinic\ acid}/g_{dry\ matter}$

[e] Maximum value observed during continuous fermentation at different dilution rates.

[f] propionic acid (3g/L).

[g] ethanol: (2.5g/L).

[h] ethanol (3g/L).

[i] ethanol (1.62g/L).

[j] pyruvic acid (1.2g/L),

[k] Aerobic growth phase followed by an anaerobic production process.