RECOVERY OF SULFURIC ACID FROM LIGNOCELLULOSIC BIOMASS HYDROLYSATE BY ELECTRODIALYSIS PROCESS

M.Tech. THESIS

by

Mr. Nikhil Kumar
(Enrolment No. 17559004)

DEPARTMENT OF BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

MAY, 2019

RECOVERY OF SULFURIC ACID FROM LIGNOCELLULOSIC BIOMASS HYDROLYSATE BY ELECTRODIALYSIS PROCESS

A THESIS

Submitted in partial fulfillment of the requirements for the award of the degree

of

MASTER OF TECHNOLOGY

in

BIOPROCESS ENGINEERING

by

Mr. Nikhil Kumar
(Enrolment No. 17559004)

DEPARTMENT OF BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

MAY, 2019

DECLARATION

I, Mr. Nikhil Kumar, certify that

- The work contained in this thesis is original and has been done by me under the guidance of Prof. Sanjoy Ghosh.
- The work has not been submitted to any other institute for any other degree or diploma.
- Whenever I have used materials (data, theoretical analysis, figures, and text) from other sources, I have given credit to them by citing them in the text of the report and giving their details in the references.

Date: 13 May, 2019 Nikhil Kumar

Place: IIT Roorkee (Enrolment No: 17559004)

CERTIFICATE

This is to certify that the thesis work entitled **"Recovery of sulphuric acid from lignocellulosic biomass hydrolysate by electrodialysis process"** submitted by Mr. Nikhil Kumar, student of the Bioprocess Engineering M.Tech. program (Session 2017-2019), in the partial fulfillment for the award of the degree of Master of Technology in Bioprocess Engineering from the Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee-247667, Uttarakhand (India), is a record of the candidate's own work carried out by him under my supervision and guidance of during the period from July 2017 to May 2019 in this institute.

It is also certified that no part of this matter has been submitted elsewhere in any other institute/university for the award of any other degree/diploma.

Date: 13 May, 2019
Place: IIT Roorkee

Yours Sincerely

Sanjoy Ghosh, Ph.D.
Professor
Biochemical Engineering Laboratory
Department of Biotechnology
Indian Institute of Technology Roorkee
E-mail ID: ghoshfbs@iitr.ac.in
Tel No: +91-1332-285424

ACKNOWLEDGEMENT

The success and final outcome of this thesis required guidance and assistance from many people and I am extremely fortunate to have received all this during the duration of my thesis work. I take this opportunity to convey my gratitude to all of them.

I thank God, the all-powerful, my parent, guardian, brother, sisters and friends, who bestowed strength, courage and perseverance to undertake the present course of study and complete it successfully.

I thank Prof. Sanjoy Ghosh for giving me the opportunity to perform this thesis work in his lab and express my profound gratitude and deep regards to him for his exemplary guidance, monitoring and constant encouragement throughout the course of this thesis. The blessing, help and guidance given by him from time to time shall carry me a long way in the journey of life on which I am about to embark.

I offer my sincere thanks to Prof. AK Sharma, Head of the Department and Prof. P. Roy, former Head of the Department, for providing all the infrastructural facilities of the department for successful completion of this thesis. I also extend my sincere thanks to Dr. S. Datta and Dr. B. Choudhury, for conducting the activities related to the Master Thesis Project in such an organized and student friendly manner. I am also thankful to the faculty and staff of the department for their consistent help and guidance during the thesis work.

I would like to give my special thanks to Mr. Pritam Singh, Research Scholar for his help and support at every single step of this thesis work and the extra pains taken by him to familiarize me with the practices of the laboratory and for the valuable information provided by him during the period of this assignment.

At last but not least, I wish to thank all the members of the biochemical engineering family for their assistance and making my stay during the thesis work meaningful, comfortable and enjoyable. In all carrying out the thesis work, I learnt the nuances of biochemical engineering from the practical and application point of view.

Date: 13 May, 2019

Place: IIT Roorkee

Nikhil Kumar (Enrollment No: 17559004)

M.Tech. Bioprocess Engineering Student

Department of Biotechnology, IIT Roorkee

E-mail ID: nk738684@gmail.com

Abbreviations

LCB	Lignocellulosic biomass
ED	Electrodialysis
I	Current (Amp)
V	Voltage
R	Resistance (ohm)
TMP	Trans membrane potential
ΔC	Concentration gradient
ΔE	Electric potential gradient
ΔT	Temperature gradient
EMF	Electromagnetic force
CD	Current density
M	Molarity

TABLE OF CONTENTS

List of Figures

List of Tables

Abstract

Lignocellulosic biomass is the source of pentose (xylose) and hexose (glucose) sugars and found abundantly on the earth but the extraction of these sugars is the main concern of today's researcher. There are mainly two popular methods for extracting these sugars: enzymatic hydrolysis and acid hydrolysis. The enzymatic hydrolysis is the traditional method which uses pretreatment followed by enzymatic treatment but the generation of toxic compounds during pretreatment, half-life of enzyme, enzyme titer value and its production time has limited this process to be scaled-up. On the other hand the acid hydrolysis is a chemical process which can extract these sugars within much lesser time in comparison to enzymatic hydrolysis with negligible or no amount of toxic compounds generated. The only disadvantage of this technology is the need to neutralization of acid before going for fermentation. So if a technology can be developed to recycle this acid then it will be most economical, ecofriendly, easily scalable and zero-waste technology. Here an acid recovery technology called electrodialysis has been attempted for acid removal from acid hydrolysate of lignocellulosic biomass. This technology is an electrically driven process in which ions move from one compartment to another through the ion exchange membrane in the presence of electric field. This technology is able to recover more than 95% of sulfuric acid from the lignocellulosic biomass hydrolysate. Although, this electrodialysis process is a power consuming process, fast acid recovery and proper utilization of the generated gases (hydrogen and oxygen) can overcome this power cost.

Chapter 1

Introduction

Lignocellulosic biomass is the most plentiful biomass on earth which is used as food, fuel, chemicals and biomaterials. Broadly, this type of biomass contains maximum agricultural residues such as sugarcane bagasse, rice straw, wheat straw, corn stover, forestry waste, fruit and vegetable waste etc. It is mainly composed of cellulose, hemicellulose and lignin. The composition of these three components of LCB mainly depends on type of biomass, cultivation time and climate conditions. Hemicellulose is the second most abundant polysaccharide which sacchrifies to give xylose as a major backbone which bind to glucose, galactose, arabinose and sugar acids [1]. Hemicellulose generally constitutes of 20%-30% of lignocellulose plant biomass, 15%-30% of softwoods and 7%-10% of hardwoods. Valorization of the hemicellulose into its basic components is the basis principle of biorefinery, using its all component without generating waste. In literature it is found that many valuable products such as bioethanol, xylitol produced from valorization of hemicellulose [1]. However, hemicellulose is converted into fermentable sugars which can be converted into these products by suitable organism after fermentation. Cellulose can also be converted into simple sugars mainly by thermochemical method or enzymatic method. Kans grass is in the frontier area of research in laboratories, pilot scale, demonstration and industrial scale. Variety of byproducts can be formed under this biorefinery concept which can be accessible to daily uses. For conversion of cellulose and hemicellulose, pretreatment is unavoidable which allows this polymer to depolymerize into monomers of sugar [2].Dilute acid hydrolysis is the most common methods for conversion of hemicellulose and cellulose into simple sugar. Pretreatment of lignocellulose which contains cellulose and hemicellulose into liquid and solid residue is depicted in figure 1.1.

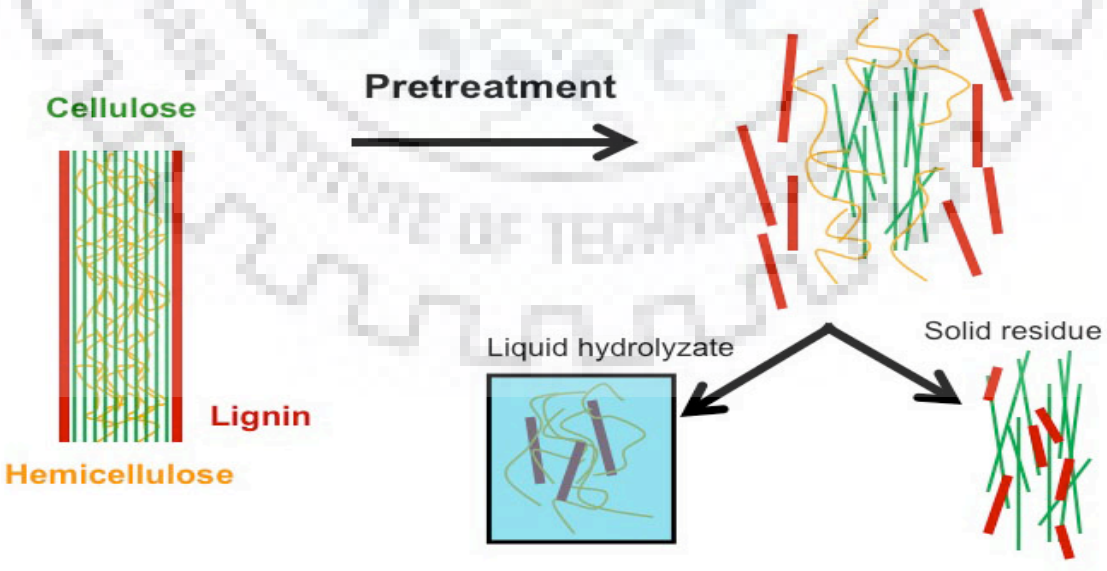


Figure 1.1: Pretreatment of lignocellulosic biomass

Sustainability is the key factor for any technology development program. Biorefinery is one of them; it has high elasticity for degradation of each component of lignocellulosic biomass for economic, social and environmental development. Conventional methods of producing product are now replaced with biorefinery concept because of its high value added biochemicals such as biofuel [3].

Due to extensive growth of population there is need of more edible foods which generates enormous amount of agricultural waste and forest residue is also treated as same. If these compounds are not properly discarded or managed for reutilization, they can cause serious environment and pollution concern and also adversely affect the economic condition of the country [4]. However, these waste materials have huge potential to use as feedstock for pretreatment and generate value added products such as biochemicals, biofuels, enzymes and food [5]. Actually, annual production of lignocellulosic biomass which is used as energy source in industry and day to day life of people is 50×109 tons [6]. Lignocellulosic biomass is studied for their conversion into high economic value products [7]. Cell wall of the biomass is made of hardwood that's why it is very hard to recalcitrance of lignocellulosic biomass for the depolymerization of cellulose and hemicellulose into fermentable simple sugars such as glucose, xylose, arabinose and others. These monomers are used as carbon source for microbial growth and product formation of commercial importance during fermentation [8].

Pretreatment of biomass is an important process to remove the fermentable sugars from lignocellulosic materials. Often, pretreatment is performed to hemicellulose removal or lignin removal. There are many methods for biomass pretreatment such as physical, chemical and biological or combination of them to maximize the accessible fermentable sugar for enzymatic treatment.

Milling is the physical treatment process (mechanical process) in which the structure of lignocellulosic biomass loses its crystallinity and increase the accessibility of enzyme for reaction [9]. This process is performed without using chemicals which generate toxic substances and considered as environment friendly [9]. However, this process consumes high power required by the machines and consequent high energy costs [9]. During a ball-milling process, biomass is break down into small uniform particle size with the help of ball inside a cycle machine [10]. Literature reported that the milling time increases the amount of sugar (glucose, 89.2±0.7 % and xylose, 77.2±0.9 %) after 4h of milling [11].

In chemical pretreatment method, acid pretreatment is one of the mostly used chemical methods for lignocellulosic biomass. This method usually helps in depolymerize the hemicellulose fraction of the cell wall of lignocellulosic biomass which eventually increases the accessible area for cellulolytic enzyme reaction. In this acid treatment process the hydrolysate is in contact with dilute acid under high temperature and concentrated acid under low temperature. The hemicellulose and cellulose fraction of biomass is depolymerized mainly into pentose (C5) (xylose, arabinose) and hexose (C6) (Glucose, galactose and mannose) sugars respectively along with inhibitory compounds.Multiple methods are applied to detoxify the hydrolysate before

fermentation and further this hydrolysate is converted into value added products such as xylitol, ethanol through fermentation [18, 19].

In biological treatment selective delignifying microorganism is used for degrading the lignin. Generally, white fungi are used for delignification of lignin and hemicellulose then this biomass is used for enzymatic hydrolysis which in turn produces high amount of fermentable sugar [20, 21]. In delignification, there should be aseptic environment which is low energy and capital intensive process for the growth of fungus and enzyme reaction. Pretreatment of sugarcane baggase showed increase in pulp yield, kappa number and viscosity pretreated [21].

Many treatment methods are used but acid treatment is conventional method which results in highest percentage of removal of pentose sugar present in biomass [22]. However, during treatment some undesirable components are generated such as sulphuric acid, acetic acid, furans, furfurals and extractive from raw material which are growth inhibitors [24], required one more processing step that is detoxification. After the pretreatment and detoxification hydrolysate is suitable for microorganism to convert each type of sugar into value added products. To minimize the effect of these inhibitory compounds different methods are evolved, but the efficiency of each method is depend on composition of hydrolysate and microorganism used [25]. In detoxifying process inhibitors are removed but sugar loss is huge. In the case of overliming large amount of gypsum is produced during the detoxification through $Ca(OH)_2$ [26]. However it might be an economical way but loss of sugar and environment problems are there. There is an alternate way to detoxify using electrochemical separation in which no sugar loss exists. Phenolics are produced in the form of radicals in the presence of electrochemical gradient which provides sufficient oxidation potential to form radicals [27]. Another way is membrane separation in which ion exchange membrane or others pressure based membrane are used those can separate the acid from hydrolysate at lower cost.

Fermentation is core of any biochemical industry. Second generation sugars from the agricultural wastes and forestry residues are used as carbon source for production of biofuel and biochemicals. When xylose is used as carbon source, it shows comparative fewer yields than glucose. Fermentation of xylose is carried out for the production of bioethanol, xylitol under various conditions.

In order to improve the productivity of products different strategies are proposed such as different configuration of reactor (continuous stirred tank reactor, fluidized bed reactor, membrane reactor, and airlift reactor), operating conditions (batch, fed batch and continuous process), mobility (free and immobilized cells) or genetically modified cells. For example carvalho et al (2003) reported that in stirred tank reactor using entrapped Ca-alginate [23], xylitol is produced from hemicellulose hydrolysate. In that study, the fermentation parameters for batch were initial cell concentration 1.4g/l, at pH6.0 and after 120 hr of fermentation xylitol production was reached47.5g/l with $Y_{p/s}$ of 0.81g/g and productivity of 0.40 g/l/h.

Saccharomyces yeasts are mostly used microorganisms in the ethanol industry. However, *Saccharomyces* uses the same metabolic pathway for the consumption of glucose and xylose and thus creates substrate competition for him. By targeting its metabolic pathway many studies are

performed to improve the C5 sugar consumption rate using this organism. There are two ways to improve the strain: first is to survey in new potential fields to get new organisms such as *C. shehatae, P.stipitis, Enteroramus dimorphus* and second is to modify that gene which helps in increasing the C5 sugar consumption.

Although, *Pischia stipitis* is the most popular organism for the xylose fermentation to ethanol and other value added products, many companies are opted different organisms for fermentation. American agricultural commodity giant Cargil screened over around thousands of fungus strain. These modified yeast strain are employed for high yield biochemical product lactic acid and is considered an attractive platform for other metabolic products.

Hemicellulose and cellulose are the basic components of lignocellulosic biomass and serve as building block for biochemical sustainable products. However, production of value added products from lignocellulosic biomass is technological solution for sustainability because they are produced from renewable sources of biomass. But, the scale-up of this process has become a big deal for process engineers to mimic. Industries are not able to reach up to the target to produce renewable chemicals from the LCB but efforts are underway to achieve it.

Chapter-2

Research Background

In past few decades, biotechnology has gained tremendous height in research. However, it has been found that basic science got more attention than applied science such as bioprocessing in terms of funding. It is now important for the development of biotechnology that symmetric growth in basic and applied areas is crucial. Bioprocessing is the broad area of research in biotechnology which deals with the production of biochemicals, food and nutraceuticals. These product need to be purified for their specific application. Bioseparation engineering is the systematic study of design and employing of engineering principles for the large scale purification of products. It can be classified into two types:

1. Reactive bioprocessing
2. Extractive bioprocessing

Reactive bioprocessing is a technique in which separation follows biological reaction where as extractive bioprocessing depends on bioseparation [28]. Figure 2.1 shows the difference between reactive and extractive bioprocessing.

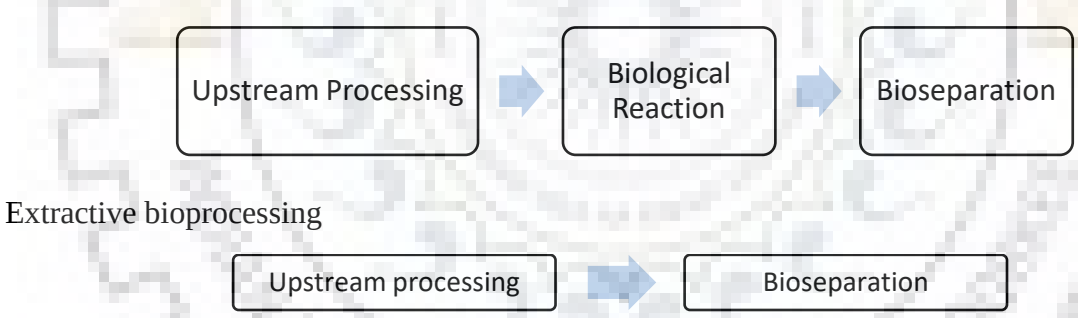


Figure 2.1: Types of bioprocessing

2.1 Economic importance of bioseparation

Purification of biomolecules from their feedstock is technically expensive and difficult task so this could become critical factor for commercialization of product. In many processes, bioseparation cost is major cost of the total cost [29]. Bioseparation cost of different biomolecules is given in figure 2.2.

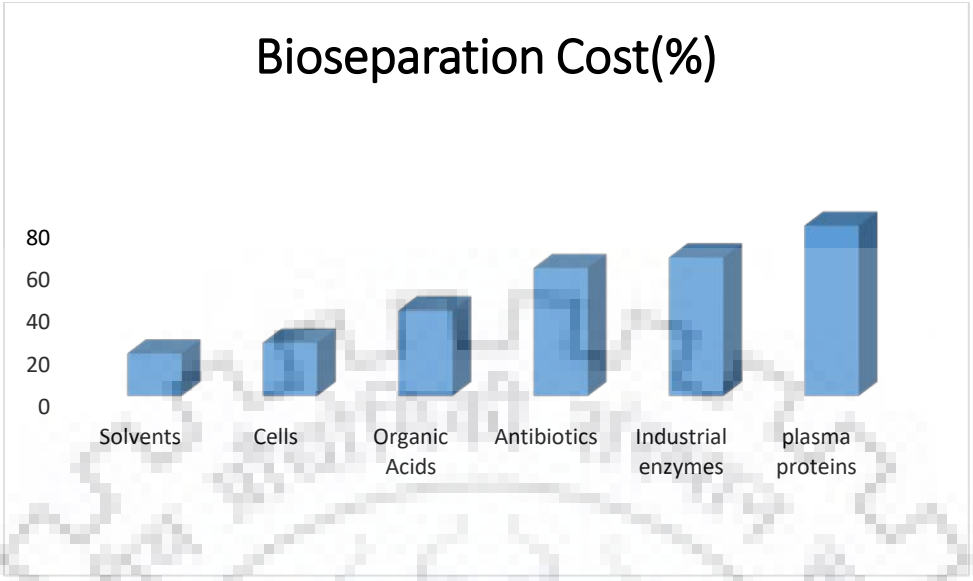


Figure 2.2: Bioseparation cost of different biological products

2.1.1 Basis of bioseparation

Biological molecules are separated from feed based on following parameters [30].

- Size. e.g. filtration, membrane separation, centrifugation
- Density: e.g. centrifugation, sedimentation, floatation
- Diffusivity: e.g. membrane separation
- Shape: e.g. centrifugation, filtration, sedimentation
- Polarity: e.g. extraction, chromatography, adsorption
- Solubility: e.g. extraction, precipitation, crystallization
- Electrostatic charge: e.g. adsorption, membrane separation, electrophoresis
- Volatility: e.g. distillation, membrane distillation, pervaporation

2.2 Membrane based separation

In recent years membrane based separation has become one of the emerging technologies. The word membrane comes from the Latin word; “membrana” which means skin. Today’s word “membrane” has been extended to describe a thin flexible sheet or film, acting as a selective boundary between two phases because of its semi permeable properties. It is used for different types of separation such as particle liquid separation, particle solute separation, solute-solvent separation and solute-solute separation. Membranes exist in solids and liquids. It functions as a separation agent that is very selective based on the difference of diffusivity coefficient, electric current or solubility. The two phases separated by the membrane, i.e., the feed and permeate; could be present in the liquid or in the gaseous state. The driving force that was necessary for the transport was a trans membrane pressure gradient (TMP) (ΔP), a concentration gradient (ΔC), an

electrical potential gradient (ΔE), or a temperature gradient (ΔT). A schematic drawing which illustrates the membrane separation process is given in figure 2.3.

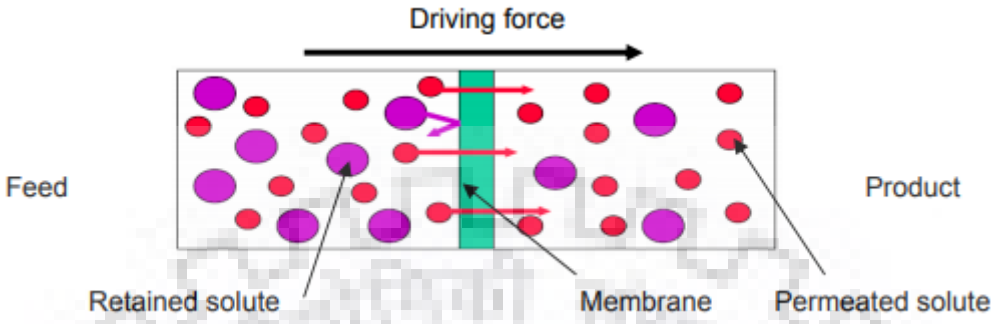


Figure 2.3: Membrane separation process with unidirectional driving force

The principal advantageous of membrane processes compared to other separation processes are low energy consumption, simplicity and environmental friendliness. Membrane-based separation is a result of different rates of transfer between each substance in the membrane and not a result of phase equilibrium. Therefore, there is no need to add additive materials such as extractors and absorbers to precede the separation. So we can then say that membrane technology is "clean technology", in which no additive materials are needed.

Advances in membrane technology, especially in novel materials, can make this technology even more competitive in comparison to traditional, energy intensive, environmentally undesirable and costly processes. There are six major membrane processes that are widely used in industrial applications. They are classified based on various driving forces, some use pressure difference (micro filtration, ultra filtration, reverse osmosis, piezodialysis), while others use other driving forces such as concentration difference (gas separation, pervaporation, liquid membrane and dialysis), thermal (membrane distillation, thermo osmosis) and electric (electro-dialysis). The key membrane performance variables are selectivity, permeability and durability.

2.2.1 Types of Membrane

1. Micro porous membranes
2. Homogenous membranes
3. Asymmetric membranes
4. Electrically charged membranes
5. Liquid membranes

2.3 Chemistry behind membranes

Among these membranes, electrically charged membranes are one of the most advanced separation membranes. These are necessarily ion-exchange membranes which consisting of highly swollen gels that carrying fixed positive or negative charges. These are mainly used in the electro dialysis. The basic applications of the ion exchange membrane process are based on the Donnan membrane equilibrium principle and have been given attention to solve two important

environmental problems, for the recovery and enrichment of valuable ions, and the removal of undesirable ions from aqueous solution. Electrically charged membranes are used together in the separation of hydrochloric acid- glucose.

There are two types of ion exchange membranes on the basis of ions present on their matrix. Membranes which contains negatively charged groups such as $–SO^{-3}$, $–COO^{-}$, $–PO_3^{-2}$,$–HPO_3^{-3}$, $–C_6H_4O^{-}$ and cation can pass but anions are rejected, are called cation exchange membranes. While, another membrane which contains positive charged groups such as $–NH_3^{+}$, $–NRH_2^{+}$, $–NR_2H^{+}$, $–NR_3^{+}$, $–PR_3^{+}$,$–SR_2^{+}$ and anion can pass through it but cations are rejected, are called anion exchange membranes [31-32]. For manufacturing of ion exchange membrane, it totally depends on the resins used for making matrix structure. Varying the type of matrix polymer and functional group present there, it is possible to produce different configurations of ion exchange membrane [33]. Figure 2.4 shows the internal structure of cation and anion exchange membranes.

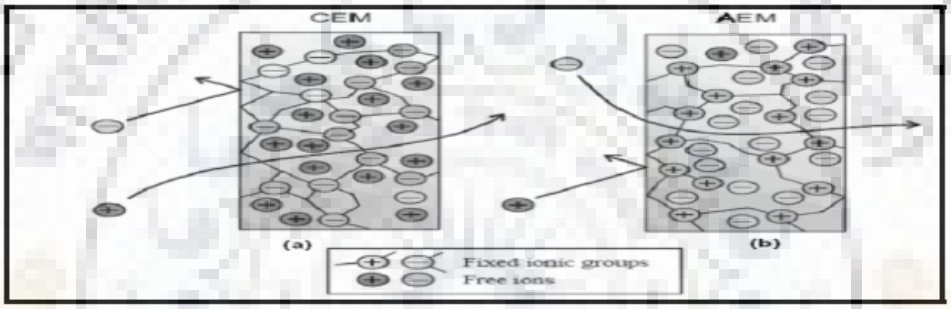


Figure 2.4: Cation and anion exchange membranes and their internal structure

When these ion-exchange membranes are incorporated with electric potential through platinum electrodes, whole system becomes electrodialysis. It is an electrically driven membrane separation process using ion exchange membranes to separate ions from the solution. It is widely used in the industry of desalination of brackish water, production of edible salt from sea water, desalination of cheese whey [24]. It is also applicable in the separation of organic acid such as oxalic acid and formic acid and malic acid or to recover galacturonic acid from sugar beet pulp's pectin hydrolysate [25] and in food processing desalting of soy sauce or fruit juice [26]. Electrodialysis is composed of anion and cation exchange membranes in an alternate manner separated by spacer placed between cathode and anode forming an individual separate compartment in which one is concentrate and other is dilute compartment. Spacer helps in separating and mixing of solution [27].

Properties of ion exchange membranes should be known before selecting it for separation purpose. These properties are listed in table 2.1.

Table 2.1: Properties of ion exchange membrane [34]

Property	Effect
High perm selectivity	highly permeable to counter-ion, but should be impermeable to co-ions
Low electrical resistance	low electrical resistance and thus there will be less potential drop during electro membrane processes
Good mechanical stability	a low degree of swelling or shrinking in transition from dilute to concentrated ionic solutions
High chemical stability	stable over a pH-range from 0 to 14 in the presence of oxidizing agents
Low degree of water transport	electro osmosis, the movement of water as a result of current flow, tends to reduce the efficiency of the system
Inertness	deteriorate in the presence of chemical and biological agents
Low cost	most important single item of capital investment for electro dialysis

2.4 Physical chemistry of electrodialysis

When electrolyte dissolved in water, it dissociate into cations and anions. The movement of ions is in opposite direction of electric field and thus circuit is formed and current is flowing. The driving force for this ion motion is electromotive force (EMF). Ions are moving from one chamber to another with specific velocity, this velocity is proportional to the potential gradient in the field.

$$\mathrm{u} = \mathrm{mz}\,\frac{dE}{dx}$$

$$Where\ u\ =\ x\ direction\ velocity\ (cm/sec)$$

$$X\ =\ distance\ in\ x\ direction$$

$$E\ =\ potential\ (volt)$$

$$m\ =\ ionic\ mobility\ (cm2/volt/sec)$$

$$Z\ =\ valence\ (eq/mole)$$

$$The\ flux\ (J)\ is\ the\ product\ of\ ions\ velocity\ and\ concentration\ of\ hydrolysates$$

$$\mathrm{J\ (mol/cm2/sec)} = \mathrm{uC}$$

$$\text{Current density I(amp/cm2)} = \mathrm{F}\sum(\mathrm{Z\,J})$$

$$where\ F\ =\ Faraday's\ Constant\ (96500\ amp\ sec/eq)$$

$$Acid\ removal\ rate\ = \frac{\mathrm{ZQ(Ci - Co)mole}}{\mathrm{sec}}$$

$$where\ Ci\ =\ input\ acid\ concentration$$

$$Co = output\ acid\ concentration$$

$$Q = Flow\ rate\ of\ acid\ solution$$

$$\frac{Acid\ removal\ rate}{\text{Membrane area}} = \frac{\text{ZQ(Ci – Co)}}{\text{n A}}\ \text{eq/sec} \quad (1)$$

$$n = No\ of\ membranes\ present\ in\ the\ ED\ unit$$

$$A = Effective\ area\ of\ single\ membrane$$

$$Acid\ removal\ rate\ per\ unit\ membrane\ area\ with\ current\ density\ as\ follows$$

$$Acid\ removal\ rate = \frac{\eta\ \text{I}}{\text{F}} \quad (2)$$

$From\ equation\ 1\ and\ 2$

$$\frac{ZQ\ (Ci - Co)}{\text{n A}} = \frac{\eta\ \text{I}}{\text{F}}$$

$$I(A) = \frac{FZQ\ (Ci - Co)}{\eta\ \text{n A}}$$

2.5 Performance parameters: Performance parameters are explained as following.

2.5.1 Current

From the above equation acid removal rate is proportional to current. So when current increases for fixed membrane area the acid removal rate will also increase.

2.5.2 Voltage

According to ohms law, $I = \frac{V}{R}$

Where I = Current (A)

V = Voltage (V)

R = Resistance (ohm)

When voltage increases current also increases due to linear relationship between current and voltage however resistance of the ED unit varies slightly but it doesn't affect much on the current and voltage ratio so removal efficiency will increase when voltage increases.

2.5.3 Temperature

When there is increase in temperature, resistance of the ED unit decreases so the small change in voltage causes a huge change in current. At high currents there is an increase in flux when temperature increased [37].

2.5.4 Flow rate

By keeping other parameters constant, if there is an increase in the flow rate, the molecules will get lower time to stay or their residence time will decrease so acid removal rate will decrease.

2.5.5 Limiting current density

It is the current which is passing through ED unit at which water molecules will ionize and suddenly current will increase cubically. Its unit is Amp/m^2. The cubical graph between voltage and current can be shown in the figure 2.5 in which there curve is divided into three regions below limiting current, on limiting current and above limiting current.

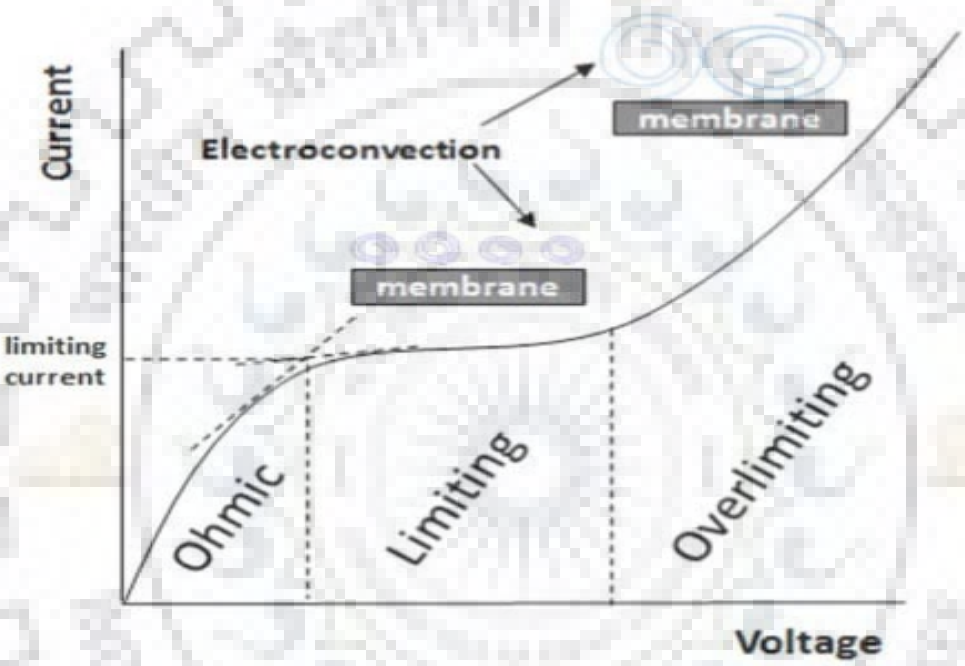


Figure 2.5: Voltage and current curve for any ionic solution which representing different limiting region

This curve in figure 2.5 has 3 regions those are elaborated as following

2.5.5.1 Ohmic region

This region follows ohms law where when we increase voltage, current also increases linearly at some extent. It means there is no increase in number of ions and ions are separated through ion exchange membrane only. In this region not much electrical energy is required.

2.5.5.2 Limiting region

In this region current will not increase when you increase voltage because all ions are already used in electro convection.

2.5.5.3 Over-limiting region

This region starts after limiting region in which current increases very rapidly with small change of voltage because suddenly the number of ions increases due to lysis of water molecules into H^+ and OH^-. At anode half reaction it generates oxygen gas and cathode half reaction it generates hydrogen gas.

$2H_2O \rightarrow 4H^+ + O_2 + 4e^-$ (Oxidation Reaction)

$4H_2O + 4e^- \rightarrow 2H_2 + 4OH^-$ (Reduction Reaction)

$2H_2O \rightarrow 2H_2 + O_2$ (Balanced Equation)

2.6 Limitation(s) of electrodialysis

- Membrane fouling in which the particles having size greater than the pore size block the pores and get deposited on the surface of the membrane. Accumulation of biomass particles and other biomolecules inside the membrane or membrane surface is called membrane fouling. It adversely affects these two parameters: flux drop and electrical resistance [38].
- Loss of water in form of oxygen and hydrogen if system is operated above limiting current.
- Energy requirement is there.

Chapter-3

Objective of Project

If our major focus is on the protection of environment and development of strong economy then waste management can become an important research area for scientists. Waste sulphuric acid is one of them produced by different industries such as leather industries, pharmaceuticals and ethanol industry. In these industries pretreatment is one of major area where sulphuric acid is used for degradation of lignin. In order to produce ethanol from lignocellulosic biomass, the acid hydrolysis process is needed at the first place. However, the sugar produced had to separate from sulphuric acid because the microorganism used in fermentation process could not sustain in high acidic medium. After the separation and recovery of the sulphuric acid, the sugars are suitable for further refinement and processing.

Cost of the sulphuric acid and chemicals which are used for neutralizing sulphuric acid in hydrolysate are the main concern of researchers in this century. However, now a days industries are using only one technique i.e. neutralization for the treatment of waste acid residue in which salt is generated and precipitated. But this method is not the recommended by researchers due to adverse effect on environment. Alternate methods are crystallization, liquid -liquid extraction, and high performance chromatography and membrane technology. In crystallization there is low yield of product with lot of waste and costly chemicals are being used while in liquid-liquid extraction is an energy intensive process and generates harmful product. A new downstream process is needed to be developing which can give high yield and fold of purification.

Only the membrane technology can overcome all these constraints such as environmental issue, economical constraint and purification and it is well suited for pollution control too. The advantages which differs membrane technology from other separation technologies are simple process, ecofriendly and less consumption of energy. When this membrane separation process is incorporated with electric potential, it becomes electrodialysis that is used for the treatment of lignocellulosic biomass hydrolysate and the separation efficiency of ionized ions increased many folds.

Electrodialysis is the physical process in which ions are moving due to electric fields which is generated through electrical energy, through ion exchange membranes. In early time, the evidences of ED are found in the purification of urine and blood. This ion exchange membrane is different from other membranes such as ultrafiltration, microfiltration and reverse osmosis. For ionic movement there is one type of driving force working called electromotive force. This technology has capacity to separate ionic compounds from hydrolysate and concentrated in another chamber and it can be reusable for same purpose. There is no requirement of costly chemicals to operate. Further, ED has capability to complete this separation technique with zero pollution as it generates hydrogen and oxygen gases which are ecofriendly.

Despite of advantages there are some disadvantages which can limit the separation and operation methodology of separation technologies using ED. Fouling is the major problem which has become the most important barrier in ED processes. The fouling increases the resistance of membrane by increasing the width of cake layer which reduces the efficiency of the

electrodialysis process. These fouling conditions and alterations in membrane can reduce the performance and there is need of change of membrane in this case. People are taking care of membrane and avoiding fouling because cleaning and replacing of membrane is very costly process. However, cleaning of membrane can also reduce the performance of membrane by increasing the pore size or decreasing the membrane permeability for ions. Another problem is that separation of fermentable sugar from lignocellulosic biomass hydrolysate using ED should provide minimum power consumption over maximum effective area and no sugar loss. These conditions are not met yet because separation efficiency proportional to the membrane area and current density as well as concentration of acid in hydrolysate plays an important role in separation.

3.1 Project goals

a) To determine the effects of sulphuric acid concentration in feed stream, the voltage supplied and the feed flow rate on separation of acid from lignocellulosic biomass hydrolysate.

b) To characterize an ED by means of the determination of the optimum working condition with minimum energy consumption.

c) To obtain maximum ethanol productivity from lignocellulosic biomass hydrolysate using *Z. mobilis*.

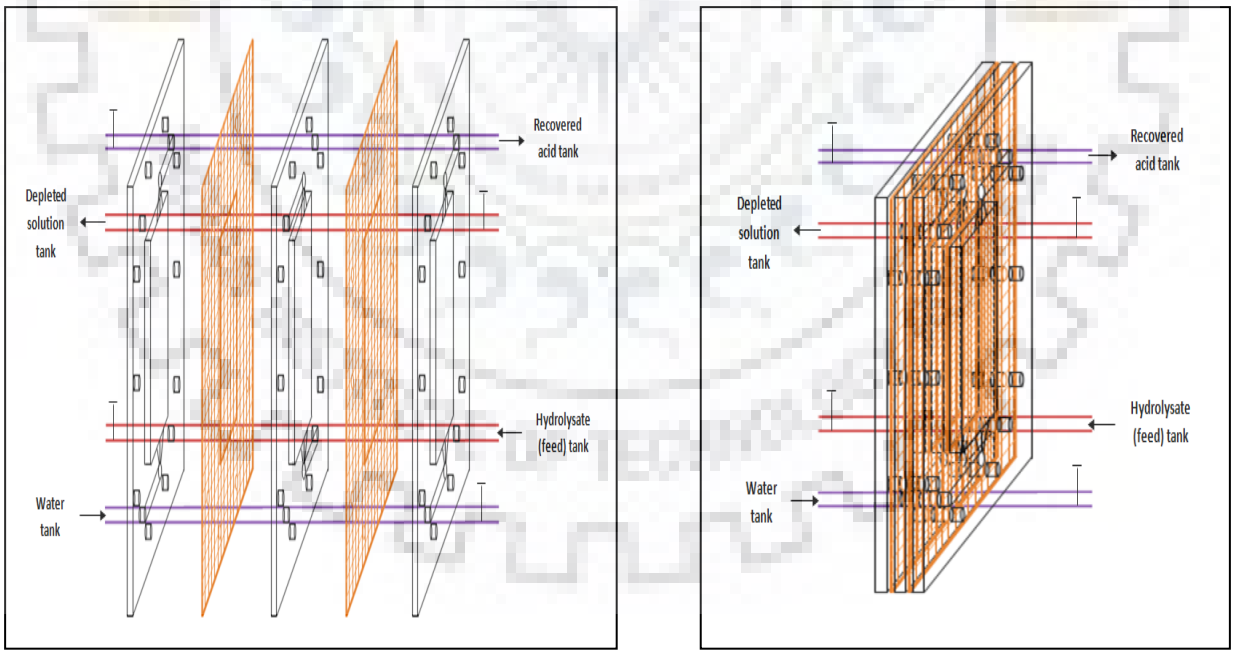
Chapter-4

Methodology

4.1 Materials and chemicals used

a) Acid solution
b) Water
c) Electrolyte
d) Power supply unit (0-16V)
e) Platinum electrode
f) Channelized fiber sheets
g) Pipes (38mm diameter)
h) Silicon rubber sheet
i) Electrical clamp
j) Peristaltic pump
k) Phenolphthalein as indicator
l) Biuret for titration

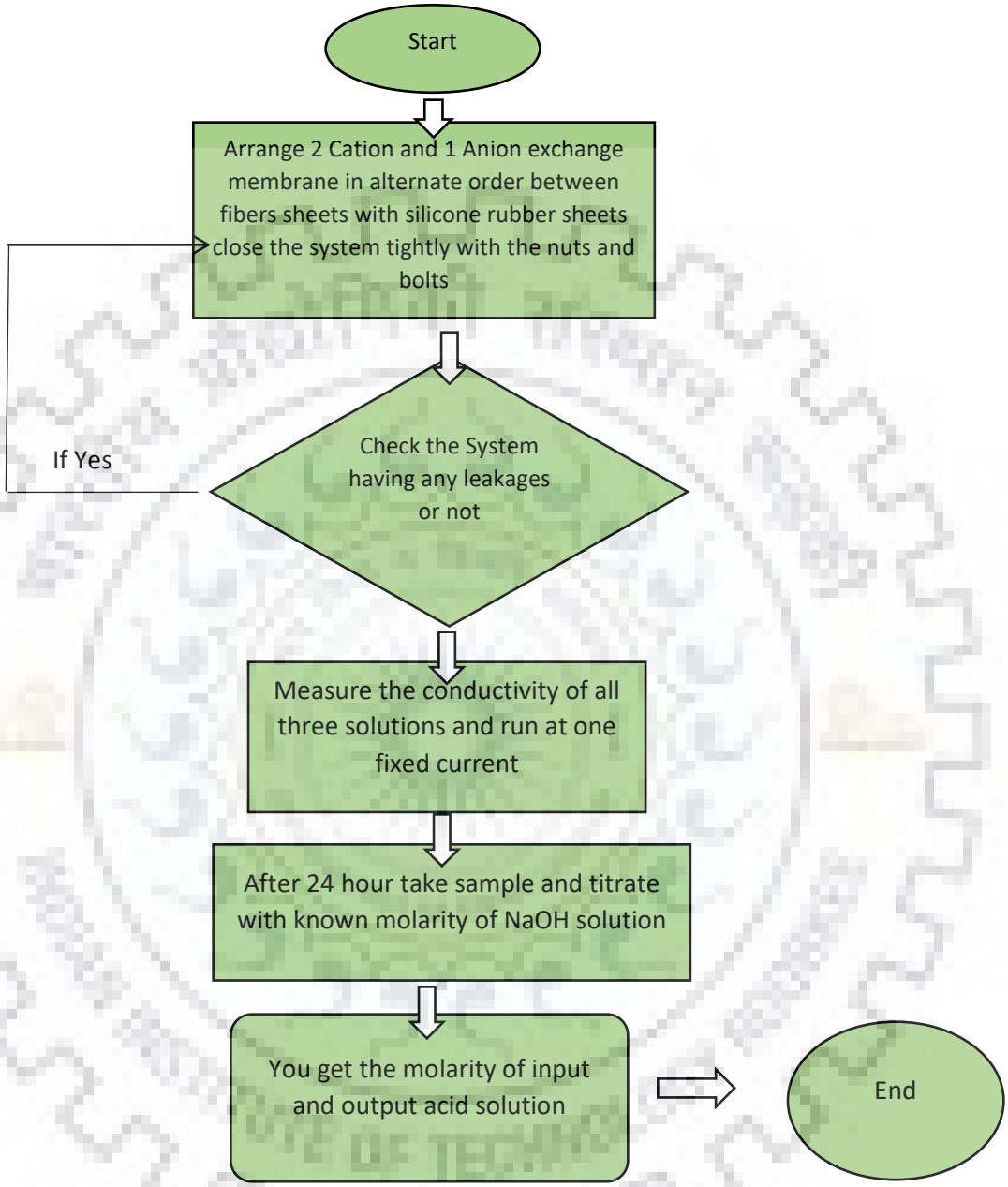
4.2 Structure and channels of electrodialysis unit: These are shown in figure 4.1. Figure 4.1a shows the expanded structure of the unit with channels and figure 4.1b shows the compressed view.



(a) (b)

Figure 4.1: Structure and channels of electro dialysis unit (a) Expanded view (b) Compressed view

4.3 Flowchart of overall experiment



4.4 Lignocellulosic biomass hydrolysate preparation

Lignocellulose biomass contains the enormous amount of fermentable sugars into it. These sugars can be extracted by different pretreatment methods in which sulphuric acid treatment is the one. We have developed a novel fractional acid hydrolysis technology in our laboratory that can extract more than 90% (w/w) of the fermentable sugars (glucose and xylose) with negligible amount of toxic compounds produced. The table 4.1 shows the amount of sugars extracted from kans grass biomass.

Table 4.1: Composition of extracted fermentable sugars (Xylose and Glucose) from Kans grass biomass through acid hydrolysis

Sugar	Hydrolysis process	Xylose		Glucose		Furfural		HmF	
		mg/ml	%g/gds	mg/ml	%g/gds	mg/ml	%g/gds	mg/ml	%g/gds
Xylose + Glucose	Acid hydrolysis	20.18	20.81	31.05	32.02	0.10	0.27	0.03	0.07

Inhibitory concentrations:

Furfural (1mg/ml [38]; 0.50-4mg/ml [39])

HmF (1-5mg/ml [39])

4.5 Experimental procedure

- Start with the preparation of hydrolysate, electrolyte and deionized water solution in mill-q water.
- Keep the concentration of sulphuric acid same in hydrolysate and electrolyte while the while deionized water has no acid.
- Prepare a solution of 0.1M NaOH which will be used for neutralization of sample those will be collected at different time intervals.
- There is also two platinum electrodes which are connected through power supply. Power supply is having the variable voltage and variable current supply.
- Attach all inputs to the ED unit from these solutions and output depends on the operation mode i.e. continuous or recycles.
- Now inputs are connected to ED unit passing through peristaltic pumps which are used for maintaining the uniform flow rate of hydrolysate, electrolyte and deionized water.
- Acid separation is started at fix current through ED unit.
- Take 1-1 ml sample of all 3 solutions and analyze it.

4.6 Analyzing methods

Samples are analyzed using titration method in which 1 ml of sample has been taken and neutralized with 0.1 NaOH. For detecting the point of neutralization, an indicator is used named phenolphthalein which gives pink color when reached to the neutralization. It remains colorless in acid solution. Its reaction with NaOH is given below.

$H_3in^+ \rightarrow H_2in \rightarrow in^{2-} \rightarrow in(OH)^{3-}$ where in represents the indicator.

The sugars (xylose and glucose) were analyzed with the help of HPLC.

4.7 Electrodialysis unit design in recycle mode

In the recycle mode the input and output remains in the same collection tank. That means there are only three collection tanks, one for hydrolysate, one for electrolyte and one for deionized water. Its design has been shown in figure 4.2.

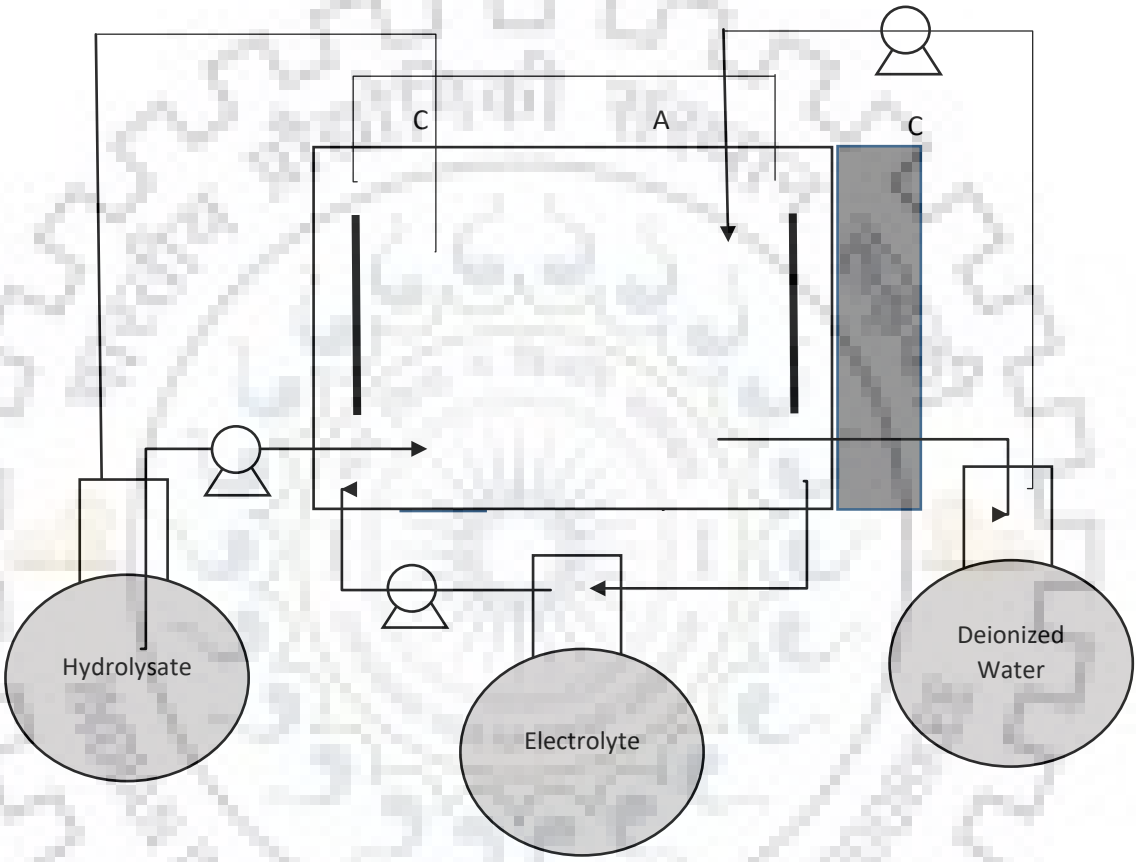


Figure 4.2: Design of ED unit in recycle mode for the separation of acid from hydrolysate

4.8 Principal of electrodialysis

Ions are moving due to electric potential. Sulphate ions are moving towards anode side by crossing the anion exchange membrane but could not cross the cation exchange membrane and their concentration will increase in concentrate tank and similarly H^+ ions move towards cathode by crossing the cation exchange membrane but could not cross the anion exchange membrane and their concentration will decrease in dilute tank. So when we operate this procedure for fixed time, it can remove the acid from the solution.

4.9 Modes of operation

There are two modes of operating the ED unit.

1. Below limiting current

2. Above limiting current

4.9.1 Acid separation below limiting current

When electrodialysis unit operate below limiting current, it works as shown in figure 4.3. However, there is no ionization of water molecules so it would not make much difference as without current. At this stage only diffusion occurs and some amount of ions separates due to electric potential but it is not efficient to separate acid in comparison of its operation above limiting current.

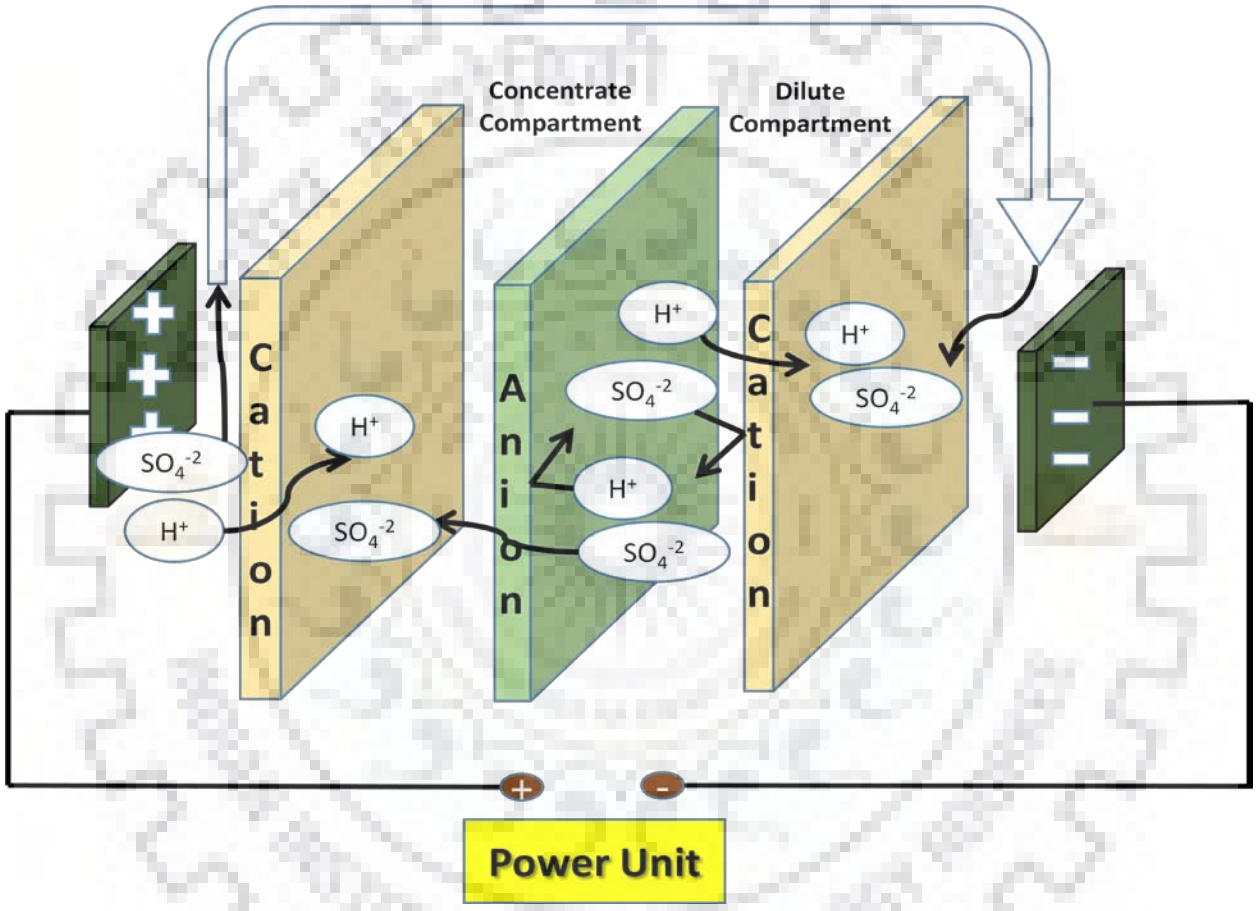


Figure 4.3: Movement of ions in the ED unit when operating below limiting current

4.9.2 Acid separation above limiting current

When ED unit is operated above limiting current, the water molecules get ionized into H^+ and OH^- ions. Water lysis also occurs in concentrate and dilute compartment but there is no generation of hydrogen and oxygen gas here. The water molecules transfer from dilute to concentrate compartment and in result the dilute chamber becomes concentrated and concentrate chamber becomes diluted as represented in figure 4.4. However, in electrolytic compartment water loss occurs in the form of gases (H_2 and O_2) at their respective anode and cathode. Further, the electrons ($4e^-$) generated during reaction travels from one to another electrode and complete the circuit to flow the current.

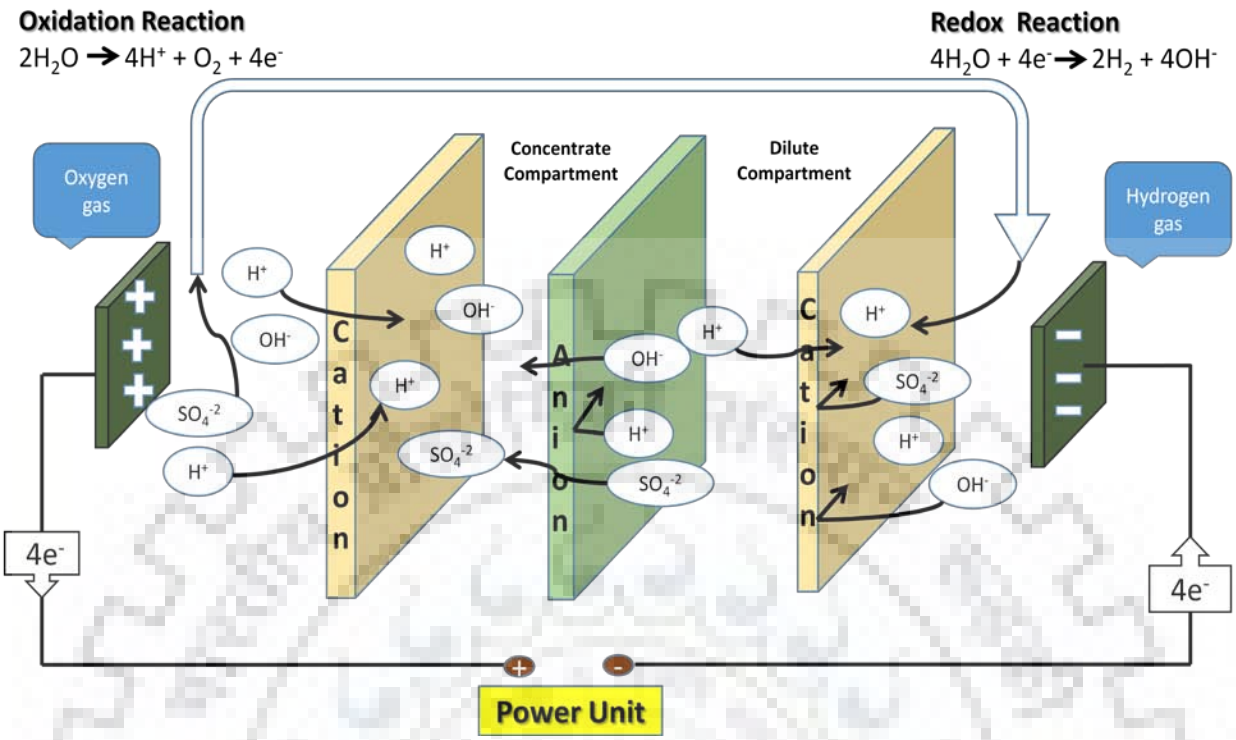


Figure 4.4: Movement of ions in the ED unit when operating above limiting current

4.10 Cleaning of ED equipment

After running so many experiments, membrane will be clogged so there is need of proper cleaning which is done by following methods shown in figure 4.5.

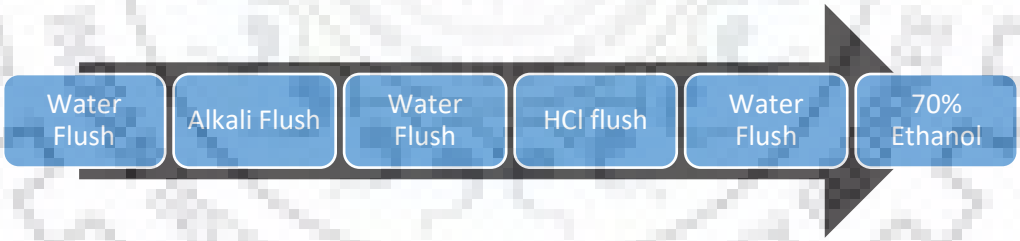


Figure 4.5: Steps for cleaning the electrodialysis unit

Chapter-5

Results and Discussion

5.1 Limiting current

Limiting current is the current at which water dissociates into hydrogen and hydroxide ions. It has essential role in separating the ions from an acidic solution as we have discussed under chapter 5.

To find out the limiting current, several experiments were conducted on feedstock solution. The feedstock solution i.e. hydrolysate contains mainly glucose, xylose and sulphuric acid. The amount of acid in hydrolysate depends on molarity of the solution which is used for treatment. Hydrogen ions are solely responsible for the conductivity of the sulphuric acid solution. Figure 5.1 represents the curve between current and voltage for different concentrations of H_2SO_4 and it also help in finding the limiting current for that specific molarity. Here the limiting current is different for different sulphuric acid molarity. Table 5.1 shows the values of limiting current in case of different H_2SO_4 concentrations.

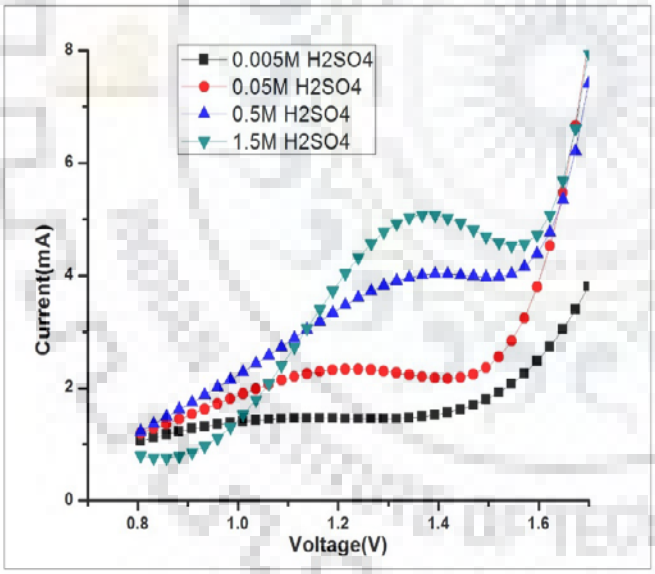


Figure 5.1: Current (mA) vs Voltage (V) curve for different concentrations of H_2SO_4

Table 5.1: Limiting current (mA) for different concentrations of H_2SO_4

Acid Molarity (M)	Limiting Current (mA)
0.005	0.75
0.05	2.2
0.5	3.95
1.5	4.5

From these results in Table 5.1 it is found that 1.5 M sulphuric acid solution is having the maximum conductivity and it is similar to our hydrolysate's molarity. So it is very easy to find out the limiting current for the acid solution at which water lysis will occur.

5.2 Operation of ED unit below limiting current

Here the current which is closer to the limiting current i.e.4.5 mA is taken for operation and it remains fixed during the whole process. When the flow rate decreases the mean residence time of molecules increases and it results in high acid removal. The removal efficiency was increased when unit was operated at low flow rate as shown in the Table 5.2. It gave around 23% acid recovery at 0.5ml/min flow rate.

Table 5.2: Effect of different flow rates on acid recovery in ED unit operating below limiting current

Flow Rate / Time	2 ml/min	1 ml/min	0.5 ml/min
0 h	1.50 M	1.50 M	1.50 M
24 h	1.35 M	1.27 M	1.16 M
Recovery (%)	9.77	15.28	22.60

5.3 Operation of ED unit above limiting current

If separation occurs at just above the limiting current where water lysis will occur then acid recovery percentage will increase and concentration of sugars (glucose and xylose) will also increase. It has been theoretical proved that two molecules of water converted into hydrogen and oxygen and no sulphuric acid has lost. To fulfill the objectives there is need of parameters optimization which will give maximum acid separation at lower cost so that process can become economically viable and sustainable developable.

5.3.1 Optimization of performance parameters

Following parameters are found to be essential in performance analysis of ED unit.

1. Acid concentration of feed solution (i.e. hydrolysate)
2. Current (Ampere)
3. Residence time (Stack volume/flow rate)
4. Voltage (V)
5. Electrolyte concentration
6. Effective membrane area (fixed in our case)

5.3.1.1 Optimization of feed concentration

Acid amount depends on the molarity of the solution which is used for hydrolysis. H^+ ions are solely responsible for the conductivity of the sulphuric acid solution. The effect of acid concentration (M) was studied on ED unit's performance. The different parameters and optimization results are listed in table 5.3 and table 5.4.

Table 5.3: Constant parameters during optimization of sulfuric acid concentration

Parameter		Value
Mode of operation		Continuous
Flow rate	Electrolyte	3 ml/min
	Hydrolysate	3 ml/min
	Deionized water	3 ml/min
Voltage		5 V
Current		2 A
No of membranes	Cation exchange membrane	2
	Anion exchange membrane	1
Effective membrane area (per membrane basis)		32 cm^2

.

Table 5.4: Removal of sulphuric acid at different hydrolysate molarity

Acid Concentration	Initial acidic molarity (M)	Final acidic molarity (M)	Acid removal (%)
1 M	1.0282	0.9222	10.30
2 M	2.067	1.961	5.12
3 M	3.0104	2.8832	4.22
4 M	4.1128	3.9644	3.6

Here at 1 M sulphuric acid concentration it gave the highest acid recovery that was taken forward to optimize other process parameters. The reason behind decreasing the acid removal efficiency might be the decrement in the diffusion coefficient with increasing acid concentration.

5.3.1.2 Optimization of flow rate

Flow rate is inversely proportional to the residence time of molecule. When the residence time increases the molecules get lesser time to move across the ion exchange membrane and as a result acid separation decreases. Due to this reason, flow rate should be kept as minimum as required. However, slow flow rate would increase the process time which will affect the process cost. The table 5.5 and 5.6 shows the different parameters and result of changing the flow rate respectively.

Table 5.5: Constant parameters for flow rate optimization

Parameter		Value
Mode of operation		Continuous
Acid molarity	Electrolyte	1 M
	Hydrolysate	1 M
	Deionized water	0 M
Voltage		5 V
Current		2 A
No of membranes	Cation exchange membrane	2
	Anion exchange membrane	1
Effective membrane area (per membrane basis)		32 cm^2

Table 5.6: Recovery of sulphuric acid at different flow rates

Flow rate (ml/min)	Initial acidic molarity (M)	Final acidic molarity (M)	Acid removal (%)
1	1.0335	0.7314	29.23
2	1.0335	0.8904	13.84
3	1.0335	0.9222	10.30
5	1.0388	0.9964	4.08

Here it gave 1 ml/min as the optimum flow rate that was further taken forward to optimize other process parameters. The flow rate below 1ml/min also gave the similar removal efficiency during experiments.

5.3.1.3 Optimization of current at fixed flow rate of 1ml/min and 1M acid concentration

Current plays a major role in separating acidic ions across the membrane. For ionization, molecules need energy which is transferred through heat or electrical energy. Here, electrical energy was provided in the form of high voltage current which ionized the water molecules and generated an electric potential to separate ions according to their charge and reduced the process time of separation. The table 5.7 and 5.8 shows the different parameter and results of changing the current respectively.

Table 5.7: Constant parameters during current optimization

Parameter		Value
Mode of operation		Continuous
Acid molarity	Electrolyte	1 M
	Hydrolysate	1 M
	Deionized water	0 M
Flow rate	Electrolyte	1 ml/min
	Hydrolysate	1 ml/min
	Deionized water	1 ml/min
No of membranes	Cation exchange membrane	2
	Anion exchange membrane	1
Effective membrane area (per membrane basis)		32 cm^2

Table 5.8: Removal of sulphuric acid at different electric currents

Voltage (V)	Current (A)	Initial acidic molarity (M)	Final acidic molarity (M)	Acid removal (%)
1	0	1.0335	1.007	2.56
1.5	0	1.0335	1.007	2.56
2.0	0.07	1.0335	0.962	6.91
2.5	0.35	1.0335	0.9464	8.43
3.0	0.68	1.0335	0.9152	11.44
4.0	1.61	1.0335	0.8632	16.48

Here the 4V and 1.61 A was found as the optimum. It was not tried with more than 4V because of the limitation of power supply unit.

5.3.1.4 Optimization of electrolyte concentration

Effect of electrolyte concentration on acid removal was also studied. The parameter and results are listed in the table 5.9 and 5.10 respectively.

Table 5.9: Parameters for electrolyte concentration optimization

Parameter		Value
Mode of operation		Continuous
Acid molarity	Electrolyte	Variable
	Hydrolysate	1 M
	Deionized water	1 M
Flow rate	Electrolyte	1 ml/min
	Hydrolysate	1 ml/min
	Deionized water	1 ml/min
No of membranes	Cation exchange membrane	2
	Anion exchange membrane	1
Effective membrane area (per membrane basis)		32 cm^2

Table 5.10: Recovery of sulphuric acid at variable electrolyte molarity

Electrolyte molarity (M)	Initial acidic molarity (M)	Final acidic molarity (M)	Acid removal (%)
0.1	1.0335	0.8120	21.43
0.5	1.0335	0.8014	22.45
1.0	1.0335	0.8752	15.31

Here 0.5 M electrolyte was found as the optimum electrolyte concentration. The decrement in acid removal may be the result of negative hindrance created between molecules with increased electrolyte concentration.

5.3.1.5 Sulphuric acid separation from synthetic hydrolysate

According to the availability, parameters which broadly affect the separation efficiency of ED unit operating above limiting current were optimized and processed to achieve a maximum acid separation from lignocellulosic biomass hydrolysate. Fixed parameters were as usual as previously used in the experiments, first effective area of membrane ($32cm^2$) and second current (just below the maximum availability i.e. 1.67 A). In the table 5.11, it is clearly seen that the sulphuric acid concentration is reduced from 1.04 M to 0.03 M in 19 h which is approximately 98% of the total acid present in hydrolysate. Eventually, the sulphuric acid which is recovered in deionized water is 97.07%, approximately same to 98%. At the same time, the glucose and xylose sugars get concentrate because of water molecules transfer from the hydrolysate to deionized water in the form of H^+ and OH^- ions.

In electrolyte, there was not much difference in acid concentration as well as electrolyte volume but in deionized water, volume difference was present due to incoming of separated acid from the hydrolysate. Volume of deionized water was changed from 158 ml to 182 ml. Table 5.11 shows the changes in volume, concentration of acid and amount of sugars in hydrolysate and table 5.12 shows the changes in volume and acid concentration of electrolyte and deionized water. Similarly table 5.13 shows the acid recovery in water, percentage sugar concentration and sugar loss from hydrolysate.

Table 5.11: Effect in hydrolysate composition when separation is done at optimized condition

Time (h)	Hydrolysate					
	Volume (ml)	**Molarity (M)**	**Glucose Concentration (mg/ml)**	**Amount of Glucose (mg)**	**Xylose Concentration (mg/ml)**	**Amount of Xylose (mg)**
0	159	1.04	4.148	659.53	4.1437	658.84
4	146	0.79	4.62	674.52	4.56	665.76
8	132	0.55	4.91	648.12	4.92	649.44
11	121	0.37	4.76	575.96	4.72	571.12
19	102	0.03	5.07	517.14	5.01	511.02

The loss of water can also be calculated with the help of following empirical equations.

$$\text{Coulomb (q)} = \text{ampere (A)} \times \text{time (t)} \quad (3)$$

$$96485 \text{ Coulomb} = 1 \text{ Faraday} \quad (4)$$

$$1 \text{ Faraday} = 1 \text{ mole electron} \quad (5)$$

During this experiment, we observed that in initial 8h time only 0.249 moles of water were lost from hydrolysate while in 19h time it increased to 0.592 moles. So this study suggests that when we increase the process time, volume of hydrolysate decreases and concentration of sugar increases simultaneously. Figure 5.2 tells how the sulphuric acid is transferring from hydrolysate to the deionized water.

Table 5.12: Change in composition of electrolyte and deionized solution

Time (h)	Electrolyte		Deionized water	
	Volume (ml)	Molarity (M)	Volume (ml)	Molarity (M)
0	**133**	0.98	**158**	0
4	**131**	1.01	**162**	0.273
8	**130**	169	**1.05**	0.483
11	**126**	0.97	**174**	0.6405
19	**122**	1.07	**182**	0.882

Table 5.13: Representing the acid recovery (%) in deionized water and sugar loss in hydrolysate

Time (h)	Acid removal (%)	% increment in sugar concentration	Sugar Loss (%)	Acid recovery (%)
0	0	Nil	No loss	Nil
4	30.24	11.37	No loss	26.74
8	56.41	18.37	1.73	49.35
11	72.92	14.75	12.67	67.40
19	98.18	22.22	21.58	97.07

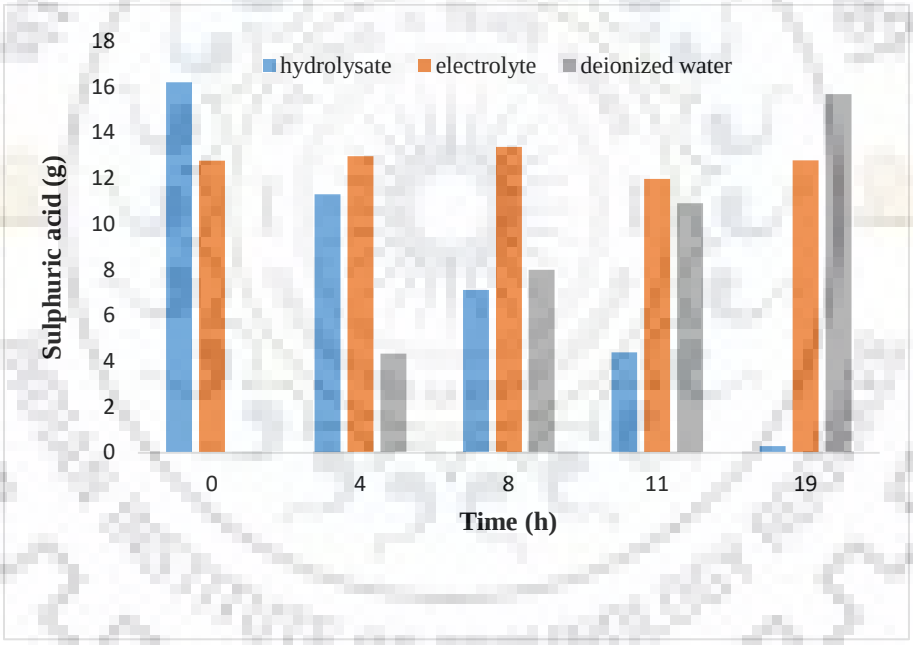


Figure 5.2: Change in amount of sulphuric acid in hydrolysate, electrolyte and deionized water during the electrodialysis process

Figure 5.3 shows that the rate of acid depletion from the hydrolysate to deionized water is very nearly same as the rate of acid gain to deionized water from the hydrolysate.

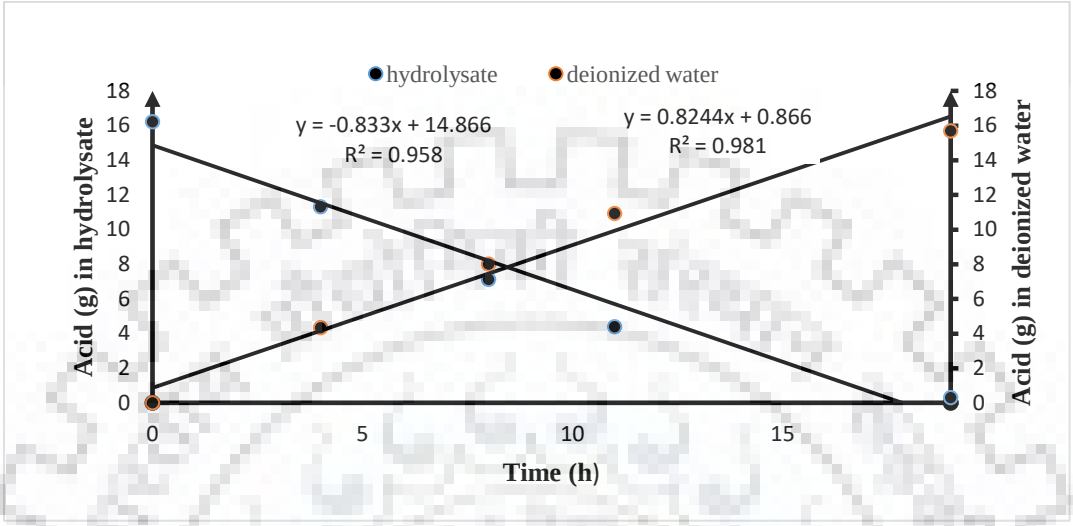


Figure 5.3: Graph representing the acid depletion rate and gaining rate in hydrolysate and deionized water respectively

Figure 5.4 shows the change in amount of water in hydrolysate, electrolyte and deionized water.

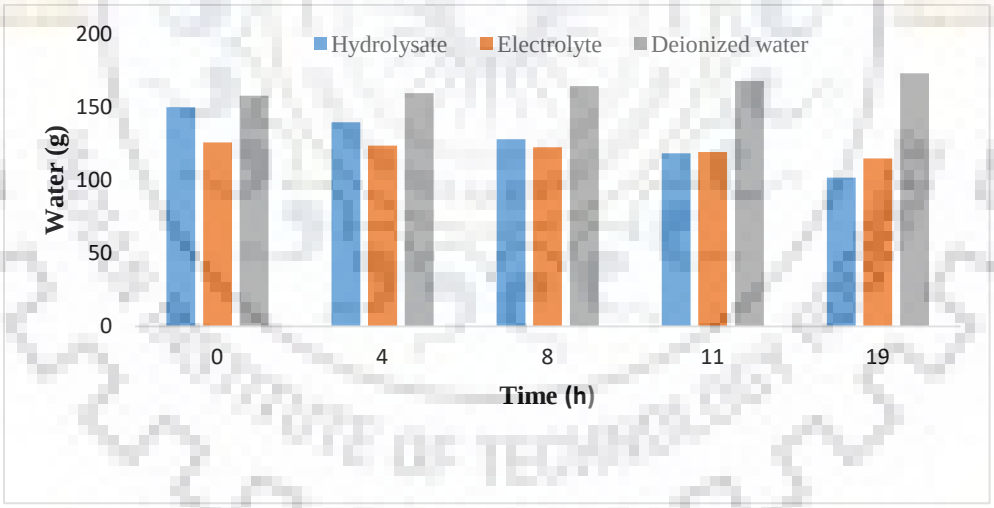


Figure 5.4: Change in the amount of water during the separation of sulphuric acid through electrodialysis

5.3.1.6 Water transport through ED membrane

Water can transport through ED membrane because of the following things.

1. Internal leakage
2. Osmosis
3. Electroosmosis

5.3.1.6.1 Internal leakage

Sometime due to pressure membrane swells and itsporosity increases which results in transportation of water molecules from one chamber to another chamber.

5.3.1.6.2 Osmosis

Water is transported due to chemical potential difference between low and high electrolyte solution.

5.3.1.6.3 Electroosmosis

This occurs when water molecules are transported with ions through ion exchange membrane and this water flux is proportional to the electric current.

Here in the figure 5.5 it is clearly mentioned that water depletion rate in hydrolysate is 3.0354 g-water/h and water gaining rate in deionized solution is 1.3098 g-water/h. These two rates are not equal because some amount of water converted into hydrogen and oxygen gas which can be calculated by equations 3, 4 and 5.

In table 5.13 that sugar loss was due to leakage in the ED unit after 8h. That leakage might occur due to diffusion of solution through membrane. In current design, membrane was sealed but some part of it was coming outside from the stack and it might be responsible for leakage.

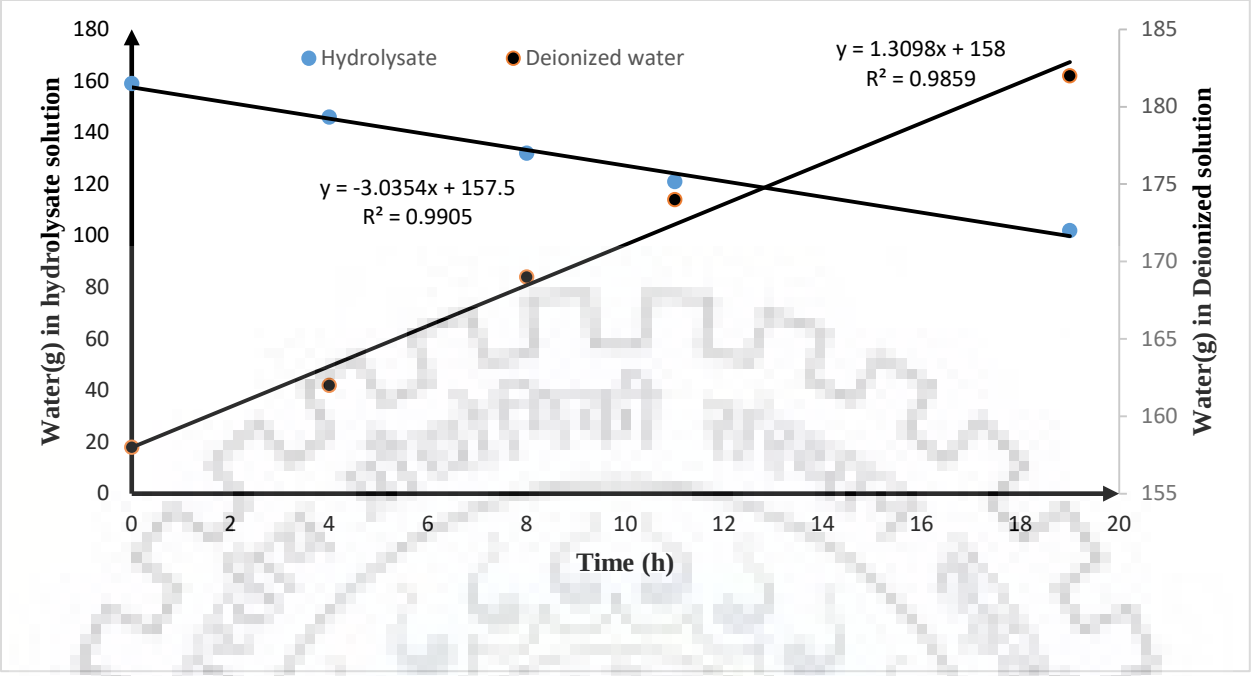


Figure 5.5: Water loss in hydrolysate and water gain in deionized water during separation process

Figure 5.6 shows the concentration of glucose sugar which is continuously increasing with time.

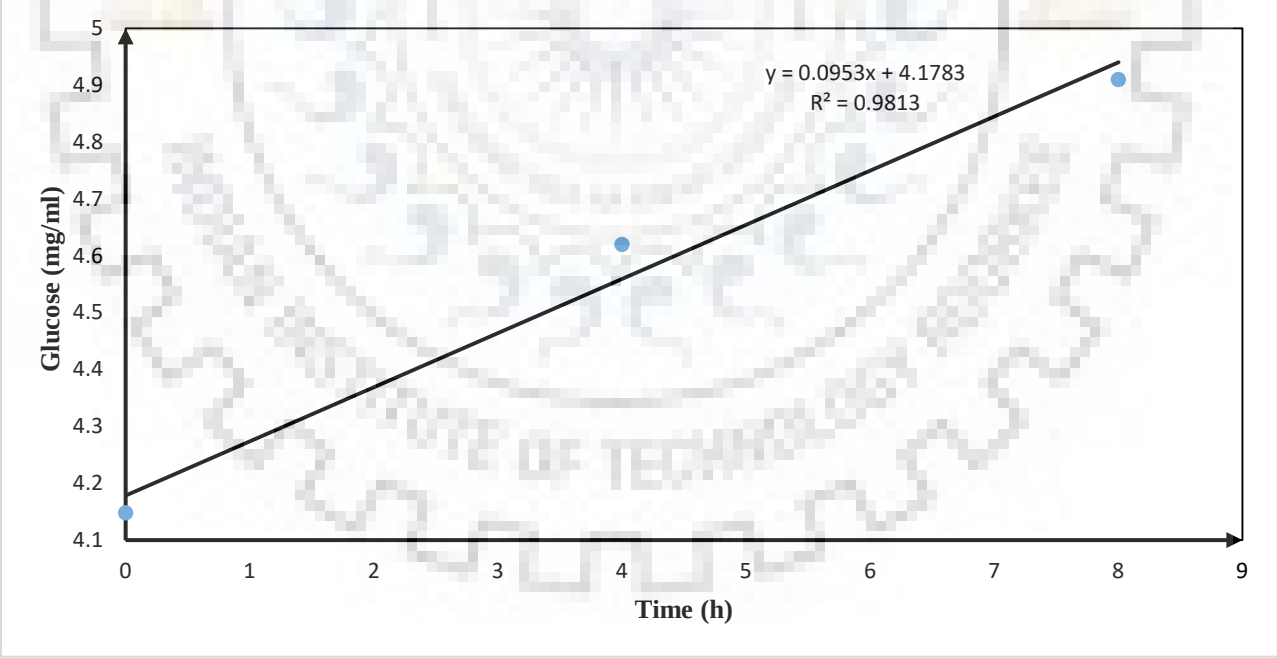
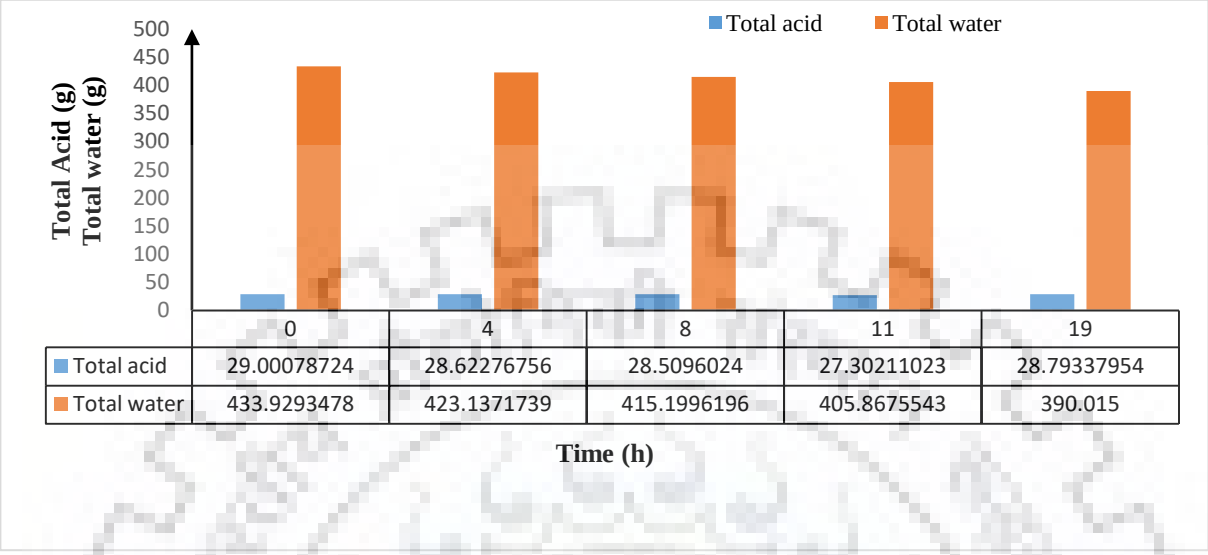


Figure 5.6: Graph representing the glucose concentration during the separation process

Figure 5.7 The mass balance on acid and water while figure 5.8 shows the mass balance on sugars in the solution.



	0	4	8	11	19
Total acid	29.00078724	28.62276756	28.5096024	27.30211023	28.79337954
Total water	433.9293478	423.1371739	415.1996196	405.8675543	390.015

Figure 5.7: Mass balance on acid and water at different times

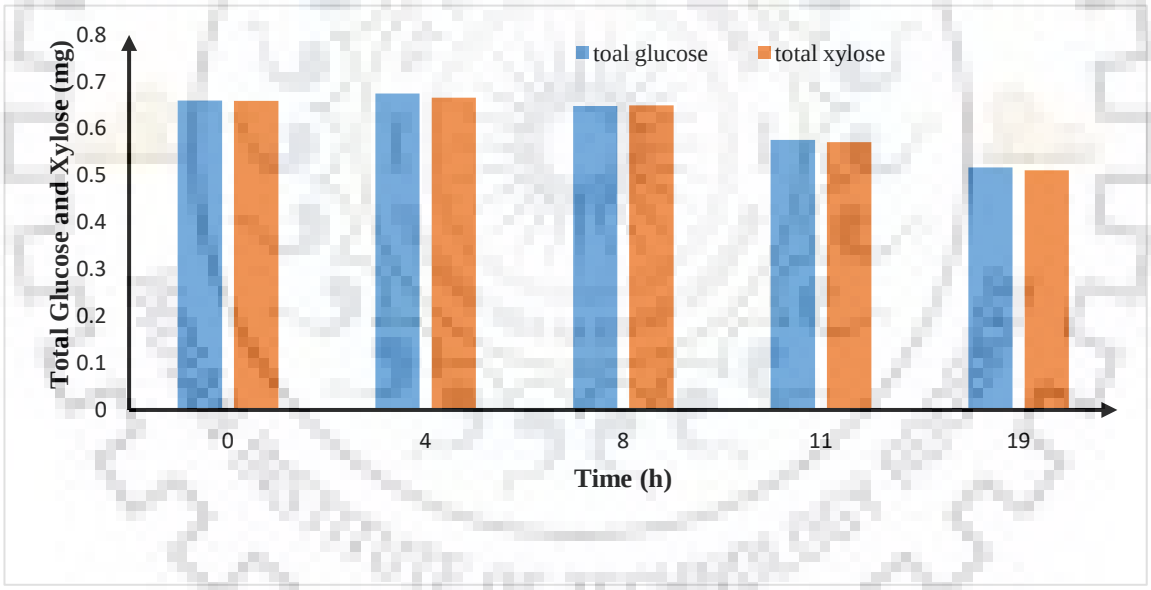


Figure 5.8: Mass balance on glucose and xylose at different times

5.4 Fermentation studies

ED removed more than 95% of the acid from the hydrolysate and very small remaining amount of acid was neutralized with calcium hydroxide followed by centrifugation. After centrifugation, the fermentable sugar (glucose in this case) was fermented using *Zymomonas mobilis* in 500 ml bioreactor. Table 5.14 and 5.15 shows the fermentation data in terms of concentration and fermentation parameters respectively. Figure 5.9 is graphical representation of fermentation data.

Table 5.14: Fermentation data in terms of concentration

Time (h)	Glucose (mg/ml)	Ethanol (mg/ml)	Biomass (mg/ml)
0	60.82	2.25	0.74
4	58.06	3.46	0.74
8	46.40	9.71	0.94
12	14.78	28.98	1.83
13.5	0.06	37.40	1.99

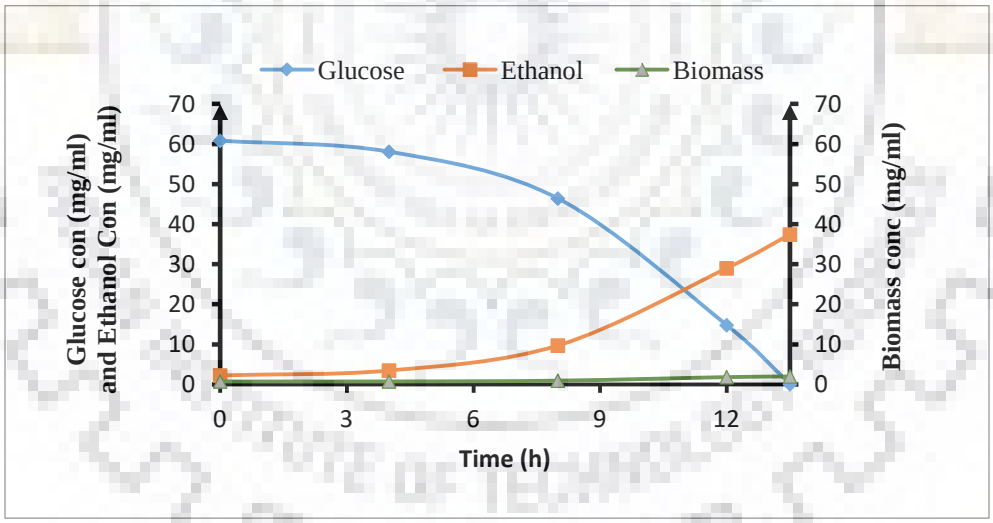


Figure 5.9: Change in the biomass, glucose and ethanol concentration (mg/ml) during fermentation

Table 5.15: Fermentation parameters

Parameters	Value
Organism	*Zymomonas mobilis*
Media composition	*Glucose (obtained from hydrolysate); Yeast extract (10g/l); KH_2PO_4 (1g/l); $MgCl_2$ (1g/l); $(NH_4(SO_4)_2)$ (1g/l)*
Operation mode	*Batch mode*
Temperature (°C)	*30*
Reactor Size (ml)	500
Working volume (ml)	150
Inoculum size (v/v)	10% (*Zymomonas mobilis)*
Initial Sugar (g/l)	60.8215 (Glucose)
Initial Biomass (g/l)	0.74
Max Ethanol (g/l)	30.3590
Ethanol Yield (g/g)	0.4941
Max Biomass yield (g/g)	0.0327
Fermentation Time (h)	13.5
Max Biomass productivity (g/l/h)	0.1474
Max Ethanol productivity (g/l/h)	2.2488

5.5 Economic analysis

Economic analysis is the backbone of any new technology. When any technology becomes environment friendly and economically feasible then it can be scaled up for large scale production. Electrodialysis is the unique method to separate the ionic species from aqueous medium and produce fuel gases such as hydrogen and oxygen to fulfill the energy requirements of society. When hydrolysate is treated with $Ca(OH)_2$, it produces gypsum and its dumping into free places creates environmental issues. The cost of neutralizing the 102 ml (1M) of hydrolysate is around 3.32 rupees whereas the cost of neutralizing the same volume of hydrolysate after treatment with electrodialysis unit is only 1.37 rupees approximately and fixed cost is one time investment and liberated fuel gases may generate extra revenue. There are no inhibitory compounds generated in whole electrodialysis process. Table 5.16 shows the economic comparison of traditional neutralization and neutralization after electrodialysis process.

Table 5.16: Economic comparison between acid separation with ED or without ED

For 102 ml (1 M) hydrolysate				
Without Electrodialysis Unit	With Electrodialysis Unit			Benefits of Electrolysis unit
1 M H_2SO_4 solution requires 7.8499 g $Ca(OH)_2$ which cost Rs3.32/-	0.03 M H_2SO_4 solution requires 0.2264 g $Ca(OH)_2$ which cost Rs 0.10/-	Variable power cost if price is 10Rs/KWh = 1.27 Rs	Fixed Cost • Membrane Cost • Power supply unit • Acrylic Sheets	• No inhibitory Compounds • Hydrogen and oxygen gas as a profit

Chapter 6

Conclusion

Electrodialysis is better process to recover acid from the hydrolysate. The objective of this project was to recover the sulphuric acid from hydrolysate using electrodialysis unit and to enhance the productivity of ethanol during fermentation economically at lower cost. This ED unit is able to recover more than 95% of sulphuric acid from lignocellulosic biomass hydrolysate (1M) when operated at 1.61A having the flow rate of 1ml/min for hydrolysate, electrolyte and deionized water. If the ED unit is coupled with fermentation vessel and continuously provides the acid free pentose and hexose sugar solutions without inhibitory compounds to the microorganism, it can enhance the productivity of ethanol during fermentation. We got 0.4991g/g ethanol yield and 2.2488 g/l/h ethanol productivity when we fermented the acid free glucose (60.8215g/l) with *Zymomonas mobilis* in 500ml bioreactor. Further, high current will result in high acid separation in less time but the power cost may not be feasible at industrial scale. However, the cost involved in recovering acid by ED process is much less than the other methods.

Chapter 7

Future prospects

The following aspects can be considered for future work. These aspects will help in commercialization of this technology.

- Development of mathematical model for electrodialysis process
- Scaling-up of the lab-scale electrodialysis unit
- Economic comparison between electrodialysis and diffusion dialysis process
- Integration of electrodialysis with diffusion dialysis process
- Integration of electrodialysis with fermenter for continuous feed supply to produce bioethanol without need of detoxification

References

1. Saha, B. C. (2003). "Hemicellulose bioconversion." Journal of industrial microbiology and biotechnology, 30(5): 279-291.
2. Canilha, L. Chandel, A. K. Suzane dos Santos Milessi, T. Antunes, F. A. F. Luiz da Costa Freitas, W. das Graças Almeida Felipe, M. & da Silva, S. S. (2012). "Bioconversion of Sugarcane Biomass into Ethanol: An Overview about Composition, Pretreatment Methods, Detoxification of Hydrolysates, Enzymatic Saccharification, and Ethanol Fermentation." Journal of Biomedicine and Biotechnology, 1–15.
3. FitzPatrick, M., Champagne, P. Cunningham, M. F. & Whitney, R. A. (2010)."A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products." Bioresource technology, 101(23): 8915-8922.
4. Cortez, L. A. B. (2010). "Bioetanol de cana-de-açúcar: P&D para produtividade e sustentabilidade."
5. Latif F. and M. I. Rajoka (2001). "Production of ethanol and xylitol from corn cobs by yeasts." Bioresource technology, 77(1): 57-63.
6. Zhao, X., Zhang, L., & Liu, D. (2012). "Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose." Biofuels, Bioproducts and Biorefining, 6(4): 465-482.
7. FitzPatrick M, Champagne P, F.Cunningham Michael, A.Whitney R (2010). "A biorefinery processing perspective: Treatment of lignocellulosic material for the production of value-added products". Bioresource Technology, 101 (23): 8915-8922.
8. Krishnan, C., Sousa, L. da C., Jin, M., Chang, L., Dale, B. E., & Balan, V. (2010). "Alkali-based AFEX pretreatment for the conversion of sugarcane bagasse and cane leaf residues to ethanol." Biotechnology and Bioengineering, 107(3): 441-450.
9. Knothe, G. (2010). "Biodiesel: current trends and properties." Topics in Catalysis, 53(11-12): 714-720.
10. Li, Y., Zhang, X., & Sun, L. (2010). "Fatty acid methyl esters from soapstocks with potential use as biodiesel." Energy Conversion and Management, 51(11): 2307-2311.
11. Ataya, F., Dubé, M. A., & Ternan, M. (2007). "Acid-catalyzed transesterification of canola oil to biodiesel under single-and two-phase reaction conditions." Energy & Fuels, 21(4): 2450-2459.
12. K. Grohmann and R. J. Bothast. "Enzymatic Conversion of Biomass for Fuels Production." Vol. 566American Chemical Society, Washington, DC: 1-52.
13. Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review." Bioresource technology, 101(13): 4851-4861.
14. Pasquini, D., Pimenta, M. T. B., Ferreira, L. H., & Curvelo, A. A. da S. (2005). "Extraction of lignin from sugar cane bagasse and Pinustaeda wood chips using ethanol–water mixtures and carbon dioxide at high pressures." The Journal of supercritical fluids, 36(1): 31-39.

15. Hideno, A., Inoue, H., Tsukahara, K., Fujimoto, S., Minowa, T., Inoue, S. Sawayama, S. (2009). "Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw." Bioresource technology, 100(10): 2706-2711.
16. Araque, E., Parra, C., Freer, J., Contreras, D., Rodríguez, J., Mendonça, R., & Baeza, J. (2008). "Evaluation of organosolv pretreatment for the conversion of Pinusradiata D. Don to ethanol." Enzyme and Microbial technology, 43(2): 214-219.
17. Bailey, M. J. (1988). "A note on the use of dinitrosalicylic acid for determining the products of enzymatic reactions." Applied Microbiology and Biotechnology, 29(5): 494-496.
18. M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers & F. Smith (1951). "A colorimetric method for the determination of sugars." Nature, 168(4265): 167.
19. Kim, S. and M. T. Holtzapple (2006). "Effect of structural features on enzyme digestibility of corn stover." Bioresource technology, 97(4): 583-591.
20. Chandel, A. K. Kapoor, R. K. Narasu, M. L. Viswadevan, V. Kumaran, S. G. S. Rudravaram, R. Kuhad, R. C. (2007)."Economic evaluation and environmental benefits of biofuel: an Indian perspective." International Journal of Global Energy Issues, 28(4): 357-381.
21. Chen, S., Zhang, X., Singh, D., Yu, H., & Yang, X. (2010)."Biological pretreatment of lignocellulosics: potential, progress and challenges." Biofuels, 1(1): 177-199.
22. Mosier. N (2005). "Features of promising technologies for pretreatment of lignocellulosic biomass." Bioresource technology, 96(6): 673-686.
23. Chandel, A. K., Singh, O. V., Narasu, M. L., & Rao, L. V. (2011). "Bioconversion of Saccharumspontaneum (wild sugarcane) hemicellulosic hydrolysate into ethanol by mono and co-cultures of Pichia stipitis NCIM3498 and thermotolerant Saccharomyces cerevisiae-VS3." New biotechnology 28(6): 593-599.
24. Anish, R. and M. Rao (2009). "Bioethanol from lignocellulosic biomass part III hydrolysis and fermentation." Handbook of plant-based biofuels: 159-173.
25. Purwadi, R., Niklasson, C., & Taherzadeh, M. J. (2004). "Kinetic study of detoxification of dilute-acid hydrolyzates by Ca (OH) 2." Journal of biotechnology, 114(1-2): 187-198.
26. Odian, G. (2004). Principles of polymerization, John Wiley & Sons.
27. Belter, P. A., (1987). "Bioseparations: downstream processing for biotechnology".
28. Carvalho, W., Santos, J. C., Canilha, L., Almeida e Silva, J. B., Felipe, M. G. A., Mancilha, I. M., & Silva, S. S. (2004). "A study on xylitol production from sugarcane bagasse hemicellulosic hydrolysate by Ca-alginate entrapped cells in a stirred tank reactor." Process Biochemistry, 39(12): 2135-2141.
29. Raja, G. (2003). “Protein Bioseparation Using Ultrafiltration: Theory, Applications and New Developments, World Scientific.”
30. Walsh, G. (2013). “Biopharmaceuticals: biochemistry and biotechnology, John Wiley & Sons.”
31. Dorfner, K. (2011). “Ion exchangers, Walter de Gruyter.”

32. Noble, R. D. and S. A. Stern (1995). “Membrane separations technology: principles and applications, Elsevier.”
33. Strathmann, H. (1995). “Electrodialysis and related processes. Membrane science and technology.” Elsevier, 2: 213-281.
34. Krol, J., (1999). "Concentration polarization with monopolar ion exchange membranes: current–voltage curves and water dissociation." Journal of Membrane Science, 162(1-2): 145-154.
35. Oztekin, E. and S. Altin (2016). "Wastewater treatment by electrodialysis system and fouling problems." Turkish Online Journal of Science & Technology, 6(1).
36. Ruiz, B., Sistat, P., Huguet, P., Pourcelly, G., Arayafarias, M., & Bazinet, L. (2007). "Application of relaxation periods during electrodialysis of a casein solution: impact on anion-exchange membrane fouling." Journal of Membrane Science, 287(1): 41-50.
37. Benneker, A. M., Rijnaarts, T., Lammertink, R. G. H., & Wood, J. A. (2018). "Effect of temperature gradients in (reverse) electrodialysis in the Ohmic regime." Journal of Membrane Science, 548: 421-428.
38. N Banerjee, R Bhatnagar, and L. Viswanathan (1981). “Inhibition of Glycolysis by Furfural in Saccharomyces cerevisiae.” European J Appl Microbiol Biotechnol 11 : 226-228.
39. L J. Jonsson and C Martin (2016) “Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects.” Bioresource Technology 199 103–112.