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ANALYZING THE SEPARATION OF HEMICELLULOSE FROM LIGNOCELLULOSICBIOMASS AND ITS CONVERSION INTO BUTYL BUTYRIC ACID AND BUTYRATE

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ABSTRACT
Lignocellulose, also known as lignocellulosic biomass, is the dry matter of plants and consists of two carbohydrate polymers, cellulose and hemicellulose, along with a polymer rich in aromatic compounds called lignin. Any biomass containing these components is classified as lignocellulosic. Hemicellulose, while the second most abundant carbohydrate biopolymer after cellulose, has significant potential for various applications. One approach to utilizing hemicellulose involves extracting and modifying it from lignocellulosic biomass, withthe aim of producing valuable chemicals like butyl butyric acid and butyrate. This study examines how key factors such as temperature, pH, and fermentation time affect the production of these chemicals. The process of extracting hemicellulose was explored, with grass (Cynodon dactylon) being a primary source due to its hemicellulose content of 24-28%. The NaOH-acetone method was chosen for its effectiveness in isolating hemicellulose from lignocellulosic materials.

Keywords: Lignocellulose, Biomass, Hemicellulose, Acid, Butyl Butyric Acid, Butyrate, Temperature, pH, and Fermentation Time

1. INTRODUCTION
Hemicellulose, a major component of lignocellulosic biomass, is a heteropolysaccharide that plays a crucial role in the structure and integrity of plant cell walls. Unlike cellulose, hemicellulose consists of various sugar monomers, including xylose, mannose, and glucose, which contribute toits amorphous and branched structure.Lignocellulosic biomass, abundantly available in energy crops, forestry byproducts, and agricultural residues, represents a viable and sustainable source for the continuous production of bio-based fuels and chemicals. Hemicellulose, a major component of lignocellulosic biomass, holds significant promise due to its polymeric structure, which can be broken down into valuable chemical compounds[1].

Hemicellulose, an intricate polymer found in lignocellulosic biomass, is often overlooked in traditional bio refining processes, which primarily focus on cellulose and lignin. Despite its complex structure, hemicellulose comprises a variety of sugar monomers such as xylose, mannose, and glucose, which can be hydrolyzed into fermentable sugars. The extraction and effective utilization of hemicellulose have garnered substantial scientific interest because of its potential to be converted into numerous valuable chemicals. Among these are butyl butyric acid and butyrate, which have significant industrial applications. Butyl butyric acid is noted for its fruity fragrance and is widely used in flavorings and fragrances, while butyrate serves as a key intermediate in the synthesis of various chemicals and as a biofuel. Developing effective ways for extracting and converting hemicellulose into these chemicals might improve the economic viability and sustainability of biorefineries, making better use of all components of lignocellulosic biomass and contributing to the advancement of green chemistry and renewable resources. [2].

To convert hemicellulose into butyric acid and butyrate through fermentation, specific microorganisms or enzymes are employed that can effectively break down the hemicellulose structure into these target compounds. These microorganisms, such as certain strains of Clostridium, or enzymes like hemicellulases, possess the unique ability to degrade the complex polymeric structure of hemicellulose into its

constituent sugars, which are then further metabolized into butyric acid and butyrate. However, this conversion process is fraught with challenges due to the intricate and heterogeneous nature of hemicellulose. The structural complexity of hemicellulose, characterized by its varied sugar monomers and branching patterns, requires highly

specialized and efficient enzymatic activity to achieve complete degradation. Additionally, optimizing the conditions for microbial fermentation, such as pH, temperature, and nutrient availability, is critical for maximizing the yield of butyric acid and butyrate. The presence of inhibitory compounds released during the pretreatment of lignocellulosic biomass can also complicate the fermentation process, necessitating the development of robust and resilient microbial strains or enzyme cocktails. Despite these challenges, advancements in biotechnology and bioengineering continue to enhance the efficiency and feasibility of converting hemicellulose into valuable bio-based chemicals[3].

Optimization of fermentation duration, temperature, and pH is essential for enhancing the yield and efficacy of butyl butyric acid as well as butyrate synthesis. The fermentation process's enzymatic or microbiological activity is significantly influenced by these variables. It is essential to identify the optimal conditions for these variables in order to guarantee the process's highest efficiency and economic viability [4].

This study's main goal is to look into the synthesis of important chemicals made from hemicellulose, particularly butyl butyric acid and butyrate, subsequent to analysing the lignocellulosic biomass hemicellulose extraction procedure. The objective of this research endeavour is to comprehensively comprehend and analyse the impacts of numerous pivotal factors influencing the fermentation process, including butyrate and butyl butyric acid, including temperature, pH, as well as fermentation duration [5].

LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass encompasses a wide range of organic materials, including agricultural byproducts, energy crops, forest residues, and horticultural debris. These materials are collectively recognized for their potential in the production of bioenergy. In the United States, lignocellulosic biomass is considered a particularly viable resource for bioenergy production, with a substantial 70% of its supply derived from agricultural byproducts.

Traditionally, lignocellulosic biomass was primarily utilized as a buffering agent during the composting process, aiding in the breakdown and stabilization of organic matter. However, in recent years, its role has significantly evolved. With the increasing emphasis on carbon-neutral and renewable energy sources, lignocellulosic biomass has emerged as a popular feedstock for adsorption deposition processes. This shift is driven by the need to develop sustainable energy solutions and reduce reliance on fossil fuels, making lignocellulosic biomass a critical component in the pursuit of renewable energy advancements[6].

LCB, comprising approximately 50% of global biomass, is not ingested by humans and possesses an energy level surpassing that of the world's fundamental energy requirements. Because of these qualities, It is a feasible feedstock for the synthesis of bioethanol, which contributes to meeting the world's energy needs. Moreover, one of the principal sources of renewable energy is the conversion of biomass derived from plants into bioethanol. This process converts solar energy into chemical energy by converting organic compounds into carbohydrates, which are both simple and complex in nature, with the assistance of carbon dioxide and water. Consequently, this process contributes to the reduction of pollution [7].

Lignocellulosic biomass (LB) is an abundant and sustainable botanical resource composed primarily of lignin, an aromatic polymer, and polysaccharides (cellulose and hemicelluloses)[8]. Chemically, every constituent exhibits a unique behaviour. As a complex composition consisting of three distinct components, lignocellulose is challenging to process. Resistance to degradation or even separation is referred to as recalcitrance. A mixture of heat, chemicals, enzymes, and microorganisms is necessary to surmount this reluctance to produce valuable, functional products. Covalently attached to lignin, these polymers composed of carbohydrates contain two distinct types of sugar monomers: those with six carbons and those with five chains. Overexploitation of resources, which contributes to a rise in global greenhouse gas emissions, worsens the detrimental effects of climate change on the environment. To fulfil the world's future energy needs, renewable resources must be used to provide energy that is both ecologically friendly and sustainable. Regarding the production of second- generation biofuels and other biobased compounds without compromising global food security, lignocellulosic biomass (LB) remains a subject of considerable interest to the international community in lieu of fossil carbon resources [2].

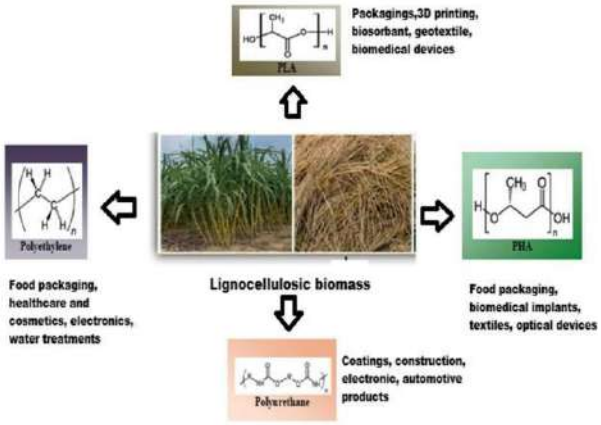


Fig.1. 1 Lignocellulose Biomass[Source: [7]]

LB presents significant potential in order to maintain global food security while acting as a replacement for fossil fuels in the production of bio-sourced chemicals, minerals, along with second-generation biofuels. The resistance of LB to enzymatic hydrolysis is a major hindrance to its valorization. which is a result of the heterogeneous, multiscale structure of plant cell walls. Thedeterminants that affect LB recalcitrance arecomplexly interconnected and challenging to disentangle. which include hemicelluloses, acetyl groups, lignin composition and concentration [2].

LIGNOCELLULOSIC BIOMASS COMPONENTS ANDSTRUCTURE

Overview of lignocellulosic biomass and its properties

Lignocellulosic biomass is a renewable and carbon-neutral resource obtained from a variety of sources, including agricultural byproducts, energy crops,forest leftovers, and grass. It consists mostly of carbohydrates (cellulose and hemicellulose) and lignin. These biopolymers are connected in intricate three-dimensional structures to varied degrees. Cellulose, the primary carbohydrate foundin lignocellulosic cell walls, creates long, aligned micro fibrils that may assemble into biggerstructures. Hemicelluloses are amorphous, branched polysaccharides that are linked to cellulose fibrils by non-covalent bonds. Lignin is acomplex, cross-linked macromolecule that is responsible for attaching the lignocellulosic matrix and strengthening cell walls. The amounts, degree of polymerization, and architectures of thesecomponents vary according to the kind of biomass. Typically, cellulose and hemicelluloses make up approximately 70% of the biomass's carbohydrate content and are firmly bonded to lignin via hydrogen and covalent interactions, which adds to the biomass's resistance to breakdown.

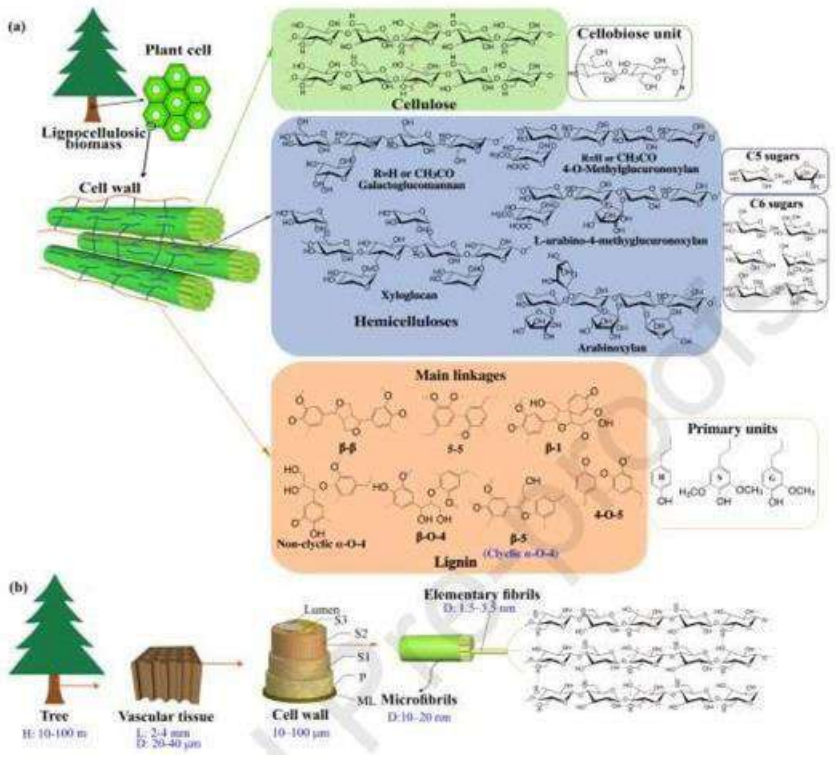


Fig. 2 presents schematic diagrams illustrating the structure of lignocellulosic biomass. In part (a), themain components of lignocellulose are shown. Part

(b) depicts the hierarchical structure of woody lignocellulose, indicating height (H), length (L), and diameter (D).

Source: [9]

The French scientist Anselme Payen initially discovered the most common naturally occurring polysaccharide, cellulose in 1838. It is a crucial structural component of plant cell walls within lignocellulosic biomass. It consists of linear chains, usually 500–1400 units long, comprising D- anhydroglucopyranose units joined by β-(1,4) glyosidic linkages. Depending on the source, the degree of polymerization varies; cellulose derived from cotton has a greater degree than cellulose derived from wood. Cereal has both crystalline and amorphous areas, and its hydroxyl groups improve both its water affinity and biodegradability. Hemicelluloses are heteropolysaccharides with shorter, more erratic chains than cellulose, making them the second most prevalent carbohydrate in lignocellulosic biomass. They are easier to break down and include different sugar units. The most prevalent aromatic polymer, lignin, aids in pathogen defence and structural support. Its composition is irregular and complicated, consisting of p-hydroxyphenyl, guaiacyl, and syringyl units connected by carbon-carbon and ether linkages. Large trunks to nanoscale cellulose fibrils make up the hierarchical structure of lignocellulosic biomass, each layer improving the mechanical qualities of the material and serving a unique purpose.[9].

Table.1. 1 Characteristics of lignocellulosic biomass components

Discriminant	Cellulose	Hemicellulose	Lignin
Composition	Three-dimensional linear molecular	Inhomogeneous with small crystalline regions	Amorphous, nonlinear

Polymers	β-Glucan	Polyxylose, Galactoglucomannan, Glucomannan	G Lignin; GS Lignin, GSH Lignin
Polymerization	10^2-10^5	Under 200	Up to 4000 [2]
Subunits	*D*-pyran glucose	L-arabinose, galactose, glucuronic acid, β-1,4-glucosidic	P-hydroksyphenylpropane, syringylpropane, guaiacylpropane
Bonds between subunits	β-1,4-glucosidic bonds	bonds – main chains; β-1,3;β-1,6-glucosidic bonds – side chains	C-C bond, ether bonds (mainly β-O-4)
Bonds between components	Without chemical bonds	Bonds with lignin	Bonds with hemicellulose [7]

Three classifications comprise lignocellulosic biomass: virgin biomass, refuse biomass, and energy crops. Plant matter is incorporated into virgin biomass. Waste biomass is a low-value byproduct that is generated by various industrial sectors, such as forestry and agriculture (e.g., sawmill and paper mill byproducts, maize stover, sugarcane bagasse, straw). Switch grass (Panicum virgatum) and elephant grass are energy crops that produce substantial quantities of lignocellulosic biomass, which is utilised as a precursor in the production of second-generation biofuel. Biofuels produced from these energy crops serve as renewable energy sources [7].

CHEMICAL COMPOSITION OF LIGNOCELLULOSIC

Biomass

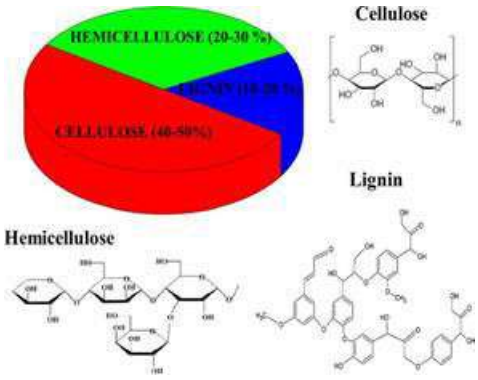


Fig.1. 2 Lignocellulosic Biomass Chemical

Composition[Source: [7]]

Lignocellulose is composed of three distinctcomponents, each possessing characteristics that impede its practical applicationLignocellulosic biomass is made up of carbohydrate polymers, such as cellulose (40–50%), hemicellulose (20–30%), and lignin (10–25%), with trace amounts of extractives, pectin, and protein [7].

Lignocellulose is predominantly composed of lignin (C9H10O2(OCH3)n), It is not a carbohydrate but a three-dimensional polymer made of phenyl propanoid molecules. Lignin is an amorphous polyphenolic compound that consists ofthree p-hydroxyphenyl groups of propanoid o- methoxylated groups—p-coumaryl, sinapyl alcohol, and coniferyl—in the form of lignin [7]. Inthe lignin polymer, the corresponding monomeric Syringyl, guaiacyl, along with p-hydroxyphenyl are the three most common phenylpropanoid units. The variability in the ratio of distinct

monomer units determines the composition of lignin, which is contingent upon the biomass source. Comparable to phenol-formaldehyde resins, lignin is a heterogeneous polymer that is abundantly cross- linked. It consists of three to four monomers, the proportion of which differs between species. Asubstantial amount of cross-linking is present. The hydrophobic nature and extreme rigidity of lignin are attributed to its aromatic composition. Lignin provides structural integrity to vegetation. Lignin isso heterogeneous and challenging to manipulate that its value is almost entirely determined as a fuel[7].

Histidine (C5H8O5)n ranks as the second most prevalent polymer. Glulоglucan, glucomannan, xylan, galactomannan, glucuronoxylan, arabinoxylan, and xyloglucan comprise this polysaccharide complex. Hemicellulose is characterised by its lack of crystalline structure, low degree of polymerisation, branching shape, and the presence of the acetyl group, which render it more amenable to thermal and chemical degradation [7]. Hemicellulose is composed of branched polysaccharides. One distinctive characteristic is that hemicellulose is covalently bonded to lignin, typically through the ferulic acid component of the lignin. This hinders the extraction of the carbohydrates necessary for the conversion of biofuels. Following cellulose, hemicellulose is the second most abundant type of carbohydrate in plants. Numerous subclasses comprise hemicellulose; these include h-glucans, galactoglucomannans, arabinoxylans, and linear mannans; glucuronoxylans, galactomannans, and glucomannans; and xyloglucans. Variations in hemicellulose content are observed among different plant taxa. Lignin and cellulose are joinedby hemicellulose, which increases the rigidity of the cellulose-hemicellulose-lignin network as a whole. Degrading hemicellulose via various pretreatments consequently led to the straightforward synthesis of cellulose units [7].

Ligandocellosic feedstock is predominantly composed of cellulose (C6H10O6)n, This is a glucose polymer that is linear and syndiotactical, joined by b-l,4-glycosidic bonds. Biocompatible, hydrophobic, and stereoregular, it is the most prevalent polymer on Earth and possesses other essential qualities as well. The crystalline and stable properties of its distinct polymer chains are evident. One primary hydroxyl unit and two secondary hydroxyl units comprise each cellulose glucosylic ring. A glucose homopolymer constitutes cellulose. Cellulolytic enzymes are utilised to extract glucose using chemical and biological processes, as its solubility in most solvents is relatively low. It is easier to extract the lignin-hemicellulose component due to the incorporation of the cellulose strands without establishing a covalent bond with them. Consequently, a multitude of hydroxyl-based chemical reactions are possible for cellulose. Significantly affecting the structure and behaviour of cellulose, these hydroxyl groups are capable of forming hydrogen bonds with other molecules. [7].

SEPARATION AND EXTRACTION OF HEMICELLULOSE

Cell walls contain hemicellulose, a type of polysaccharide that forms strong bonds with cellulose micro fibrils through the action of hydrogen bonds and the Van der Waals force. Alcohol fermentation and sorbitol reduction areprocesses facilitated by hemicellulose. These processes are indispensable in the production of food, toothpaste, cosmetics, explosives, and paper.Hemicellulose pentose finds application in the production of fluoric acid, xylose, xylitol, and feedyeast [10].

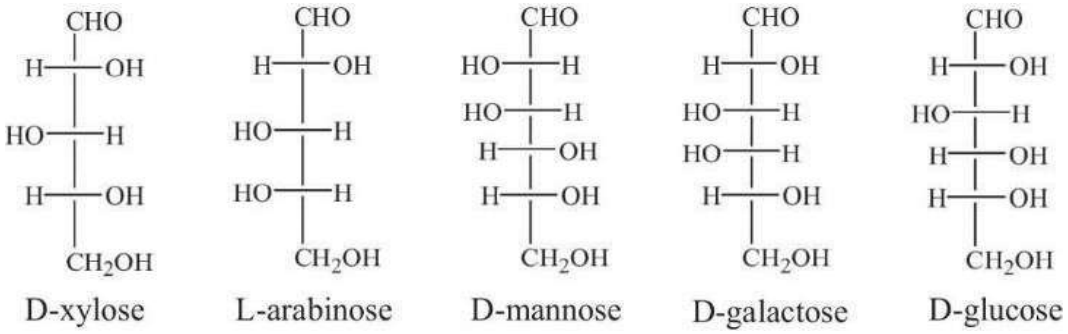


Fig.1. 3 Hemicellulose and its severalcommon sugar-based structures.
[Source: Yuchan Lu, 2021]

Moreover, xylooligosaccharides, its distinct physical, chemical, along with physiological features have led to their extensive use in the functional food along with pharmaceutical sectors as byproducts of hemicellulose breakdown. [6].

Approximately 1,800 billion tones in stockpile, On Earth, lignocellulos biomass is the most plentiful renewable resource. Given its 64 billion tons of petroleum equivalent, it is seen as a possible fossil fuel replacement. A conversion procedure is necessary to use lignocellulose biomass as a source of energy. Many technologies have been developed, such as pretreatment and bio refinery, toboost the efficiency of using biomass[2].

Hemicellulose is extracted from lignocellulose biomass using a variety of methods, including solvent extraction, steam explosion, acidic along with alkaline pretreatments, ultrasonic assisted, and microwave assisted. Separating the components of the cell wall from one another is achieved by rupturing the hydrogen bonds along with chemical bonds that link hemicellulose to lignin and cellulose. Typically, hemicellulose breaks down completely or partially during the pretreatment procedures. Furthermore, it is very hard to remove all of the hemicellulose without also causing the other ingredients to dissolve[10].

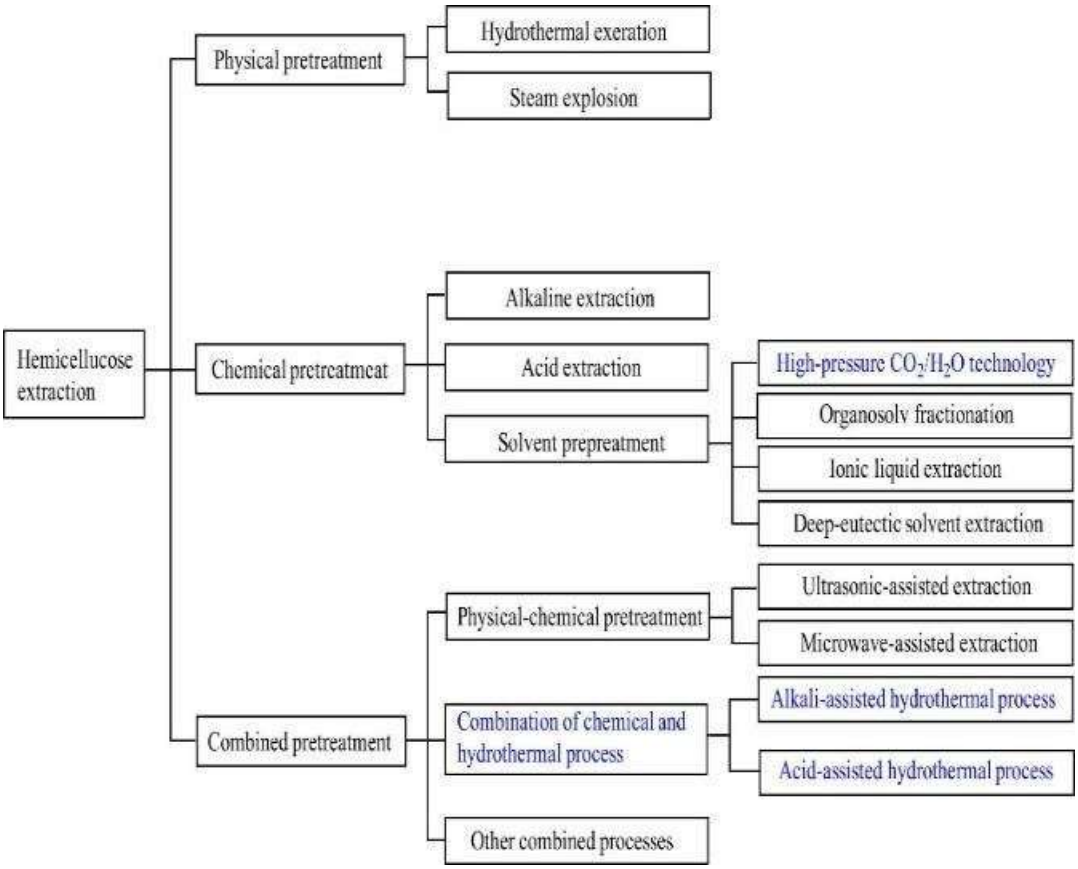


Fig.1. 4 The extraction process ofhemicellulose

[Source: Qiao He, 2021]

Chemical Pre-treatment:

Alkaline extraction

A prevalent technique utilised to extract hemicellulose from lignocellulosic biomass is alkaline extraction. The chemical bonds between lignin and hemicellulose are severed, as are the hydrogen bonds between hemicellulose

and cellulose molecules. Consequently, hemicellulose hydrolysis and cellulose hypertrophy are facilitated. Both enhance the dissolution of hemicellulose, leading to the extraction of hemicellulose with a high degree of purity. The illustration illustrates the conventional approach to alkaline hemicellulose extraction from biomass, wherein the ester link connecting hemicellulose's sugar residue in the cell wall and lignin's ferulic acid is severed [2].

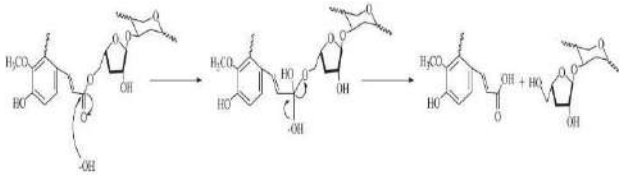


Fig.1. 5 The mechanism of alkalineextraction of hemicellulose.

[Source: Guozhi Fan, 2021]

NaOH solution is the most frequently employed inorganic alkali solutions during alkaline processes. Commercial bamboo pellets underwent a partial removal of hemicelluloses using a NaOH solution, before pulping Kraft. Without the breakdown of cellulose or lignin, around 50% of the hemicellulose was obtained. The response surface approach was used to increase the crude xylan extraction from sweet sorghum bagasse's efficiency. The optimised conditions for the crude xylan extraction process (three hours, eight6.1 degrees Celsius, one-eighth of a gramme, one-third of a gramme weight), which contained 1.7% glucose, 5.3% arabinose, and 79% xylose, were employed. By virtue of their high extraction rate, purity, and DP, hemicelluloses extracted from lignocellulosic biomass via an alkaline process are favourable for the expansion of high-value applications. Diverse industries are anticipated to adopt as-extracted hemicellulose as a consequence. As opposed to this, the conventional alkaline process generates substantial pollutants and incurs substantial expenses [11].

Acid extraction

When hemicellulose and cellulose's hydrogen bonds break down in the presence of acid, hemicellulose hydrolysis takes place. Thus, it became possible to identify the principal constituents of lignocellulosic biomass [12]. H2SO4Figure illustrates the frequent uses of HCl, HF, along with HNO3 in acid extraction.

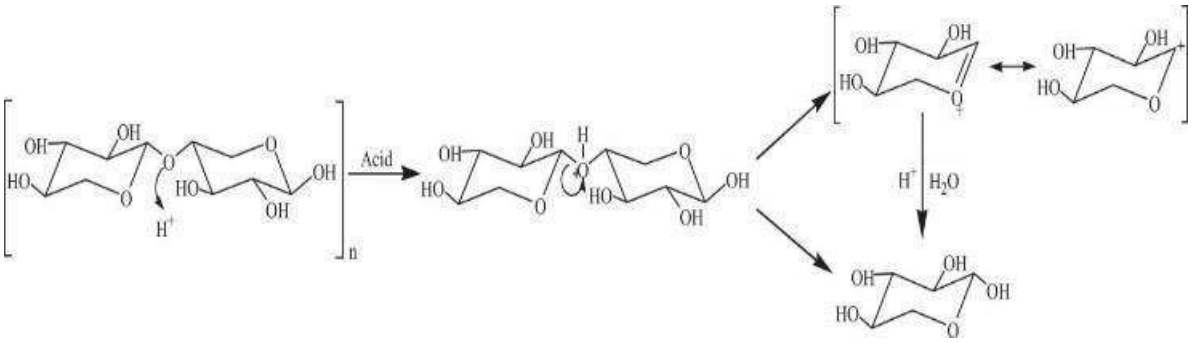


Fig.1. 6 Acid extraction of hemicellulose.[Source: Guangsen Song, 2021]

To extract hemicellulosic sugar (mainly xylose) from Miscanthus sacchariflorus Goedae-Uksae 1, a hydrothermal fractionation procedure using low acidity was developed. At 180°C for ten minutes, a 0.3 wt% H2SO4 solution produced the maximum xylose yield of 74.75%. The process of hemicellulose degradation to monosaccharides is an unavoidable outcome, and an analysis revealed that hemicellulose (primarily xylan) degraded at an86.41% rate. The efficacy of low-acidhydrothermal fractionation in transforming xylose present in the hydrolyzate into furfural without inducing additional degradation was comparable. It appeared that roughly half of the unrecovered xylose was converted to furfural. As a catalyst for the extraction of xylan, mannan, and galactan from rapeseed stalks, attenuated H2SO4 was utilised. Significant influences on the aforementioned outcomes were observed in

relation to acid concentration, reaction time, and hemicellulose removal, sugar production (xylose, glucose, and arabinose), and by-product formation (furfural, 5- hydroxymethylfurfural, and acetic acid) in the hydrolysate. Recovery of total sucrose in a 1.76 wt% H2SO4 solution at 152.6°C for 21 minutes was 85.5%. Xylan, mannan, and galactan comprised the remaining 78.9%. While acid hydrolysis does allow for the recovery of substantial quantities of sugar, the resulting hemicellulose has a relatively low yield and molecular weight due to its susceptibility to hydrolysis in acidic environments. As a consequence, the product is predominantly composed of monosaccharides as opposed to xylan. Additionally, acid pretreatment is accompanied by severe corrosion and adverse effects [11].

SYNTHESIS OF BUTYL BUTYRIC ACID AND BUTYRATE FROM HEMICELLULOSE

Specialty chemical butyric acid, which finds widespread application in the food and pharmaceutical sectors, is mostly made chemically using petroleum-based feedstock's. Concerns regarding environmental contamination resulting from chemical synthesis and the desire for bio- based goods made from renewable resources has reignited curiosity in the fermentation of butyric acid. The synthesis of butyric acid from starch along with sucrose-based substrates using Clostridium tyrobutyricum has been the focus of much study to date, including butyric acid- producing sugars such as wheat flour, cane molasses, wheat flour, Jerusalem artichoke, sweet sorghum and beetroot molasses. In fed-batch fermentation, the organism has achieved a high final titer (55.2 to 11 62.8 g/L), yield (0.38 to 0.52 g/g/g), and productivity (1.1 to 3.2 g/Lh). Commercial production of bio-based butyric acid is, nevertheless, constrained by the expenses associated with various substrates, which account for half of the total cost [1].

Butyl butyrate, Alternative name for this chemical compound is butyl butanoate; it is produced by the condensing of n-butanol as well as butyric acid It is a transparent, colourless liquid that is soluble in ethanol and diethyl ether but insoluble in water. It has a refractive index of 1.406% at 20 °C. Similar to other volatile esters, butyl butyrate emits an agreeable scent. It is employed in the flavour industry to create pineapple-flavored pleasant floral flavours. A wide range of fruits, including strawberries, bananas, pears, plums, apples, and bananas, contain it naturally [7].

Table.1. 2 Physical Properties of ButylButyric Acid & Butyrate

Properties	Butyl Butyric Acid	Butyl butyrate
Chemical formula	CH3CH2CH2CO OH	$C_8H_{16}O_2$
Molarmass	88.106 g/mol	144.214 g·mol^{-1}
Density	1.135 g/cm3	0.8692 g/cm^3 at 20 °C
Melting point	437 K	−91.5 °C (−132.7 °F; 181.7 K)
Boiling point	268 K	165 °C (329 °F; 438 K)
Solubility in water	Miscible	Insoluble[11]

Chemical Properties of Butyric Acid

Butyric acid reacts with sodium hydroxide to form butanoic acid sodium salt, which also includes water and carbon dioxide [1]

$20NaOH + 21C4H8O2 \rightarrow 20\ NaC4H6O + 4CO2 +$

$34H2O$

When the acid is handled with water, it produces ether and acetic acid. The chemical formula for thisis as follows.

$H2O + C4H8O2 \rightarrow CH3COOH + C2H6O$

Chemical Properties of Butyrate

Butyrate is a short-chain fatty acid anion that is the conjugate base of butyric acid formed via carboxy group deprotonation. It functions as an inhibitor of EC 3.5.1.98 (histone deacetylase), a metabolite, as well as a human metabolite. It is a butyric acid conjugate base [1].

Table.1. 3 Chemical Properties ofButyrate

Formula	C4H7O2
Net Charge	-1
Average Mass	87.09718
Monoisotopic Mass	87.04515

Butyl butyric acid and butyrate are produced from hemicellulose by a number of chemical reactions. Hemicellulose is a polymer found in plant cell walls that may be degraded into sugars, primarily xylose as well as arabinose [11]. These sugars may then befermented to produce a number of organic acids, including butyric acid. Here's a rundown of the method [7]:

Hemicellulose may be extracted from plant biomass such as wood or agricultural waste using hot water or dilute acid. The extracted hemicellulose contains a sugar mixture. To liberate its component sugars, the hemicellulose polymer must be degraded. This may be accomplished using acid hydrolysis, enzymatic hydrolysis, or other methods. In acid hydrolysis, sulfuric acid is widely used to break down hemicellulose into sugars. Microbes such as yeast or bacteria may ferment the resulting sugar combination, which is mostly made of xylose. These bacteria metabolise carbohydrates into butyric acid. Butyric acid is produced by microorganisms during fermentation. Butyric acid may be extracted from the fermentation broth or processed further. Butyric acid may be converted to butyl butyric acid by an esterification process. In the presence of an acid catalyst, butyric acid is reacted with an alcohol, such as butanol. The final product is butyl butyric acid. Butyrate is formed by neutralising butyric acid with a base, usually sodium or potassium hydroxide [7].

EFFECTS OF VARIOUS PARAMETER ON FERMENTATIONOF BUTYL BUTYRIC ACID AND BUTYRATE

The fermentation of butyl butyric acid as well as butyrate from hemicellulose is affected by temperature, pH, and fermentation time. All ofthese elements are critical to the efficiency and effectiveness of the fermentation process [6].

Temperature:

Temperature has a significant impact on the pace offermentation. Temperature is usually optimal for the enzymatic activity of microorganisms involvedin fermentation. Temperature ranges that aresuitable for the growth as well as

activity of the microbial strains responsible for hemicellulose conversion may be used to generate butyl butyric acid and butyrate [1]. Higher temperatures may hasten the fermentation process, but they may also denaturize the enzymes or microorganisms involved if they operate in a temperature range. Lower temperatures may cause the procedure to take longer. Finding the best temperature for fermentation is critical for enhancing output and efficiency [6].

pH:

The fermentation medium's pH has a direct impact on enzyme activity as well as microbial growth. Microorganisms involved in the conversion of hemicellulose to butyl butyric acid as well as butyrate may have pH preferences. Some microbial strains thrive in acidic settings, whereas others favour neutral pH levels. A deviation from the appropriate pH range may hinder fermentation. It may reduce enzyme activity or impact microbial growth, reducing expected product output [1].

Fermentation Time:

The amount of time that the fermentation process is permitted to continue is an important factor. The time needed to convert hemicellulose to butyl butyric acid and butyrate is governed by the microbial strains used, the beginning substrate concentration, and the given conditions (temperature, pH, and so on). Longer fermentation times do not always equal higher yields. There maybe an optimal time after which production plateaus or resources run out. Shorter fermentation times may not allow for complete conversion, lowering overall efficiency [13].

These parameters must be optimised for a successful fermentation process that produces butyl butyric acid as well as butyrate from hemicellulose. Experimentation and careful monitoring of these components will enable the optimum conditions for fermentation production and efficiency to be determined. Adjusting and managing these elements may have a significant influence on the performance of the conversion process [14].

2. RESEARCH STUDIED

Previous studies looked at a variety of approaches for extracting hemicellulose from lignocellulosic biomass, but achieving high purity and yield remains a challenge. Furthermore, for the synthesis of butyl butyric acid and butyrate from hemicellulose, it is necessary to understand the effects of temperature, pH, and fermentation duration on the conversion process.

Table.2. 1 Research Studied

Author, Year	Purpose	Parameters	Findings
Gabriel Ramos Ferreira Goncalves, et.al. 2021	Generally, tamarind seeds are discarded, producing refuse. Therefore, further research is required to determine how to be more effective with them.	Initial conditions: 6 mg.mL−1 of concentration; 24 hoursat 105 °C of temperature	The butyl butyrate esterification yields for PPL extract immobilised on ACTand FCT were 82% and66%, respectively. Residual activity wasevidenced by both biocatalysts. Following fiveiterations of utilisation, the esterification yield surpassed 86%.[15].

Zhijie Sun, et.al. 2012	Required investigation Clostridium acetobutylicum ATCC824 is produced using solvents from hemicellulosic hydrolysate concentrated at the Nano membrane level.	Concentration to 7 gl^{-1} from 0.8 gl^{-1} , Temperature: 30°C; 90°	This results in increasedresidual acids and decreased butanol concentrations. Therefore, it is imperative to enhance cellular tolerance to phenolic and butanol compounds in order to optimise the synthesis of butanol from lignocellulose hydrolysate.[5].
Nursyafiqah Elias, et.al. 2017	In order to isolate nanocellulose NC)from oil palmfrond leaves (OPFL), a technique involving acid hydrolysis, bleaching, and alkaline treatmentwill be implemented.	Temperature 70 °C time 15 h	At 4 h immobilisationtime, 76.3% butylbutyrate conversion was obtained employing 3 mg/mL CRL/CS-NCs,according to the time course reaction profile. NMR tests of pure butylbutyrate validated theester's effective synthesis [8].
T.A. Costa-Silva, et.al. 2020	Synthesis of butyl butyrate enzymatically using oil palm frond-derived magnetised nanosilica accompanied with Candida rugosa lipase.	Temperature of 50°C along with 0.5 g of enzyme concentration every six months	With 95.0% of theesterification yields,there was 15.2 g/L ofbutyl butyrate. [16].
Emmanuel Onoja, et.al. 2018	Butyl butyrate is enzymatically synthesised using Candida rugosa lipase aggregated	3 hours of incubation at 45 °C Using CRL/Gl-A-SiO2-MNPs at a molar ratio of 2:1 and 3.5	At 70 °C, the CRL/Gl- A-SiO2-MNPs exhibited superiorthermal stability incomparison to the

	on oil palm leaf-derived magnetised nanosilica.	mg/mL produced a 93.9% ester yield.	aggregated CRL, as seen by their higher D- value (152.45 minutes), half-life (45.894 minutes), Ed (125 KJ/mol), and H d (122.64 KJ/mol) and thevalues of God (11.957 KJ/mol). At room temperature, a factor of nine separated CRL/Gl-A-SiO2 MNPs with aggregated CRL[4].
Zhiping Xiao, et.al. 2018	Butyric acid is generated through the fermentation process utilising acid hydrolysate of maize husk and Clostridium tyrobutyricum. .	A hydrolysate was produced by hydrolyzing 10% solid charge of maize husk with 0.4 M H2SO4 at 110 °C for 6 hours; it contained approximately 50 g/L of total reducing sugars (glucose:xylose=1.3:1.0).	It is feasible to achievesodium butyrate concentrations exceeding 30% (w/v)via extractive fermentation [12].
Shashi Kant Bhatia, et.al. 2020	Integration of systems for the production of renewable biohydrogen from lignocellulosic biomass and other energy generation technologies	a coproduction of other energy resources, a variety of techniques for pretreating lignocellulosic biomass,modern technology tocreate and enhance biohydrogen production, and a techno-economic study of biohydrogen production from lignocellulosic biomass.	Pure culture has a high biohydrogen production, howevermost bacteria areincapable of utilising complex substrates [10].
Karolina Kucharska, et.al. 2018	In order to establish a comprehensive framework and methodology that illustrates the complete impact of a specific stage of the bioconversion process on the overall performance and efficacy of the system.	Lignocellulosic materials derived from forest management and agriculture constitute the primary sources of C-5 and C-6 sugars.	Minimising theformation or effectiveseparation of inhibitors,developing highbiofuel-producing strains through genetic engineering, and determining optimaloperating strategiesthrough the use of optimisation and modelling processes.[13].

Seong-Heon Cho, et.al. 2019	Producing Butanol bioalcohol from acidogenic products using butyric acid in two steps	Methyl butyrate was hydrogenated on these catalysts at ideal temperatures of 250 °C, 5 MPa H2, and a weight ratio of 11.2 for the catalyst to feed, which led to a 54.1% selectivity for 1-butanol.	Using this method, the MSP for 1-butanolproduced from butyricacid was $3.388% pergallon of petrol equivalent (GGE)[14].
Fengxue Xin, et.al. 2017	This inquiry pertains to the characterization of a recently discovered	butanol is produced through the fermentation of monosaccharides,	The simultaneous saccharification and fermentation at a
Clostridium species thatis both solventogenicand xylanolytic. This species is capable of generating a high ratio of butanol to ethanol byconverting acetone into isopropanol and eliminating ethanol.		including glucose, via the fermentative acetone isopropanol butanol pathway.	temperature changeproduced 2.06 g/L of butanol and 0.54 g/L of isopropanol, indicatingthat hemicellulosedirectly facilitated the largest butanolsynthesis [3].

3. MATERIALS AND METHODS

During a 4-month period, a methodological framework for isolating hemicellulose from lignocellulosic biomass was developed utilising Grass (Cynodon dactylon) and the NaOH-acetone approach:

1. *Collection and Preparation of Grass(Cynodon dactylon):*

Collect fresh Grass (Cynodon dactylon) from a consistent and well-defined location to maintain sample homogeneity. To prevent enzymatic deterioration, remove pollutants and dirt before drying the grass at an acceptable temperature.

2. *Chemicals and Reagents:*

Sodium hydroxide (NaOH), Acetone, Distilled water, Analytical grade ethanol, Hydrochloric acid(HCl) & Safety equipment (gloves, goggles, lab coat).

3. *Isolation of Hemicellulose:Preparation of NaOH Solution:*

Dissolve the correct amount of NaOH pellets in distilled water to create a 4-6% (w/v) NaOH solution. Stir the solution until all of the NaOH hasdissolved.

Extraction Process:

Weigh the dried Grass sample and add it to the NaOH solution in a specified ratio to ensure effective hemicellulose extraction (e.g., a biomass to NaOH solution ratio of 1:10 is usual). Incubate the mixture for an optimised duration of time under specific conditions (temperature and time) in order for optimal hemicellulose extraction. Consider using a heating mantle or a water bath set to a specific temperature (determined by preparatorytesting).

Filtration and Washing:

Filter the mixture using appropriate filter paper or membrane filters after the extraction time to separate the solid residue (lignin and cellulose)from the hemicellulose solution. To eliminate any remaining alkali or contaminants, wash the recovered hemicellulose with pure water.

Precipitation and Recovery:

To precipitate the hemicellulose, gradually add acetone to the hemicellulose solution while stirring. Allow time for the precipitate to settle. Filter the hemicellulose precipitate and wash it with ethanol to remove excess acetone.

Drying and Storage:

To eliminate remaining solvents, dry the isolated hemicellulose under controlled conditions. Dry the hemicellulose and store it in closed containers for further analysis and use.

4. *Duration:*

The full isolation process, from grass to hemicellulose extraction, is expected to take four months. This time span allows for careful condition optimisation and the collection of many samples at various phases for analysis.

5. *Monitoring and Analysis:*

Throughout the process, monitor and record the experimental parameters such as temperature, concentrations, and extraction efficiency on a regular basis.

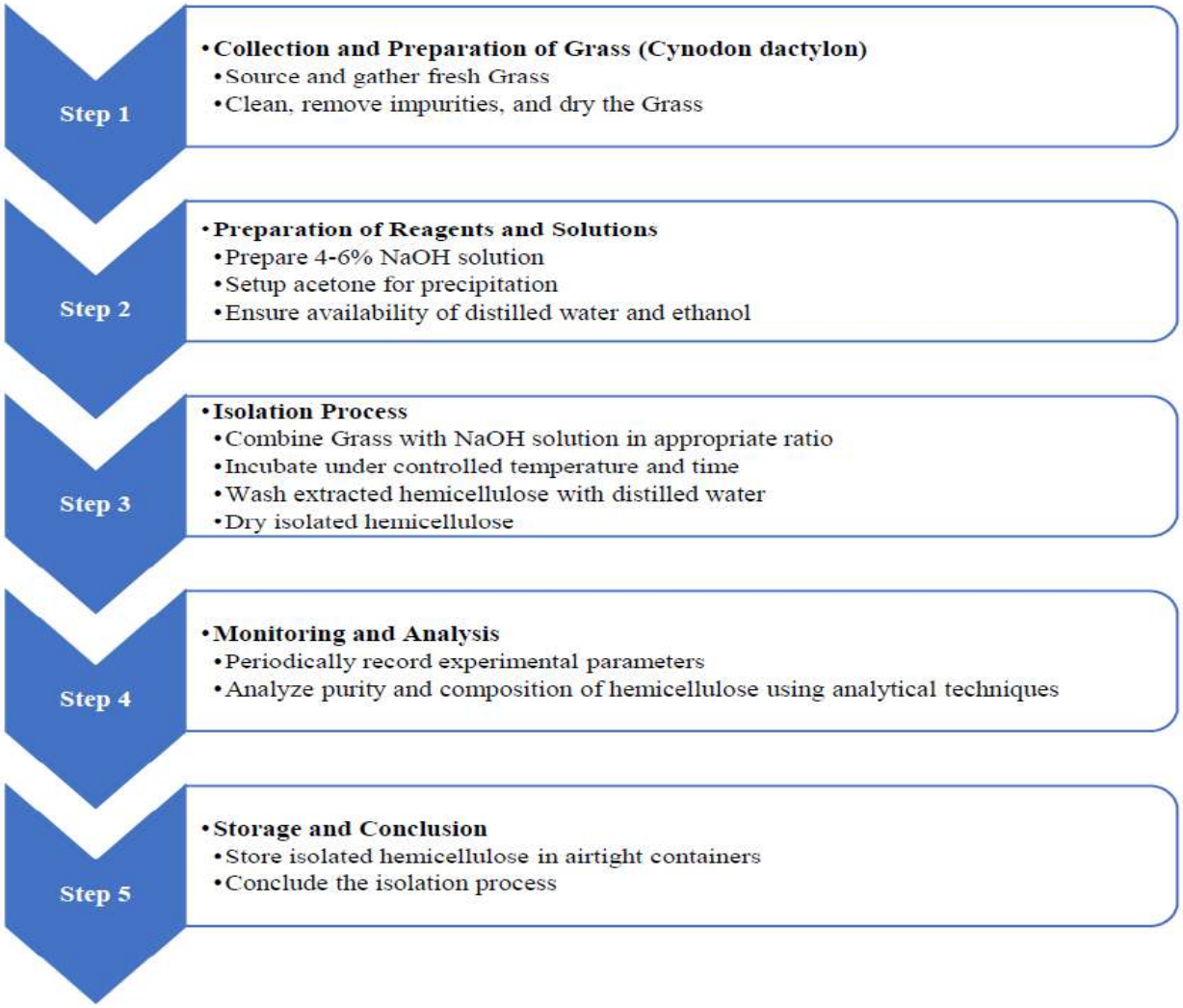


Fig.3. 1 Flowchart: Isolation of Hemicellulose from Grass using NaOH-Acetone Method

4. RESULTS

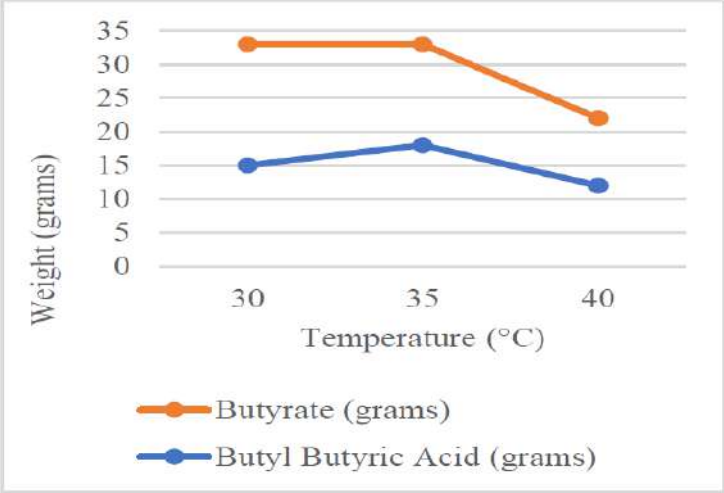
Hemicellulose extraction from lignocellulosic biomass, using Grass (Cynodon dactylon) with a hemicellulose concentration of 24-28% and theNaOH-acetone technique for isolation.

Temperature Influence on Butyl Butyric Acid andButyrate Production:

Table shows how various temperatures (30°C, 35°C, and 40°C) affect the generation of butyl butyric acid and butyrate throughout the fermentation process.

Table.4. 1 Temperature Influence on ButylButyric Acid and Butyrate Production

Temperature (°C)	Butyl Butyric Acid (grams)	Butyrate (grams)
30	15	18
35	18	15
40	12	10



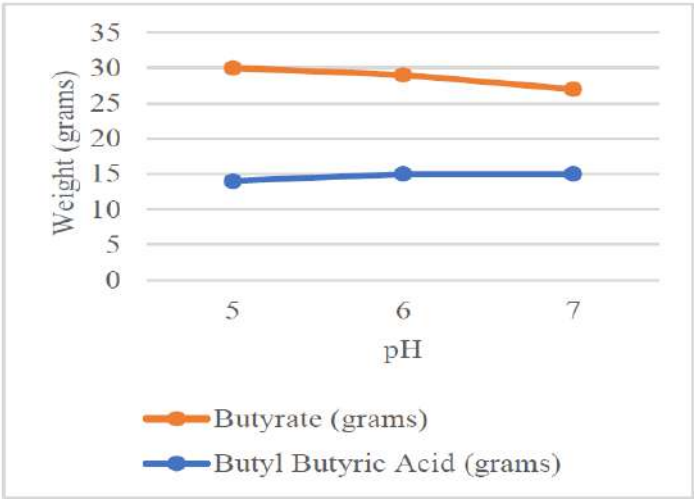
Graph.4. 1 Temperature Impact on Butyl Butyric Acid and Butyrate Production

Butyl butyric acid produced 15 grams at 30°C, whereas butyrate generated 18 grams. When the temperature was raised to 35°C, the amount ofbutyl butyric acid produced rose to 18 grams, but the amount of butyrate reduced to 15 grams. Butyl butyric acid and butyrate, on the other hand, dropped at 40°C, giving 12 and 10 grams, respectively. The results show that temperature affects the generation of butyl butyric acid and butyrate throughout the fermentation process. Based on this finding, the ideal temperature for maximising the synthesis of these chemicals varies,possibly favouring a temperature around 35°C for better yields.

pH Impact on Butyl Butyric Acid and Butyrate Yield

Table.4. 2 pH Impact on Butyl Butyric Acidand Butyrate Yield

pH Level	Butyl Butyric Acid (grams)	Butyrate (grams)
5	14	16
6	15	14
7	15	12



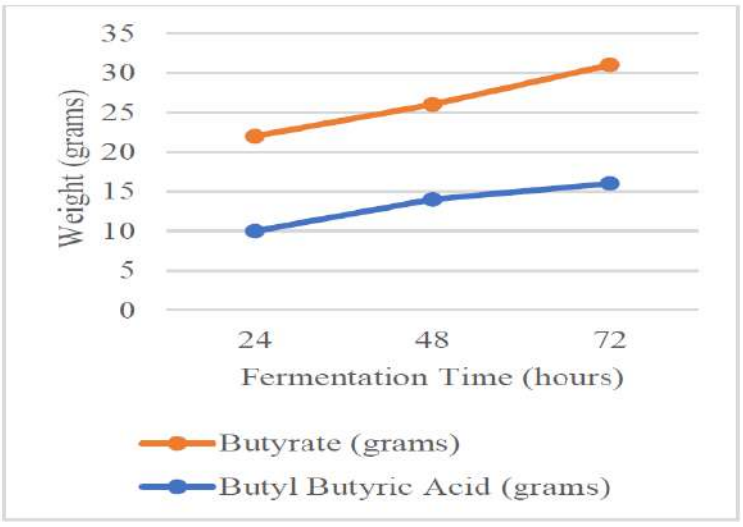
Graph.4. 2 pH Impact on Butyl Butyric Acid and Butyrate Yield

Butyl butyric acid produced 14 grams and butyrate produced 16 grams at pH 5. When the pH increased to 6, the generation of butyl butyric acid increased slightly to 15 grams, whereas butyrate reduced to 14 grams. However, at pH 7, butyl butyric acid synthesis remained stable at 15 grams, whereas butyrate production decreased dramatically to 12 grams. The results show that varying pH levels affect the generation of butyl butyric acid and butyrate during a fermentation process. It demonstrates the difference in compound yields dependent on the pH levels used.

Fermentation Time and Butyl Butyric Acid/Butyrate Production

Table.4. 3 Fermentation Time and ButylButyric Acid/Butyrate Production

Fermentation Time (hours)	Butyl Butyric Acid (grams)	Butyrate(grams)
24	10	12
48	14	12
72	16	15



Graph.4. 3 Fermentation Time-Butyl Butyric Acid/Butyrate Production

After 24 hours of fermentation, the amount of butyl butyric acid obtained was 10 grams, and the amount of butyrate produced was 12 grams. Butyl butyric acid production rose to 14 grams after 48 hours of fermentation, whereas butyrate remained at 12 grams. Butyl butyric acid and butyrate production reached to 16 and 15 grams, respectively, after 72 hours of fermentation. The data shows a relationship between fermentation time and butyl butyric acid and butyrate generation.

5. CONCLUSION

The research focused on two essential aspects: the extraction of hemicellulose from lignocellulosic biomass and next synthesis of useful compounds, especially butyl butyric acid and butyrate, utilising hemicellulose as a source. The research examined Grass (Cynodon dactylon) as a possible source of hemicellulose for the production of important materials like butyl butyric acid and butyrate. The grass, which has a high hemicellulose content ranging from 24-28%, provided itself as a feasible raw material for this study. The study includes using the NaOH-acetone technique to effectively extract hemicellulose from grass biomass. The isolation process made it simpler to separate hemicellulose from the grass's lignocellulosic structure, allowing for further downstream processing and utilisation. The isolated hemicellulose was converted, resulting in the formation of butyl butyric acid and butyrate. Specific microorganisms or enzymatic systems were used in a fermentation process to transform the hemicellulose into these desired chemical compounds. The study aimed to improve the production and efficiency of butyl butyric acid and butyrate by optimising the conversion process.

Butyrate generated 18 grams and butyl butyric acid produced 15 grams at 30°C. At 35°C, butyl butyric acid synthesis rose to 18 grams, whereas butyrate production reduced to 15 grams. Butyl butyric acid and butyrate, on the other hand, decreased at 40°C, giving 12 and 10 grams, respectively. During fermentation, temperature has an effect on the generation of butyl butyric acid and butyrate. According to this research, the ideal temperature for synthesis of these chemicals may be 35°C for higher yields. At pH 5, butyl butyric acid produced 14 grams and butyrate produced 16 grams. Butyl butyric acid increased to 15 grams when the pH approached 6, whereas butyrate declined to 14 grams. Butyl butyric acid synthesis remained constant at 15 grams at pH 7, but butyrate production dropped to 12 grams. The results show that the pH of the fermentation impacts the generation of butyl butyric acid and butyrate. It demonstrates how pH impacts compound yields. After 24 hours, 10 grams of butyl butyric acid and 12 grams of butyrate were produced. After 48 hours of fermentation, butyl butyric acid production reached 14 grams, whereas butyrate remained at 12. After 72 hours of fermentation, butyl butyric acid and butyrate production reached 16 and 15 grams, respectively. Butyl butyric acid and butyrate production are affected by fermentation time.

The experimental analysis showed a parameter that had significant effects on the generation of butyl butyric acid and butyrate. Temperature, pH, and fermentation duration were found to be essential parameters influencing the production of these chemicals. Temperature had a major impact on the generation of butyl butyric acid and butyrate within particular ranges, indicating an ideal range for increased yield. Similarly, the pH levels and fermentation length were important in affecting the amount of synthesised chemicals. The results show that fermentation temperature, pH, and duration greatly affect butyl butyric acid and butyrate production. Synthesis occurs best at 35°C. The findings highlight the need of regulating these factors to improve chemical output.

Further research will concentrate on improving process efficiency and scalability. Developing techniques to simplify the hemicellulose separation process and subsequent production of butyl butyric acid and butyrate is part of this. Efforts will be made to develop more efficient and cost-effective techniques of industrial-scale manufacturing, providing a more sustainable and economically viable process.

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