



## Conversion of Cellulosic Biomass to Bioethanol through Fermentation Using Native Microorganisms: A Review

\***ATIKU, YM; ABDULSALAM, S; MOHAMMED, J; AHMED, SI**

*Department of Chemical Engineering, Faculty of Engineering and Engineering Technology, Abubakar Tafawa Balewa University Bauchi, PMB 0248 Bauchi, Bauchi State-Nigeria.*

*\*Corresponding Author Email: [18519uaym@gmail.com](mailto:18519uaym@gmail.com)*

*Co-authors emails: [sabdulsalam@atbu.edu.ng](mailto:sabdulsalam@atbu.edu.ng); [jmohammed@atbu.edu.ng](mailto:jmohammed@atbu.edu.ng); [siahmed@atbu.edu.ng](mailto:siahmed@atbu.edu.ng)*

**ABSTRACT:** In order to produce bioethanol from cellulosic biomass, a pretreatment process is used to reduce the sample size, break down the hemicelluloses to sugars, and open up the structure of the cellulose component. The cellulose portion is hydrolyzed by acids or enzymes into glucose sugar that is fermented to bioethanol. However, this paper is a review on the conversion of cellulosic biomass to bioethanol through fermentation using native microorganisms. Information used were mainly from secondary sources; data obtained reveal that a lot of work needs to be carried out to identify sustainable native microorganism (s) and more bio-friendly processes to achieve more microbial productivity and improvement of bioethanol yield. These can go a long way in ensuring a safe, clean, economical and sustainable energy resource.

**DOI:** <https://dx.doi.org/10.4314/jasem.v27i8.7>

**Open Access Policy:** All articles published by **JASEM** are open-access articles under **PKP** powered by **AJOL**. The articles are made immediately available worldwide after publication. No special permission is required to reuse all or part of the article published by **JASEM**, including plates, figures and tables.

**Copyright Policy:** © 2023 by the Authors. This article is an open-access article distributed under the terms and conditions of the **Creative Commons Attribution 4.0 International (CC-BY- 4.0)** license. Any part of the article may be reused without permission provided that the original article is cited.

**Cite this paper as:** ATIKU, Y. M; ABDULSALAM, S; MOHAMMED, J; AHMED, S. I (2023). Conversion of Cellulosic Biomass to Bioethanol through Fermentation Using Native Microorganisms: A Review. *J. Appl. Sci. Environ. Manage.* 27 (8) 161-164

**Dates:** Received: 10 July 2023; Revised: 25 July 2023; Accepted: 14 August 2023 Published: 30 August 2023

**KEYWORDS:** Cellulosic biomass, Bioethanol, Fermentation, Microbial; Saccharification

The world's conventional energy sources might not be able to meet up with the rising energy demands (Lee *et al.*, 2019; Pothiraj *et al.*, 2015); as a result, biofuels like bioethanol have emerged as possible substitutes for the fossil fuels currently used in the transportation industry. Alvira *et al.* (2010) noted that ethanol has a wide range of uses in the chemical, pharmaceutical, and food sectors as a fuel, solvent, and raw material. It was discovered that the process economics is a key concern for the manufacture of bioethanol. Current research efforts have focused on developing commercially viable processes that can sustainably produce large amounts of bioethanol. Global energy demand has consistently expanded over the past few decades as a result of population growth and industrialization; at the moment, about 80% of this energy comes from non-renewable fossil fuel resources. (Kumar and Singh, 2016). Katoka *et al.* (2017) claimed that due to the excellent fuel qualities

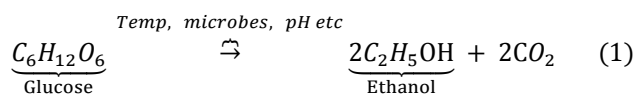
demonstrated by ethanol and its major benefits on the minimization of carbon emissions when used as fuel, ethanol production is a crucial global industrial product. The various generation feedstocks were first, second, third, and fourth generation feedstocks from which biofuels such as bioethanol are produced. The majority of first-generation biofuels come from food crops such sugarcane, wheat, barley, corn, potatoes, soybeans, sunflowers, and coconuts. The majority of second-generation biofuels are made from lignocellulosic resources like wood, straw, and agricultural waste (Naik *et al.*, 2010; Sems *et al.*, 2007). According to Lee *et al.* (2019) Since the depletion of fossil fuels, global warming, and the depletion of natural resources are currently major global concerns, it has been reported that bioethanol produced from lignocellulosic biomass continues to draw attention on a global scale as a substitute to fossil fuel. Due to their strong potential to produce

*\*Corresponding Author Email: [18519uaym@gmail.com](mailto:18519uaym@gmail.com)*

significant amounts of lipids suitable for the production of biodiesel, another biomass, algae, is introduced as the feedstock for third generation biofuels. The fourth generation of biofuels involves the application of photocatalytic reaction. (Lee *et al.*, 2019). Elliston *et al.* (2015) reported that second-generation bioethanol production involves a number of consecutive stages each with a multitude of combinations; broadly broken down into pretreatment, hydrolysis, fermentation and distillation and/or separation, the overall process for any given substrate could potentially have thousands of different permutations. In order to ascertain the most effective, economical or rapid way to produce a final product, screening various process parameters may be required. However, more research is needed to take this process beyond obtaining fermentable sugar yields, to include the effect of yeast cultures, or indeed other microorganisms, also of particular interest is the potential effect of fermentation inhibitors such as metal ions, H<sub>2</sub>S and NH<sub>3</sub> released from the biomass during processing on final alcohol yields, which may be process or substrate-specific (Elliston *et al.* 2015). The cost-effectiveness of bioethanol production through hydrolysis of starchy substrates by using enzymatic and microbial processes has been proven to be commercially viable (Kim and Dale, 2002). Many countries are interested in developing biomass as a fuel source after the energy crisis of the 1970s. However, the high greenhouse gaseous emission, deadly air pollution, unstable fossil-based energy prices and strong growth of global transportation fuel demand have boosted extensive research efforts in developing bioenergy. Bioenergy can be said to be energy derived from any fuel that originated from biomass (Lee *et al.*, 2019). Ancient techniques have been further updated and enhanced to increase production as fermentation techniques have grown in prominence over time due to their advantages in the economy and environment. Additionally, as a result of this rapid development, two major fermentation

techniques have emerged: solid state fermentation and submerged fermentation; solid state fermentation is used by many industries, including pharmaceuticals, food, textile, and others, to produce metabolites of microorganism using solid support rather than a liquid medium whereas submerged fermentation mostly utilizes free flowing liquid substrates during bioethanol production such as molasses, broths (Subramaniyan and Vimala. 2012). The overall efficiency of processes designed to convert lignocellulosic biomass to bioethanol depends on the composition of such material. Lignocelluloses mainly consist of cellulose, hemicellulose, and lignin which are bonded together by covalent bonding, various intermolecular bridges, and van der Waals' forces forming a complex structure, making it resistant to enzymatic hydrolysis and insoluble in water (Ayeeni *et al.*, 2013). Lignocelluloses continue to be investigated as a source of fermentable sugars for bioethanol production due to their availability. Biomass is the major primary energy source contributing up to 78 % of Nigeria primary energy supply (Foyle *et al.*, 2007; Edirin and Nosa, 2012). Lignocellulosic biomass includes all plants and plant derived materials, including agricultural crops and trees, wood and wood residues, municipal residues and other agriculture residue materials (Ayeeni *et al.*, 2013). Application of microorganisms in their individual form in the saccharification process usually results in partial conversion of the cellulosic material to fermentable sugar which results in low yield of bioethanol. Therefore, this paper focus more on the biochemical conversion pathway for the production of bioethanol from the cellulosic biomass.

*Fermentation:* Fermentation is a process for converting both complex substrates and simple molecules using microbes like fungus and bacteria. Equation (1) illustrates how complicated substrates are biochemically transformed into simple products.



The reaction in Equation (1) takes place in the absence of oxygen and is called fermentation (Reece *et al.*, 2014). Fermentation works best when the yeast and glucose solution is kept warm and during fermentation enzymes become ineffective if the temperature exceeded 65°C. Any extreme of temperature during fermentation, either high or low, produces minimal concentrations of ethanol. This is partly because yeast does not grow well in temperatures much lower than 20°C or much higher than 40°C (Meenakshi and

Kumaresan, 2014). The hydrolysis process, however, performs best at temperatures of about 47°C; if the temperature drops too low, the enzymes will not the digest material (Meenakshi and Kumaresan, 2014). In addition, during this metabolic breakdown, several additional compounds for example acetic acid beside the usual products of fermentation, such as carbon dioxide and alcohol are released. These additional compounds are called secondary metabolites that range from several antibiotics to peptides, enzymes

and growth factors (Machado *et al.*, 2004), they are also called 'bioactive compounds' because they possess biological activity. Recently, researchers have demonstrated that many of these secondary metabolites are industrially and economically relevant. They have been used in various industries such as pharmaceuticals and food (Daverey and Pakshirajan, 2009). The emergence of these industries has brought about the amplification of techniques used in the laboratory on a large scale; this has presented a number of problems, since the creation of a controlled environment for microorganisms need to be carried out with utmost adherence to parameters and processes. Adverse conditions may result in the production of unwanted compounds instead of the bioactive compound of interest; therefore, the development of techniques such as solid-state

fermentation and submerged fermentation has led to industrial-level production of bioactive compounds such as bioethanol (Subramaniyam and Vimala, 2012). The presence of the ethanol produced from the glucose fermentation during simultaneous saccharification and fermentation has the possibility of inhibiting the fermentation reaction. Fermentation is used in all production of alcoholic drinks: for stronger alcohol, such as whiskey and vodka, these need to be distilled after fermentation to increase the concentration of ethanol in the fermented mixture. This is due to the fact that once the concentration reaches about 18% by volume, ethanol poisons the yeast and inhibits metabolic activity. (Renge *et al.*, 2012). Figure 1 shows the schematic diagram of fermentation of cellulosic biomass for biofuel such bioethanol production.

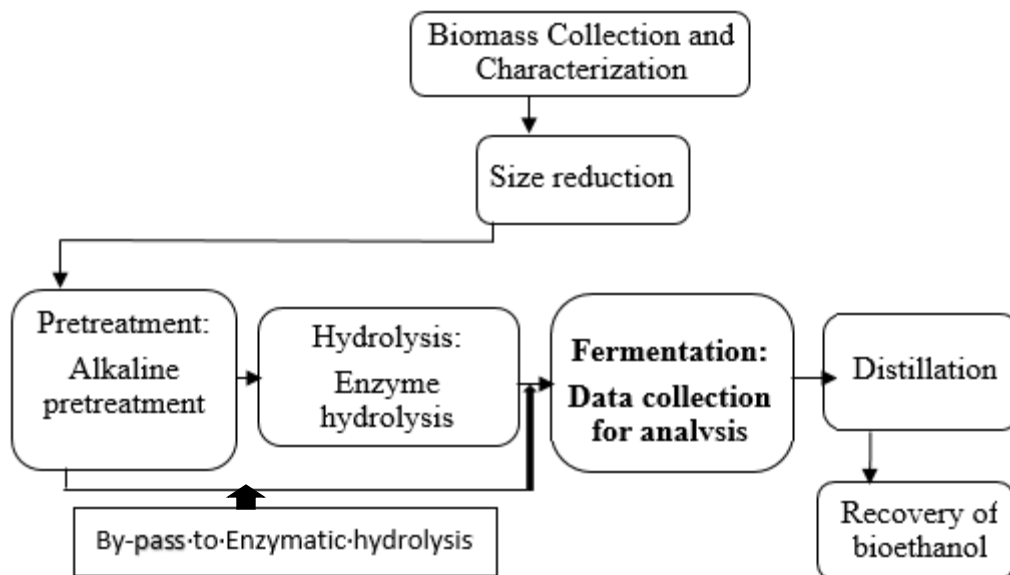


Fig 1: Schematic Diagram for the Production of Bioethanol

**Techniques of Fermentation:** Solid state fermentation and submerged fermentation are typically used for the industrial-scale manufacture of bioactive chemicals such as bioethanol hence, indicates how cellulosic biomass is converted to bioethanol through fermentation.

**Solid state fermentation:** Bran, bagasse, and paper pulp are just a few examples of the solid substrates used in solid state fermentation. The main benefit of adopting these substrates is their high nutrient content paired with their simplicity in recycling as substrates; the substrates are used very slowly and steadily in this fermentation approach, allowing for the use of the same substrate for a longer fermentation period. Consequently, this method encourages the controlled release of nutrients. (Subramaniyam and Vimala,

2012). Solid state fermentation is best suited for fermentation techniques involving fungi and other microorganisms that require less moisture content. However, the current paradigm is focused more towards solid state fermentation (SSF); this is due to the simplification of the engineering requirement (low capital and operational expenditures) and the reduced potential for microbial contamination prior to the addition of yeast (Elliston *et al.*, 2015). In the case of second-generation biofuels, more realistic results could be obtained if solid, 'real world' substrates were used. However, this adds its own unique set of problems to high-throughput (HTP) screening. One such difficulty is reliably, repeatedly and rapidly dosing small quantities of solid material. However, it cannot be used in fermentation processes involving organisms that require high water activity, such as

bacteria, SSF is a fermentation method used by several industries like the pharmaceuticals, food, textile and many more to produce metabolites of microorganisms using a solid support in place of liquid medium. The support used is especially grain brans and other substances alike, the main advantage of such methods is that it produces a minimum amount of waste and liquid effluent thus not very damaging to the environment (Ghosh, 2016). In addition, SSF has been used in food industry for various purposes like enzyme production, organic acid production, flavours, colours and many more (Ghosh, 2016).

*Submerge fermentation/ liquid fermentation:* Unlike SSF, submerge fermentation uses free-flowing liquid substrates like molasses and broths, through submerge fermentation bioactive chemicals get secreted into the fermentation broth. Because the substrates are used up so quickly, nutrients must be constantly supplied or added. For microorganisms like bacteria that need a high moisture content, this fermentation method works well. This method has the added benefit of making product purification simpler. The primary use of submerge fermentation is the extraction of secondary metabolites that need liquid usage (Subramaniyam and Vimala, 2012). Additionally, the simultaneous saccharification and fermentation process may be inhibited by the presence of the ethanol produced during the fermentation of the glucose. All alcoholic beverages are produced by fermentation, albeit stronger alcohols like whisky and vodka require distillation to enhance the amount of ethanol in the fermented mixture. This is due to the fact that when the concentration of ethanol rises to roughly 18% by volume, it poisons the yeast and causes it to stop functioning. (Renge *et al.*, 2012).

*Substrates for Fermentation:* It is crucial to choose the proper substrate for fermentation because the end product differs greatly depending on the substrate. Additionally, fermentation processes must be tailored specifically for each substrate to ensure an efficient conversion process. This is mainly because each substrate affects an organism's response differently. Additionally, productivity and the rates at which particular nutrients are utilized vary depending on the substrate. Kucharska *et al.* (2018) reported that residues from sawmills, the forest and paper industry, waste paper and wastes coming from agriculture, that is cereal straw, corncob and corn straw, potato haulms, parts of sugar beets, residue from sunflowers and rapeseed oil pressing are the main sources of lignocellulosic materials for bioethanol production. Some common substrates used in submerged fermentation are soluble sugars, molasses, liquid media, fruit and vegetable juices, and sewage/waste

water (Subramaniyam and Vimala, 2012). Cellulosic biomass such as maize cob which has been predicted to be the replaceable raw material for bioethanol production, is the right source of energy due to the fact that it is both renewable and available in commercial quantities across the globe (Hsu *et al.*, 2011). Conversion of cellulosic biomass to glucose and other fermentable sugars has been considered in the recent time, which is an attractive pathway for bioethanol production (Gaspar *et al.*, 2005; Sims *et al.*, 2010). Bioethanol produced from lignocellulosic biomass is already used in many countries such as Brazil, USA and Sweden (Hsu *et al.*, 2011; Sujit and Manas, 2018).

*Biomass Residues and Waste:* As opposed to biomass that is specially cultivated for energy purposes, biomass residues and waste are generated as by-products when the desired raw products are planted, processed and consumed (Speight and Singh, 2014). In addition, to be more specific, biomass residues can be divided into primary, secondary, and tertiary groups (Lee *et al.*, 2019). Primary residues mostly are generated during the plantation of target food crops and forest products in the field, such as corn stalks, stems, leaves and straw. The secondary residues are produced when the food crops are processed into final form of products. Woodchips, coffee husk, rice hulls, sugarcane bagasse and palm kernel cake are examples of agricultural and food processing wastes. Tertiary residues, on the other hand, become available after a biomass-derived product has been consumed by human and/or animals, and these residues might be present in the form of municipal solid waste (Chen *et al.*, 2015; Li *et al.* 2018) and are then converted to sewage sludge or wastewater. Among the biomass residues and waste, wood and agricultural residues (primary and secondary biomass residues), waste cooking oils (tertiary biomass residues) and microalgae biomass have showed their promising potentials (Lee *et al.*, 2019).

*Wood and agricultural residues for bioethanol production:* Waste products from sawmills and timber processing operations, such as sawdust, wood chips, and abandoned logs, can be utilised as feedstocks for biofuels. (Ragauskas *et al.*, 2006 For instance, wood waste and sawdust produced by the paper and saw industries can be used as boiler fuels and feedstocks for the manufacturing of ethanol Lee *et al.* (2019) reported that straw has accounted for 72.2% of the biomass energy resources in China, on the other hand in Nigeria solid biomass and municipal solid waste are major sources of energy and they account for about 80% of total primary energy consumed (Ben-Iwo *et al.*, 2016). The straw is referred to the residues or by-products of the harvesting food crops such as rice,

wheat, corn, beans, cotton and sugar crops (Zeng *et al.*, 2007; Pattanaik *et al.*, 2019). Corn stover such as stalks, cobs, and leaves, has been also reported to show potential to be converted into fermentable sugars for bio-butanol production (Lee *et al.*, 2019). While in tropical countries, sugarcane residues, particularly sugarcane bagasse and leaves, can be a good candidate for the economic utilization of residual substrates for the production of bioethanol and other biofuels such as biochar (Chandel *et al.*, 2012). Palm kernel press cake, a residue obtained from palm oil extraction, demonstrated its use to produce bioethanol via fermentation process (Jorgensen *et al.*, 2010).

**Enzymes Production:** The main method for producing different enzymes is fermentation; when fermented on the right substrates both fungi and bacteria produce a wide variety of enzymes that are extremely beneficial. Enzyme manufacturing involves both submerged

fermentation and solid-state fermentation, due to the need for a larger water potential, submerge fermentation is typically used to produce bacterial enzymes. However, solid state fermentation is favoured when fungi's enzymes need to be extracted because it uses little water (Subramaniyam and Vimala. 2012). Submerge fermentation is used to manufacture more than 75% of commercial enzymes, which is mostly due to the fact that it facilitates the use of genetically modified organisms more than solid state fermentation does. In solid state fermentation and submerge fermentation, the metabolism displayed by microorganisms differs, and the influx of nutrients and outflow of waste materials need to be carried out depending on these metabolic parameters. Additionally, even a small departure from the parameters will have an unpleasant outcome (Subramaniyam and Vimala. 2012). Figure 2 is the diagram showing the mechanism of enzyme action.

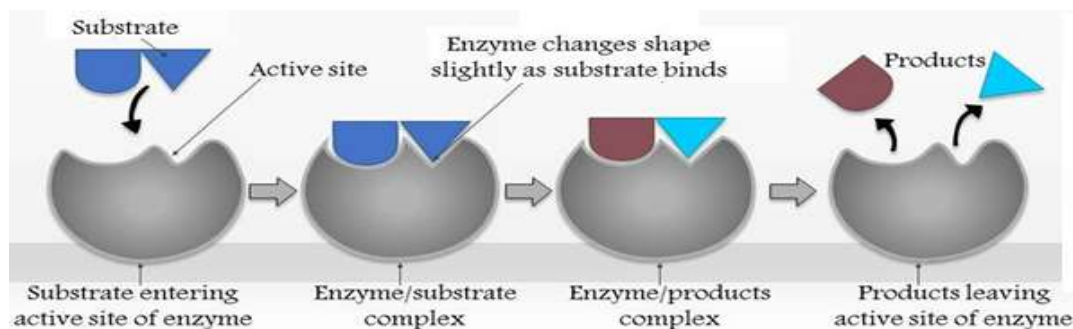


Fig 2: The Mechanism of Enzyme Action, Source: (Gaurab, 2020)

**Fungal enzymes:** The fungi of the genus *Aspergillus* have yielded numerous enzymes with commercial significance; in fact, such is the importance of this genus that it has been investigated as a model organism for the manufacture of fungal enzymes. *A. niger* is also by far the most important fungal source of enzymes. (Chen *et al.*, 2015). Species of *Actinomucor*, *Amylomyces*, *Aspergillus*, *Monascus*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizopus*, and *Ustilago* are reported for many fermented foods and alcoholic beverages (Tamang *et al.*, 2016). The metabolic differences between solid state fermentation and submerge fermentation have a direct impact on the productivity of the fungus (Nampoothiri *et al.*, 2004). Fungal enzymes such as *Pichia kudriavzevii* a yeast specie is a naturally occurring proteins that can cause certain chemical reactions to occur in plants, for instance in their structural and storage polysaccharides (Ayhan, 2019). Ghosh *et al.* (2019) reported on the isolation and partial evaluation of a potential indigenous yeast strain *Pichia kudriavzevii* from a traditional rice beer—"Gora" prepared by the Koloj Tribes of Tripura, the finding revealed that bioethanol

(alcohol) percentage in the rice beer "Gora" measured 6.40 % (v/v) after fermentation. In addition, Oberoi *et al.* (2012) reported on the ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant *Pichia kudriavzevii* HOP-1. Ethanol concentration of 24.25 g/l was achieved using *P. kudriavzevii* during simultaneous saccharification and fermentation from alkali-treated rice straw after the fermentation period. A wide range of agricultural and forestry residues, as well as energy crops, are being considered as substrates for bioethanol production (Jansen *et al.*, 2017; Khoo, 2015). These lignocellulosic feedstocks have different chemical compositions, which further depend on factors such as seasonal variation, weather and climate, crop maturity, and storage conditions (Kenney *et al.*, 2013). Despite this variability, common features of substrates composition and biomass deconstruction methods generate several generic challenges that have to be addressed in the development of yeast strains for second-generation bioethanol production (Jansen *et al.*, 2017)

**Bacterial enzymes:** Many different enzymes, including cellulase, xylanase, L-asparaginase, and amylase, have been produced by bacteria. It was once thought that submerged fermentation was the most effective way to produce enzymes from bacteria. Recent research has revealed that solid state fermentation is more effective than submerge fermentation for the generation of bacterial enzymes, with the main cause being the metabolic variations. A

variety of intermediate metabolites build up during submerge fermentation, which reduces enzyme activity and production effectiveness. (Subramaniyam and Vimala. 2012).

**Biomass Conversion Pathways:** Pathways are used in biomass conversions into useful products. Figure 3 is the diagram showing various conversion pathways for biomass.

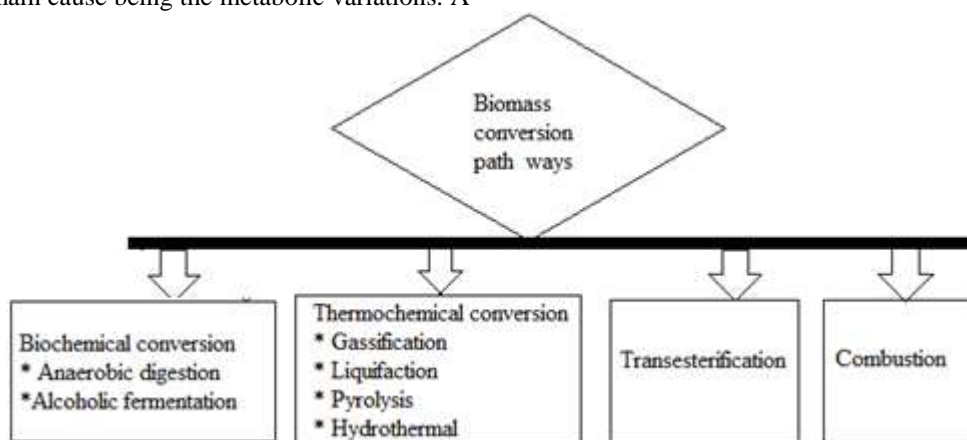


Fig 3: Block Diagram Showing Various Conversion Pathways for Biomass

**Biochemical conversion:** Biochemical conversion encompasses the utilization of the yeast and/or specialized bacteria /yeast to convert biomass or waste into useful energy. The classical process options are anaerobic digestion, alcoholic fermentation and photo-biological techniques which lead to different biofuels production (Lee *et al.*, 2019). After pretreatment the mixture is then fermented; sugars are converted to ethanol by using microorganisms such as, bacteria, yeast or fungi (Salman, 2019).

**Anaerobic digestion:** Utilizing the biomass to its full potential will enhance the economic, sustainable, and environmentally friendly aspects of microalgae bio-refineries because the biomass contains high concentrations of nutrients (such as carbohydrates, proteins, and lipids). (Sialve *et al.*, 2009). Anaerobic digestion of the biomass residue left over after the biodiesel synthesis process is one method for maximizing nutritional extraction. In anaerobic digestion, microorganisms turn used up microalgae biomass into biogas, which primarily contains CH<sub>4</sub> and CO<sub>2</sub> with minute amounts of H<sub>2</sub>S and H<sub>2</sub>O. Additionally, anaerobic digestion can handle wet biomass with a moisture content of up to 90%. The biogas has an energy content of 20–40% of the lower heating value of the biomass. (Brennan and Owende, 2010). However, there are three major phases in anaerobic digestion, namely hydrolysis, fermentation, and methanogenesis. Hydrolysis breaks down

complicated biomolecules in the biomass into simple biomolecules, and fermentation uses the simple biomolecules to alcohols, acetic acid, fatty acids that are volatile, and H<sub>2</sub> and CO<sub>2</sub> gas mixture. Methanogens metabolized this gas mixture producing biogas comprising CH<sub>4</sub> (60–70%) and CO<sub>2</sub> (30–40%) (Cantrel *et al* 2008). In the methanogenesis phase, the operating pH plays an important role in increasing the ratio of CH<sub>4</sub> in the biogas. In addition, as fermentation process proceeds, ammonia (NH<sub>3</sub>) concentration (nitrogen waste secreted by the microbial communities) increases causing pH to also increase. Increase in pH (0 to 14) during fermentation results in the dissolution of CO<sub>2</sub> in the fermentation broth, and this enhances the CH<sub>4</sub> concentration in the biogas. The high methane (CH<sub>4</sub>) content is desirable as it results in greater energy content of the biogas. Apart from pH, higher operating temperature (15 to 52°C) also encourages microbial activity and CH<sub>4</sub> production using spirulina maxima biomass improved CH<sub>4</sub> productivity and volatile solids reduction by 35% (Lee *et al.*, 2019). Furthermore, the challenge facing anaerobic digestion is the low concentration of biomass in the feed stream, a concentrating step for microalgae biomass was essential for optimum operation of the anaerobic digester. Also, when the biomass feed stream was too diluted, the microbial communities were washed out due to lack of digestible nutrients. Another issue is the recalcitrant nature of microalgae cell walls which delays the hydrolysis

process. To tackle this, cell disruption can be carried out on the microalgae biomass to break down the cell walls. This way, the nutrients inside the microalgae cells will become available for hydrolysis and subsequent uptake by the microbial communities. The greater the availability of short-chain nutrients, the higher the CH<sub>4</sub> yields in the biogas. Cell disruption methods are broadly divided into three categories, namely physical (e.g. microwave, ultrasonication, and bead milling), chemical (e.g. acid/alkali treatment), and enzymatic methods (Gunerken *et al.*, 2015). The low carbon to nitrogen (C/N) ratio of microalgae biomass (from 4.16–7.82) also presents an issue for anaerobic digestion. If the C/N ratio is less than 20, a nutrient imbalance resulted in the anaerobic microbial community and causes the release of NH<sub>3</sub> as nitrogen waste. High concentrations of NH<sub>3</sub> can inhibit the methanogens and promote the accumulation of volatile fatty acids in the digester. In addition, the low C/N ratio can be remedied by co-digesting microalgae biomass with other waste streams such as pig manure, cow manure and paper waste (Lee *et al.*, 2019).

*Alcoholic fermentation:* Alcoholic fermentation of biomass residues containing fermentable sugars, which are produced when cellulose and hemicellulose components of biomass are transformed into fermentable sugars in the presence of bacteria and fungi like yeast, can produce bioethanol. For instance, significant concentrations of starch, glycogen, and cellulose (greater than 50% dry weight) have been found in microalgae species like *Chlorella*, *Chlamydomonas*, *Scenedesmus*, *Dunaliella*, and *Spirulina*. The basic materials required for the manufacture of bioethanol are these complex polysaccharides. The polysaccharides are hydrolyzed into simple sugars since the bacteria have trouble metabolising them before being fed as a substrate into the reaction vessel. The most popular hydrolysis processes make use of enzymes, acid, and alkali. Alkaline hydrolysis is more practical since alkaline pretreatment chemicals are less caustic than acidic reagents like sulfuric acid and sulfite, whereas acid treatment is quick and inexpensive but the acidic environment may transform the sugars into undesired forms. Enzymatic therapy, in contrast, is effective and does not produce unwanted byproducts, although enzymes are more expensive and longer to use. For the shared goal of improving enzyme digestibility of lignocellulosic biomass, cell disruption techniques can be used prior to hydrolysis to boost the efficacy and shorten the length of hydrolysis by alkaline pretreatment. (Gunerken *et al.*, 2015). The crude alcohol (10–15% ethanol) formed must undergo a concentration step using distillation and the remaining solid residue can still be processed into valuable

products using liquefaction, gasification, or microwave-assisted pyrolysis. Genetic engineering of microalgae strains has been researched to improve yields of valuable metabolites (Lee *et al.*, 2019). Furthermore, important issue regarding alcoholic fermentation is the ability to grow under strictly anaerobic condition(s). For example, many yeasts that ferment sugars (including pentoses) to ethanol, only do so when limited amounts of oxygen are provided, while over aeration resulted in an increased respiration and suboptimal ethanol yields (Antonius *et al.*, 2006). Conversion of cellulosic biomass through thermochemical technology as opposed to biochemical conversion involves high-temperature (500-1400°C), atmospheric pressure 33 bar and with low/absent of oxygen chemical reformation process. The aforementioned thermal conversion process requires bond breaking and reforming of organic matter such as lignocellulosic biomass into biochar (solid), synthesis gas and highly oxygenated bio-oil (liquid). Within thermochemical conversion, there are three main process alternatives available which are gasification, pyrolysis, and liquefaction. The selection of conversion type can be affected by the nature and quantity of biomass feedstock, the preferred type of energy, for example; end use conditions, environmental principles, financial circumstances and project precise aspects (Goyal *et al.*, 2008; Ahmad *et al.*, 2016 and Sansaniwal *et al.*, 2017). The biochemical conversion unlike the thermochemical conversion is cost effective, low energy utilization during conversion and environmentally friendly.

*Biomass Collection for Conversion Process:* Transporting biomass to the production sites is necessary for its biochemical conversion into fuels like bioethanol. Biomass can either be farmed or collected from a variety of sources. After harvest, biomass is often processed into bales, pellets, and briquettes, all of which require size reduction of the biomass. The mechanical preprocessing phase of size reduction is crucial for improving the bulk density and flow properties of the particles for transportation. In order to create pellets or briquettes with a higher density, biomass is typically pulverised to particles between 3 and 8 mm in size. Particle size, particle size distribution, shape, surface area, density, and the energy efficiency of the mill utilised are crucial factors in determining how effective size reduction (Miao *et al.* 2011). Storage of biomass is necessary to maintain an uninterrupted supply for the continued manufacture of biofuels due to the lack of a constant supply of biomass feedstocks. Although studies reveal that terpenes are released from wood as a result of exposure to direct heat from sunshine, research show that outdoor storage of wood chunks is a regularly

used strategy. (Rupar and Sanati, 2005). Figure 4 is the block diagram of the biomass management functions.

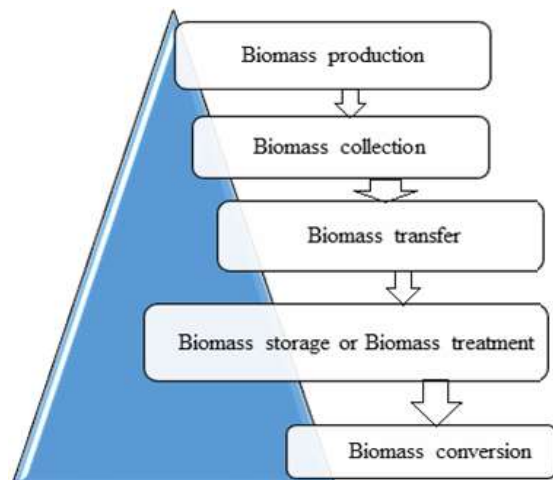


Fig 4: Block Diagram of the Biomass Management Functions

However, large silos and specially constructed facilities are used for biomass storage to protect feedstock from the effects of weather, rodents, and microbial growth. Microbial growth during storage causes loss of substrate and also has the potential to result in self-ignition due to exothermic reactions. Therefore, it is required to maintain dry conditions to allow little microbial activity in the biomass during storage. Field drying post-harvest is a common method for drying in sunny regions (Miao *et al.* 2011).

**Biomass Pretreatment for Conversion:** In the biochemical conversion yields of biofuels, pretreatment is crucial. Pretreatment converts complex biomass structures into oligomeric building blocks. Hydrolysis and fermentation further decompose these oligomers into monomeric molecules. By destroying and solubilizing the hemicellulose and lignin structures in biomass, pretreatment improves product yields. The degree of polymerization, moisture content, accessible surface area, lignin content, and the crystallinity of the cellulose are important factors influencing the conversion of lignocellulose. (Chang and Holtzapple, 2000). By (1) eliminating hemicellulose, increasing mean pore size, and allowing enzymes and hydrolysis to enter more easily, pretreatment aims to disturb the lignocellulosic structure; and (2) removing or redistributing lignin to lessen its "shielding" effect. (Alvira *et al.* 2010; Tsegaye *et al.*, 2019). In addition, pretreatment processes will ideally achieve the following:

- i. High yields for multiple crops, sites ages, and harvesting times
- ii. Highly digestible pretreated solid
- iii. Minimum amount of toxic compounds

- iv. Operation in reasonable size and moderate cost reactors
- v. Nonproduction of solid-waste residues
- vi. Effective at low moisture content
- vii. Obtains high sugar concentration (from hydrolysis)
- viii. Fermentation compatibility (minimal production of inhibitors)
- ix. Lignin recovery
- x. Minimum heat and power requirements

*Major pretreatment classes of agricultural residues:*

Pretreatment can be divided into four main categories: mechanical, chemical, physicochemical, and biological. Pretreatments that are primarily related to the synthesis of bioethanol include chemical, physicochemical, and biological processes. At this stage, mechanical pretreatment is covered because it is relevant to the majority of biomass conversion process trains; mechanical milling uses grinding to reduce crystallinity and particle size. The degree of polymerization is reduced while the specific surface area increases. You can use a variety of milling technologies, including vibro energy milling, ball, hammer, roller, and colloid milling. (Alvira *et al.* 2010). Coupled with other pretreatment, milling can increase hydrolysis yield for lignocellulose by 5–25 % and reduces digestion time by 23–59% (Delgenes *et al.* 2003). There are limits to effectiveness, size reduction below #40 mesh does not improve hydrolysis yield or rate (Chang and Holtzapple, 2000); power requirements are large, which will limit economic feasibility (Hendriks and Zeeman, 2009). The various components of the aforementioned pretreatment classes can be listed below:

-Chemical pretreatment

- i. Acid pretreatment – concentrated and dilute
- ii. Alkali pretreatment – NaOH, Ca(OH)<sub>2</sub>, or ammonia

-Physicochemical pretreatment

- i. Thermal processes include liquid hot water and steam pretreatment
- ii. Steam explosion
- iii. Ammonia explosion and CO<sub>2</sub> explosion
- iv. Other physicochemical methods include the wet oxidation

-Biological pretreatment

- i. brown and white soft-rot fungi pretreatment

**Chemical Pretreatment:** Making lignocellulosic biomass susceptible to enzymatic reactions (saccharification) with reasonable processing costs is the goal of chemical pretreatment with alkaline or acids. Pretreatment can efficiently overcome both chemical and physical barriers and improve the enzymatic digestibility of biomass if the right chemical reagents/catalysts are applied. Some



frequently observed outcomes of chemical pretreatment include decrease in lignin content, increase of surface area, and decrease in crystallinity of the biomass. (Kim *et al.*, 2015).

*Alkaline pretreatment of lignocellulosic biomass:* Alkaline pretreatment techniques utilising various chemicals were investigated with the common goal of enhancing the enzyme digestibility of lignocellulosic biomass. These include, among others, ammonia, calcium hydroxide, and sodium hydroxide. Table 1 provides a summary of typical alkaline pretreatment procedures. Because alkaline pretreatment chemicals are less caustic than acidic reagents like sulfuric acid

and sulfite, they are conducted under less strenuous settings. When they were immersed in sodium hydroxide or ammonium hydroxide, some of them were discovered to be at room temperature. The requirement for expensive materials and distinctive designs to withstand corrosion and severe reaction conditions may be eliminated by such solutions. Alkaline pretreatment appears to be significantly more effective than woody ones at delignifying grass species (Kim *et al.*, 2015, Kim *et al.*, 2018; Modenbach, 2013). Table 1 shows some of the alkaline pretreatment technologies and their operating conditions.

**Table 1:** Some Alkaline Pretreatment Technologies and their Operating Conditions

Catalysts	Reaction types and conditions	Major effects
Sodium hydroxide	0.5–10.0% NaOH 60–180°C, 5–60 min; 10–30% Solid loading	50% hemicellulose dissolution, 60–80% delignification, difficulty in recovery of NaOH.
Sodium carbonate	1–30% Na <sub>2</sub> CO <sub>3</sub> 60–180 °C, 5–60 min; 10–30% Solid loading	20–40% hemicellulose dissolution, 40–60% delignification, easier recovery than NaOH.
Ammonium, ammonium hydroxide	5–30% ammonia, 30–210 °C, 5–60 min, high pressure (2–17 atm), Solid loading: 10–50% gaseous ammonia	10–50% hemicellulose solubilization, 0–80% delignification Profound swelling Lignin removal or modification.
Anhydrous-gaseous ammonia	Gaseous ammonia 25–80°C, - 72 h Solid loading: approx. 50% (moisture - 50%)	No need for washing Low liquid loading (only - 50% moisture is enough) Mild reaction condition.
Liquid-anhydrous ammonia	70–90 °C, - 5 min, high pressure (15–20 atm), anhydrous ammonia, Solid loading: 60–90%	No hemicellulose dissolution. No lignin removal. Rapid Evaporation and liquefaction of ammonia.
Lime	25–130 °C, 1 h–8 weeks 0.05–0.15 g (Ca(OH) <sub>2</sub> water)/g of biomass Solid loading: 5–20%	20–40% hemicellulose dissolution. 60–80% delignification, de-acetylation. Low energy requirement.

Source: Kim *et al.* (2015)

However, the extensively studied chemical pretreatment technique for biomass known as alkali pretreatment is based on the solubilization of lignin in the alkali solution. In addition to other alkaline reagents, sodium, potassium, calcium, and ammonium hydroxides are widely used for alkali pretreatment. Sodium hydroxide was shown to be the one of these that worked the best (Baruah *et al.*, 2018; Kim *et al.*, 2016). The intermolecular ester linkages between hemicelluloses and lignin are broken during the alkali pretreatment process via a saponification reaction. The cellulose comes into touch with the enzymes as a result of the solubilization of lignin and hemicellulose fragments in the alkali solution Sun *et al.* (2016). Alkali pretreatment also modifies the lignocellulosic structure by causing cellulose swelling, which lowers the degree of polymerization and crystallinity while increasing internal surface area (Behera *et al.* 2014). Additionally, Shen *et al.* (2017) demonstrated the efficacy of sodium hydroxide pretreatment as a way to improve the anaerobic digestion process. A greater methane production of 205.86 mL g<sup>-1</sup> VR at 3% NaOH concentration, which was 53.99% higher than the untreated VR, was discovered when the

pretreatment parameters for vinegar residue (VR) were optimized. Calcium hydroxide, often known as lime, has also been examined and proven to be a simple and efficient alkaline pretreatment method because Ca(OH)<sub>2</sub> is so cheap and secure to handle. According to a study on the lime pretreatment of maize cob residue to increase biogas generation, the pretreatment speeds up the digestion process by eliminating lignin and produces biogas at a rate that is twice that of untreated maize cobs. Furthermore, alkali pretreatment is less effective for hardwoods and more effective for biomass with low lignin content, such as herbaceous crops and agricultural leftovers (Baruah *et al.*, 2018).

*Acid pretreatment of lignocellulosic biomass:* Based on the glucosidic linkages between hemicellulose and cellulose's acid susceptibility, lignocellulosic biomass (LCBs) are subjected to acid pretreatment. Long cellulose and hemicellulose chains are broken down into sugar monomers by hydrogen ions produced by the acid catalyst (Baruah *et al.*, 2018). Acid pretreatment can be used as concentrated acids (30–70%) at low temperatures (100°C) or as dilute acids

(0.1-10%) at high temperatures (100-250°C). Additionally, both inorganic acids like sulfuric acid, phosphoric acid, nitric acid, and hydrochloric acid and organic acids like formic acid, maleic acid, and oxalic acid are used. Although the pretreatment with concentrated acids can significantly speed up the conversion of sugar (by more than 90%), most concentrated acids are highly poisonous and corrosive, necessitating considerable operational and maintenance expenditures. They also promote unintentional cellulose breakdown, which produces inhibitory chemicals like furfurals, 5-hydroxy methyl furfural, phenolic acids, and aldehydes (Baruah et al., 2018). Ion exchange resins, activated charcoal or tin oxides, calcium hydroxide over liming, and neutralisation procedures are used in chemical detoxification to either make the inhibitor chemicals inert or lower their concentration (Li et al., 2018). The biological approaches, on the other hand, rely on utilising bacteria like *Rhodococcus* sp. YHY01,

*Streptomyces coelicolor*, and many others to affect the inhibitors in a similar way (Bhatia et al., 2016, Bhatia et al. 2017).

**Table 2:** Common Gasoline Bioethanol Blends Available in Various Countries

Countries	Common vehicles	Flexible fuel vehicles (FFVs)
USA	E10	E85
Canada	E10	E85
Sweden	E5	E85
India	E10	-
Australia	E10	-
Thailand	E10	-
China	E10	-
Columbia	E10	-
Peru	E10	-
Paraguay	E7	-
Brazil	E20, E25	Any blend available

E7, E10, E20, E25; E85 = percentages of bioethanol in the blends  
Source: Balat, (2007)

**Table 3:** Review of Pertinent Literature on bioethanol Production

Author (S)	Title	Summary of Findings
Boonchuay et al. (2021)	Production of Bioethanol from Cellulose-Rich Corn cob Residue by the thermotolerant <i>Saccharomyces cerevisiae</i> TC-5	It was observed that 7.5 % (w/v) solid loading after 72 h produced 20.92 g/L of ethanol after the completion of the fermentation experiment through batch and fed-batch simultaneous saccharification and fermentation (SSF)
Efeovbokhan et al. (2019)	Production of bioethanol from hybrid of cassava pulp and peel using microbial and acid hydrolysis	The gelatinized cassava pulp from the samples hydrolysed with <i>Aspergillus niger</i> consistently produced more reducing sugar than the controls samples not hydrolysed with <i>Aspergillus niger</i> . Highest ethanol yields of 54.8% and 33.1% were obtained from heat pretreatment variety and cassava peel respectively.
Orji et al. (2016)	Bioethanol production from corn cob hydrolyzed by cellulose of <i>Aspergillus Niger</i> using <i>Zymomonas mobilis</i> and <i>saccharomyces cerevisiae</i> isolated from palm wine.	The findings also revealed that the cellulosic hydrolysate yielded 1.78 g/L sugars which produced 9.10 g/L ethanol after fermentation using <i>Zymomonas mobilis</i> in comparison to ethanol yield of 8.20 g/L using <i>Saccharomyces cerevisiae</i> .
Melekwe et al. (2016)	Bioethanol production potentials of corn cob, waste office paper and leaf of <i>Thaumatococcus daniellii</i> .	Separate hydrolysis and fermentation method were adopted for the study. Hydrolysis of the feedstock was carried out at H <sub>2</sub> SO <sub>4</sub> concentration of 6 M, 9 M and 13 M at 100°C for 60 min. Hydrolysates obtained were fermented at 30°C for 72 hours using <i>Saccharomyces cerevisiae</i> , optimal concentration of H <sub>2</sub> SO <sub>4</sub> for hydrolysis of the tested feedstock was 9M. The finding revealed a highest ethanol yields of 16.8 g/L at 48-hour fermentation period from corn cob.

**Bio-ethanol:** Among the biofuels, bio-ethanol is the most extensively studied biofuel to date and has gained good attention as sustainable biofuel. Mabee and Saddler (2009) reported that bioethanol production and application is estimated to reduce greenhouse gas emissions, improve agricultural economy, enhance rural employment, and increase national security. Bioethanol has higher octane number, broader flammability limits, higher flame speeds, and higher heats of vaporization than gasoline,

which allow for higher compression ratio, shorter burn time, and leaner burn engine. A major problem with ethanol is its water solubility and azeotropic mixture formation with water, limiting separation during distillation, consequently intensifying the cost of the separation process. Other major disadvantages include lower energy density than gasoline, low vapor pressure (making cold starts difficult), and toxicity to ecosystems (Balat, 2007). Bioethanol, on the other hand, has a 35% oxygen content, which lowers particle

and NO<sub>x</sub> emissions. As it has a reasonable antiknocking value, it improves combustion efficiency. For use in the current internal combustion engines, it can be mixed with gasoline in a variety of ratios, ranging from 5% to 85-100%, with 85% (E85, or 85% ethanol by volume in gasoline-bioethanol) mixes being utilised in flexible fuel vehicles (FFVs). Table 2 lists the numerous bioethanol petrol mixes that are used in various nations across the world. Sulphur emissions from pure bioethanol vehicles have completely vanished, and carbon monoxide emissions from gasoline-powered vehicles that include bioethanol in place of lead are hardly detectable (Goldemberg et al., 2008). The types of materials used to make bioethanol vary depending on the location and the accessibility of the feedstock. The two biggest producers of bioethanol worldwide are the USA and Brazil. In Brazil and the USA, cornflour and cane molasses serve as the substrates for the manufacture of bioethanol (Almeida et al., 2007). Other substrates include cassava, wheat, and sugar beetroot. The use of food products like maize and cassava for the production of bioethanol, however, has an impact on both the supply and the price of these staple commodities. Additionally, microbial contamination might occur during the storage of high concentration sugar substrates. The typical petrol bioethanol blends that are offered in different nations are listed in Table 2. Table 3 also contains a review of earlier relevant material on the manufacture of bioethanol. The aforementioned authors as shown in the reviewed papers (Table 3) worked on the different substrates and techniques for the production of bioethanol.

*Conclusion:* The rapid rise in demand for alternative energy sources like bioethanol has necessitated the use of fermentation techniques. With its extensive range of uses and several advantages, fermentation has established itself as a sustainable method of producing bioethanol (biofuels). However, more work needs to be done to fully utilise the potentials of native microbial isolates and agricultural residues for fermentation-based conversion to bioethanol due to variations among various biomass conversion pathways and difficulties in having native microbial strains capable of hydrolyzing the lignocellulosic biomass.

## REFERENCES

Ahmad, AA; Zawawi, NA; Kasim, FH; Inayat, A; Khasri, A (2016). Assessing the gasification performance of biomass: a review on biomass gasification process conditions, optimization and economic evaluation. *Renewal. Sustainable. Energy Rev.* 53:1333–1347.

Almeida, JRM; Modig, T; Petersson, A (2007). Increased Tolerance and Conversion of Inhibitors in

Lignocellulosic Hydrolysates by *Saccharomyces Cerevisiae*. *J. Chem. Technol. Biotech.* 4: 340–349.

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. 101, 4851-4861.

Antonius, JA; Van Maris, A; Derek, A; Abbott, A; Eleonora, BA; Joost -Van Den, B. A; Marko, KA; Marijke, AH; Luttkik, AH; Wouter-Wisselink, AW; Alexander -Scheffers, A; Johannes, P; Van Dijken, A; Jack, TP (2006). Alcoholic Fermentation of Carbon Sources in Biomass Hydrolysates by *Saccharomyces Cerevisiae*: *Current Status*, 90: 391–418

Ayeni, AO; Banerjee, S; Omoleye, JA; Hymore, FK; Giri, BS; Deskmukh, SC; Pandey, RA; Mudliar, SN (2013). Optimization of Pretreatment Conditions Using Full Factorial Design and Enzymatic Convertibility of Shea Tree Sawdust, *Biomass and Bioenergy*. 48, 130-138

Ayhan, D (2019). Bioethanol Production (Fungal Enzymes), Sila Science, University Mah, Trabzon Turkey. Doi: <https://doi.org/10.1036/1097-8542.900123>.

Baruah, J; Nath, BK; Sharma, R; Kumar, S; Deka, RC; Baruah, DC; Kalita, E (2018). Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-added Products. *Frontiers in Energy Res.* doi:10.3389/fenrg.2018.00141: 1-50

Balat, M (2007). Global Bio-Fuel Processing and Production Trends, *Energy Exploration Exploitation* 3: 195-218

Behera, S; Arora, R; Nandhagopal, N; Kumar, S (2014). Importance of Chemical Pretreatment for Bioconversion of Lignocellulosic Biomass. *Renewable Sust. Energy Rev.* Doi: 849 10.1016/J.Rser.2014.04.047: 1-25.

Ben-Iwo, J; Manovic, V; Longhurst, P (2016). Biomass Resources and Biofuels Potential for the Production of the Transportation Fuels in Nigeria, *Renewable and Sust. Energy Rev.* 63 172-192,

Bhatia, SK; Kim, J; Song, HS; Kim, HJ; Jeon, JM; Sathiyarayanan, G (2017). Microbial Biodiesel Production from Oil palm Biomass Hydrolysate Using Marine *Rhodococcus sp.YHY01*. *Biore Technol.* 233: 99-109.

Bhatia, SK; Lee, BR; Sathiyarayanan, G; Song, HS; Kim, J; Jeon, JM (2016). Medium Engineering for Enhanced Production of Undecylprodigiosin

- Antibiotic in *Streptomyces Coelicolor* Using Oil Palm Biomass Hydrolysate as a Carbon Source. *Biores. Technol.* 217: 141-149
- Brennan, L; Owende, P (2010). Biofuels from microalgae: A Review of Technologies for Production, Processing, and Extractions of Biofuels and Co-products. *Renewable Sust. Energy Rev.* 14:5 57–77
- Boonchuay, P; Techapun, C; Leksawasdi, N; Seesuriyachan, P; Hanmoungjai, P; Watanabe, M; Srisupa, S; Chaiyaso, T (2021). Bioethanol Production from Cellulose-Rich Corn cob Residue by the Thermotolerant *Saccharomyces cerevisiae* TC-5. *J. Fungi*, 7(547): 1-10.
- Cantrell, KB; Ducey, T; Ro, KS; Hunt, PG (2008). Livestock Waste-to-Bioenergy Opportunities. *Biores. Technol.* 99: 1-11
- Chang, V; Holtzapfle, M (2000). Fundamental Factors Affecting Biomass Enzymatic Reactivity. *Appl. Biochem. Biotechnol.* 1: 5–37.
- Chen, D; Yin, L; Wang, H; He, P (2015). Reprint of: Pyrolysis Technologies for Municipal Solid Waste: A Review, *Waste. Manage.* 37: 116-36
- Daverey, A; Pakshirajan, K (2009) Production of Sophrolipids by the Yeast *Candida Bombicola* Using Simple and Low-Cost Fermentative Media. *Food Res. Inter.* 42: 499-504,
- Delgenes, JP; Penaud, V; Moletta, R (2003). Pretreatments for the Enhancement of Anaerobic Digestion of Solid Wastes. *Chemic. Information* 34(13): 1-12.
- Edirin, BA; Nosa, AO (2012). A comprehensive Review of Biomass Resources and Biofuel Production Potential in Nigeria, *Res. J. Engineer. Appl. Sci.* 1(3): 149-155.
- Efeovbokhan, VE; Egwari, L; Alagbe, EE; Adeyeni, JT; Taiwo, OS (2019). Production of Bioethanol from Hybrid of Cassava Pulp and Peel Using Microbial and Acid Hydrolysis, *Biores. Communication* 14(2): 2596-2609.
- Elliston, A; Wood, LP; Soucouri, MJ; Tantale, RJ; Dicks, J; Roberts, IN; Waldron, KW (2015). Methodology for Enabling High-Throughput Simultaneous Saccharification and Fermentation Screening of Yeast Using Solid Biomass as a Substrate, *Biotechnol for Biofuels*. Doi 10.1186/s13068-014-0181-z: 1-9.
- Foyle, T; Jennings, L; Mulcahy, P (2007). Compositional Analysis of Lignocellulosic Materials: Evaluation of Methods Used for Sugar Analysis of Waste Paper and Straw, *Biores. Technol.* 98: 3026-3036
- Ga'spa' r, M; Juha'sz, T; Szengyel, Z Re'czey, K (2005). Fractionation and Utilization of Corn Fibre Carbohydrates, *Process Biochem* 40: 1183-8
- Gaurab, K (2020). Enzymes: Properties and Mechanism of Enzyme Action, Online Biology Notes, Retrieved June 18, 2021: 1-5.
- Ghosh, S; De Mandal, S; Nath, N; Kaipeng, D; Rahaman, L; Nachimuthu, SK; Sharma, BK (2019). Isolation and Partial Evaluation of a Potential Indigenous Yeast Strain *Pichia kudriavzevii* from a Traditional Rice Beer—"Gora" Prepared by the Koloï Tribes of Tripura, *Advances in Microbiolo.* 9: 824-841.
- Ghosh, JS (2016). Solid State Fermentation and Food Processing: A Short Review. *J. of Nutrition and Food Sci.* 6: 1-20.
- Goldemberg, J; Coelho, ST; Guardabassi, P (2008). The Sustainability of Ethanol Production from Sugarcane. *Energy Policy* 6: 2086–2097
- Goyal, HB; Seal, D; Saxena, RC (2008). Bio-fuels from Thermochemical Conversion of Renewal Resources: A Review, *Renewable Sust. Energy Rev.* 12(2): 504-517.
- Günerken, E; D'Hondt, E; Eppink MHM; Garcia-Gonzalez, L; Elst, K; Wijffels, RH (2015). Cell Disruption for Microalgae biorefineries. *Biotechnol Advancement*, 33:243–60
- Hendriks, ATWM; Zeeman, G (2009). Pretreatments to Enhance the Digestibility of Lignocellulosic Biomass. *Biores Techno.* 100: 10–18.
- Hsu, C; Chang, K; Lai, M; Chang, T; Chang, Y; Jang, H (2011). Pretreatment and Hydrolysis of Cellulosic Agricultural Wastes with a Cellulase-Producing *Streptomyces* for Bioethanol Production, *Biomass and Bioenergy* 35(5): 1878-1884
- Jansen1, AM; Bracher, MJ; Papapetridis, I; Maarten, D; Verhoeven, MD; Bruijn, H; DeWaal, PP; Van Maris, AJA; Klaassen, P; Jack T; Pronk, JT (2017). *Saccharomyces Cerevisiae* Strains for Second-Generation Ethanol Production: From Academic Exploration to Industrial Implementation *J. Yeast Res.* 17: fox044.
- Jorgensen, H; Sanadi, AR; Felby, C; Lange N; Fischer, M; Ernst, S (2010). Production of Ethanol and Feed by High Dry Matter Hydrolysis and Fermentation of Palm Kernel Press Cake. *Appl. Biochemical and Biotechnol.* 161: 318–32.

- Katoka, F; Tulashie, SK; Setsoafia, DD (2017). Production of Bioethanol from Liquid Waste from Cassava Dough during Gari Processing, *Taylor and Francis Group*. Doi: 10.1080/1759-727769.2017.1329491: 1-10.
- Kenney, KL; Smith, WA; Gresham, GL (2013). Understanding Biomass Feedstock Variability. *Biofuels* 4(1): 111–27.
- Khoo, HH (2015). Review of Bio-Conversion Pathways of Lignocellulose to Ethanol: Sustainability Assessment Based on Land Footprint Projections. *Renewable Sust. Energy Rev.* 46 (C):100–119.
- Kim, JS; Lee, YY; Kim, TH (2015). A Review on Alkaline Pretreatment Technology for Bioconversion of Lignocellulosic Biomass. *Biores. Technol.* 1-15
- Kim, JS; Lee, YY; Kim, TH (2016). A Review on Alkaline Pretreatment Technology for Bioconversion of Lignocellulosic Biomass. *Biores. Techno.* 199: 42-48
- Kim, S (2018). Enhancing Bioethanol Productivity Using Alkali-Pretreated Empty Palm Fruit Bunch Fiber Hydrolysate, *Hindawi Biomed Research Int.* Article I. D 5272935, 1-15
- Kim, S; Dale, BE (2002). Allocation Procedure in Ethanol Production System from Corn Grain I. System Expansion. *Inter. J. Life Cycle Assess.* 7: 237-243,
- Kucharska, K; Rybarczyk, P; Holowacz, I; Lukajtis, R; Glinka, M; Kaminski, M (2018). Pretreatment of Lignocellulosic Materials as a Substrates for Fermentation Process: Review, *Molecules*, Doi: 10.3390/molecules 231192937.
- Kumar, D; Singh, V (2016). Dry-grinded Processing Using Amylase Corn and Superior Yeast to Reduce the Exogenous Enzyme Requirement in Bioethanol Production, *Biotechnol. Biofuels*: 9-228
- Lee, SY; Sankaran, R; Chew, K KW; Tan, CH; Krishnamoorthy, R; Chu, D; Show, P (2019). Waste to Bioenergy: A Review on the Recent conversion Technologies, *BMC Energy Rev.* <https://doi.org/10.1186/s42500-019-0004-7>: 1-22
- Li, J; Shi, S; Tu, M; Via, B; Sun, FF; Adhikari, S (2018). Detoxification of Organosolv-Pretreated Pine Prehydrolysates with Anion Resin and Cysteine for Butanol Fermentation. *Appl. Biochemical and Biotechnol.* 1-19. doi: 10.1007/s12010-018-2769-4.
- Mabee, WE; Saddler, JN (2009). Bioethanol from Lignocellulosics: Status and Perspectives in Canada. *Bioresource. Technol.* 101(13):4806–4813.
- Machado, CM; Oishi, BO; Pandey, A; Soccol, CR. (2004). Kinetics of *Gibberella fujikori* Growth and Gibberellic Acid Production by Solid State Fermentation in a Packed-Bed Column Bioreactor, *Biotechnol. Progress*, 20(5): 1449-1453
- Meenakshi, A; Kumaresan, R (2014). A Review of Production of Ethanol from Corn *Int. J. of Engineering Sci. Research Technolo.* 3(1): 1-5.
- Melekwe, EI; Lateef SA; Ana, GE (2016). Bioethanol Production Potentials of Corn Cob, Waste Office Paper and Leaf of *Thaumatococcus daniellii*, *British J. of Appl. Sci. Technol.* 17(4): 1-10.
- Miao, Z; Grift, TE; Hansen, AC (2011). Energy Requirement for Comminution of Biomass in Relation to Particle Physical Properties. *Ind. Crop Prod* 33(2): 504–513
- Modenbach, A (2013). Sodium Hydroxide Pretreatment of Corn Stover and Subsequent Enzymatic Hydrolysis: An Investigation of Yields, Kinetic Modeling and Glucose Recovery, Ph.D. Dissertation at the University of Kentucky, [https://uknowledge.uky.edu/bae\\_etds/17/](https://uknowledge.uky.edu/bae_etds/17/).
- Naik, SN; Goud, VV; Rout, PK; Dalai, AK (2010). Production of First and Second Generation Biofuels: A Comprehensive Review. *Renewable Sust. Energy Review.* 14: 578–97
- Nampoothiri, KM; Tomes, GJ; Roopesh, K; Szakacs, G; Nagy, V; Soccol, CR; Pandey, A (2004). Thermostable Phytase Production by *Thermoascus aurantiacus* in Submerged Fermentation, *Appl. Biochemistry and Biotechnol.* 118: 205-214
- Oberoi, HS; Babbar, N; Sandhu, SK; Dhaliwal, SS; Kaur, U; Chadha, BS; Bhargav, VK (2012). Ethanol Production from Alkali-Treated Rice Straw via Simultaneous Saccharification and Fermentation using Newly Isolated Thermotolerant *Pichia kudriavzevii* HOP-1, *J. Indust. Microbiol. Biotechnol.* 39: 557–566
- Orji, J; Aleke, K; Chika, E; Anthonia, N; Ikechukwu, M; Stanley, E; Emmanuel, U (2016). Bioethanol Production from Corn Cob Hydrolysed by Cellulase of *Aspergillus Niger* Using *Zymomonas Mobilis* and *Saccharomyces Cerevisiae* Isolated from Palm Wine, *Int. J. Curr. Res. Biosc. Plant Biol.* 3(1): 39-45.
- Pattanaik, L; Pattnaik, F; Saxena, DK; Naik, SN (2019). Biofuels from Agricultural Wastes, Centre for Rural Development and Technology, Indian Institute of

- Technology Delhi, New Delhi, India. *Research Gate Publishers*, DOI: 10.1016/B978-0-12-815162-4.00005-7: 103-122.
- Pothiraj, C; Arun, A; Eeyeni, M (2015). Simultaneous Saccharification and Fermentation of Cassava Waste for Ethanol Production. *Biofuel Res. J.* 5:196-202
- Ragauskas, AJ; Nagy, M; Kim, DH; Eckert, CA; Hallett, JP; Liotta, CL (2006). From Wood to Fuels: Integrating Biofuels and Pulp Production. *Industrial Biotechnol.* 2: 55–65
- Reece, JB; Urry, LA; Cain, ML; Wasserman, SA; Minorsky, PV; Jackson, R (2014). *Campbell Biology (Tenth edition)*. Boston: Pearson. Renewable Resources: A Review. *Renewable Sust. Energy Rev.* 12: 504–17
- Renge, VC; Khedkar, SV; Nikitar, N (2012). Enzyme Synthesis by Fermentation Method: A Review, *Scientific Rev. and Chemical Communications* 2(4): 585-990
- Rupar, K; Sanati, M (2005). The Release of Terpenes during Storage of Biomass. *Biomass Bioenergy* 28:29–34,
- Salman, Z (2019). Waste to Energy Conversion Routes, *Bioenergy Consult*, Retrieved November 26, 2019.
- Sansaniwal, SK; Pal, K; Rosen, MA; Tyagi, SK (2017). Recent Advances in the Development of Biomass Gasification Technology: A Comprehensive Review. *Renewable Sust. Energy Rev.* 72 (C): 363–384
- Shah, TA; Tabassum, R (2017). Enhancing Biogas Production from Lime Soaked Corn Cob Residue, *Int. J. Renewable Energy Res.* 8(2): 761-766
- Shen, J; Zhao, C; Liu, G; Chen, C (2017). Enhancing the Performance on Anaerobic Digestion of Vinegar Residue by Sodium Hydroxide Pretreatment. *Waste Biomass Valorization.* 8, 1274 1119-1126
- Sialve, B; Bernet, N; Bernard, O (2009). Anaerobic Digestion of Microalgae as a Necessary Step to Make Microalgal Biodiesel Sust. *Biotechnol. Adv.* 27:409–16.
- Sims, R; Mabee, W; Saddler, JN; Taylor, M. (2010). An Overview of Second-Generation Biofuel Technologies. *Biore. Technol.* 101-1570
- Speight, JG; Singh, K (2014). Environmental Management of Energy from Biofuels and Biofeedstocks, Hoken: John Wiley and Sons, Inc; doi.org/10.1002/9781118915141
- Subramaniam, R; Vimala, R (2012). Solid State and Submerge Fermentation for the Production of Bioactive Substances: A Comparative Study, *Int. J. Sci. Nature*, 3(3):280-486
- Sujit, KM; Manas RS (2018). Bioethanol Production from Corn and Wheat: Food, Fuel, and Future, Chapter Three (3), DBT-IOC Center for Advance Bio-energy Research, Faridabad, India, <http://dx.doi.org/10.1016/B978-0-12-813766-6.00003-5>.
- Sun, S; Sun, S; Cao, X; Sun, R (2016). The Role of Pretreatment in Improving the Enzymatic Hydrolysis of Lignocellulosic Materials. *Biores. Technol.* 199, 49-58
- Tamang, JP; Shin, D; Jung, S; Chae, S (2016). Functional Properties of Microorganisms in Fermented Foods: A Review, *Frontiers in Microbiology*, doi:10.3389/fmicb.2016.00578.
- Tsegaye, B; Balomajumber, C; Roy, P (2019). Microbial Delignification and Hydrolysis of Lignocellulosic Biomass to Enhance Biofuel Production, an Overview and Future Prospect. *Bull. National Res. Center:* 1-16
- Watanabe, Y; Shimada Y; Sugihara, A (2001). Tominaga Y. Enzymatic Conversion of Waste Edible Oil to Biodiesel Fuel in a Fixed Bed Bioreactor. *J. American Oil Chemist Soc.* 78(2):703–707.
- Zeng, X; Ma, Y; Ma, L (2007). Utilization of Straw in Biomass Energy in China. *Renewable Sust. Energy Rev.* 11:976–87