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Review Paper

Ethanol production from livestock manure: A review

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Abstract

As a result of drastic increase in population and industrialization, the demand for biofuels globally, particularly bioethanol is incessantly increasing. Common crops like sugarcane, corn and cassava are not able to satisfy the worldwide requirement of ethanol production because of their key importance of food and feed for humans and animals. Thus, interest is shifting to animal manures and other agricultural wastes as major lignocellulosic biomass feedstocks for production of bioethanol. Agricultural wastes are abundant, renewable and cost effective. Ethanol produced from agricultural wastes, particularly animal manures might be a likely technology however the process has a number of challenges such as conveyance and handling of biomass, and effectual pre-treatment techniques for complete delignification of lignocellulosics. Proper methods of pre-treatment can increase the quantities of fermentable sugars after enzymatic hydrolysis, thus improving the whole process efficiency. Availability of lignocellulosics as alternative feedstock, and improvement of technology has resulted to the emergence of several bio-conversion methods like separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bio-processing (CBP). In order to convert glucose and any other sugar to ethanol, it needs those fermentation technologies mentioned earlier to make the whole process cost effective. Those bio-conversion technologies are direct fermentation methods where biomass feed stocks are pre-treated, hydrolysed and fermented to ethanol. This review paper explains those available technologies for ethanol production from livestock manure and other major agricultural materials which include their benefits, limitations and possible effects on the environment.

Keywords: Ethanol, animal manure, lignocellulosic, biomass, fermentation, hydrolysis.

Introduction

Ethanol exists as a pure, colourless, inflammable, oxygenated hydrocarbon and it has a chemical formula of C_2H_5OH. Ethanol may well be applied as a fuel for transportation in a minimum offour ways: anhydrous ethanol (100% ethanol), hydrous ethanol (95% ethanol and 5% water), anhydrous ethanol-gasoline blends (10 - 20% ethanol in gasoline), and as raw material for ethyl *t*-butyl ether (ETBE)[1]. One of the advantages of bioethanol over conventional fuels is the derivation from renewable which include agricultural crops (cereals, sugar beet, maize, etc.) and animal wastes. The adoption of bioethanol as a fuel for road transport will reduce green gas emission which account for about 22% of total greenhouse gas emissions. The fuel crops used in bioethanol production will absorb the CO_2emittedduring combustion for plant growth[1].

Ethanol, in case of fuel leakage, is let alone likely to catch fire and explodes. The application of bioethanol in older engines could help in the reduction of carbon monoxide (CO) amount generated by the vehicle, thereby enhancing the air quality[2]. The making and application of biofuels has great potential in reducing greenhouse gas emissions, specifically with the development of bioethanol from agricultural crops and animal wastes[3]. The heavy dependence on oil producing countries will be reduced by blending bioethanol with gasoline. Bioethanol is biodegradable and far less toxic than fossil fuels. The latent heat of vaporization of ethanol (855MJ/kg) is higher than that of petrol (293kJ/kg). Ethanol has higher octane number (99) than petrol (80-100), preventing pre-ignition when ethanol is used. Ethanol burns more completely during combustion process, thereby reducing hydrocarbon emission drastically which is usually lower than petrol[1].

Manures from animal have been effectively utilized as fertilizers for centuries, and poultry manure especially has since been recognised as possibly the best suitable of these natural fertilizers due to its high amount of nitrogen. Manures can also produce other vital plant nutrients and function as a soil amendment with the addition of organic matter. The persistence of organic matter varies with various environmental factors such as temperature, drainage, rainfall, etc. The presence of organic matter in soil also develops the retention of moisture and nutrient. Livestock manures comprise lignocelluloses, polysaccharides, proteins and different organic materials. The conversion of these agricultural waste materials into value-added products has been acknowledged as a good-looking alternate waste management solution[1].

Manure from livestock remains a readily available waste biomass source and it comprises a range of nutrient elements such as Nitrogen (N), Phosphorus (P) and Potassium (K), which a few crops could directly absorb[1]. Also, integrating the decomposed plant and animal materials into the soil from manure can significantly decreases oil erosion risk and improve water retention capacity. Therefore, livestock manure is commonly employed directly as an improvement for soil, and occasions for developing energy from the manure are frequently overlooked[4].

Increasing environmental worries combined with higher prices of energy have in recent times resulted into a better concentration in the application of manure to yield viable bio energy that are economical. Manure could be burned directly to generate heat, changed to bio-oil, or produced into flammable syngas[5]. Manure could be alternatively digested an aerobically to methane, which has corresponding heating value to ethanol[6]. Though, the residual cellulose in manure is insufficiently utilized normally by anaerobic digestion. Manure that is digested an aerobically actually has more good structural properties (less hemicellulose and related or more cellulose) compared to other lignocellulosic biomass, such as switchgrass[7].

Technically, ethanol might be produced biologically from manure by glucose fermentation resulting from enzymatic hydrolysis of cellulose. The use of animal manure for generation of energy is turning into a prominent way of alternative disposal, though generating energy from livestock manure has generally been in the form of biogas production. This paper therefore aims to present a review of the available technologies for ethanol production from three livestock manures (cow, pig, poultry) and other major agricultural materials.

Feedstocks for ethanol production

The available feedstocks (or materials) used for producing ethanol can largely be categorized as i. sugar-based feedstock, ii. starch-based feedstock, and iii. lignocellulosic-based feedstock. However, ethanol is presently made mostly from traditional crops like corn, sugar cane, wheat, cassava and sorghum. These crops are used in countries like USA, Brazil, France, England, India, Germany, Thailand, Spain, Nigeria, etc. The feedstock depends on the locality and the prevailing agricultural product[8]. Most recent ethanol production methods employ biomass feedstocks that are more freely degradable, e.g. cereals as in corn or any grain, sugar cane juice, etc.

Nevertheless, use of eatable crops solely for making biofuel struggle with producing feed and food. The ethanol made from sugar-based (sugarcane) and starch-based (corn) feedstocks are categorized as the first generation ethanol, ethanol produced from lignocellulosic feedstock (rice straw, corn stover and other biomass materials) are the second generation ethanol and the third generation ethanol are ethanol from algae biomass.

Sugar-based feedstock

The key sugar-based feedstocks for production of ethanol comprise sugarcane, sugar beet and sweet sorghum with feedstock yields of 62-74, 54-111 and 50-62 tons/ harespectively[9,10]. They are commonly utilized in France, India, Brazil and Germany[11]. They also consist mostly of glucose, fructose, and sucrose as their major components[12]. The extraction of these fermentable sugars is through milling followed by ethanol fermentation. Ethanol is then separated eventually from the stream of products through distillation followed by dehydration. Sugarcane molasses/black strap made from processing of sugarcane, aqueous juice released from sweet sorghum stalks and sugar beets were used as raw materials in the production of ethanol. The proximate composition of sugar-based and starch-based feed stocks for production of ethanol is presented in Table-1[13,14]. Sugarcane molasses contains sucrose of about 31% and inverted sugar of 15%[15]. Hence, concentration of sugar in sugarcane molasses need to be diluted to about 14%-18% in advance of fermentation to enable the optimal development of fermenting micro-organism. The extracted sugar beet juice composes 16.5% of sucrose while for sweet sorghum, stalks constitutes major stock of sugar which are pressed mechanically to regain about 12% - 22% of sugar juice concentration that are fermented directly by yeast (*Saccharomyces cerevisiae*)[13,16].

Starch-based feedstock

The starch-based feedstocks commonly used in the production of ethanol such as corn, wheat and cassava, are the most engaged in Europe, North America including tropical countries. Starch, as a glucose polymer, can be split into smaller molecules of glucose through the activity of enzymes like α-amylase and gluco-amylase[11]. Starch is indirectly fermented by yeast. After grinding the grains and removing starch, starch is hydrolysed into glucose with the use of α-amylase and glucoamylase[17]. At that point, glucose is fermented to ethanol. The approximate configuration of starch-based feedstock is presented in Table-1. Milling, liquefaction and saccharification by enzymes are the three main methods involved largely in the bio-conversion of starch-based feedstock to acquire fermentable sugars. Wet and dry milling processes are employed in the commercial conversion of Corn grain into ethanol. Wet milling method involves soaking of Corn grain inside water to separate grain into starch, fibre and germ containing distinct processing of each fractionated constituent. Dry milling method thereby includes processing the entire grain while remaining constituents are divided at the process completion.

Amid the countries of the world that produce ethanol from sugar-based and starch-based feedstocks, USA produces about 40,000,000,000 litres of ethanol from corn and wheat while Brazil about 25,000,000,000 litres from sugar cane. China also produces about 3000,000,000 litres from corn/cassava/rice, while Canada about 2,000,000,000 litres from corn/wheat, India

about 1,000,000,000 litres from sugarcane/molasses and France about 1,000,000,000 litres from wheat, sugarcane and sugar beet, while Germany about 750,000,000 litres from wheat, sugarcane and sugar beet and Australia about 500,000,000 litres from sugar cane. Apart from the United States and Brazil being the two major producing countries of the world, these other countries mentioned later are the rest nations producing substantial bioethanol. It is essential for the recovery of transitional products and to incorporate the fermentation of pulp/bagasse using the procedure, in order to sustain the production of ethanol from sugar-based and starch-based feedstocks and to develop process energy economics.

First generation ethanol produced from food crops have a number of limitations knowing fully well its direct influence on food production in relation to cost and quality of food and soil used for the growth of crop while giving only inadequate reduction benefits of greenhouse gas emission [18]. Presently there is considerable attention on promoting the concept of a cellulosic bioethanol (Second generation) which employs lignocellulosic biomass. Second generation ethanol made from lignocellulosic biomass, non-food crops, wastes from industries and municipal areas led to more reductions of greenhouse gas and in any way does not contend with food crops for agricultural land.

Lignocellulosic-based feedstock

Lignocellulosic biomass signifies a favourable raw material for production of bioethanol that is renewable naturally. Lignocellulosic biomass can be described as the decomposable part of products, wastes and remnants from biological source of agriculture (containing animal and vegetable components), forestry and related industry. Lignocellulosic feedstocks involves cellulosic biomass, for example, energy crops (such as: switch grass, miscanthus) and agricultural and wood residues (such as: straw, grasses, woodchips/sawdust, corn stovers, sugarcane bagaseses, agricultural wastes, paper, etc.)[19]. Apart from being a source of energy, biomass remains as well as a likely raw material for makingchemicals[20].Such as biomass symbolises a source of renewable energy, it could be possibly used with no the depletion of reserves. Nevertheless, the structures of lignocellulosic biomass constitute challenges for conversion technologies. An operative technology for conversion needed to be developed to allow the lignocellulosic biomass processing which has an appropriate intricate and resilient structure and permit the effective utilization of all biomass parts. The relative fractions of the diverse lignocellulosic biomass parts vary significantly subject to the source. Developing the technical and economic paths for producing bio-based compounds has now become very essential to encourage competition of bio-industry in the market.

Lignocellulosic biomass contains carbohydrate polymers (as in cellulose and hemicellulose), lignin and a minor residual portion of extractable acid, salts and minerals. Cellulose has a crystalline structure as same polymer of glucose subunits (cellobiose) while hemicellulose an amorphous structure as a different polymer of pentose sugars, and lignin is very crystalline and inflexible part of biomass. Cellulose and hemicellulose naturally contain two-third part of the dry mass and differs by the biomass feedstock type. The composition of cellulose, hemicellulose and lignin of diverse renewable feedstocks are presented in Table-2[21,22]. The three biomass components could be transformed to a number of value added products via diverse paths. There are various recent reports on the highest level of development in biofuel and production of biochemical and different feedstock used for this developing bioindustry[23].

Table-1: Approximate composition of sugar-based and starch-based feedstocks[11].

Cassava (% w)	Corn Grain (% w)	Sugar beet Juice (% w)	Sugarcane Molasses (% w)	Sweet Sorghum (% w)	Wheat Grain (% w)
Starch (77.0 – 94.0)	Starch (72.0)	Water (65.6)	Water (18.9)	Cellulose (8.7)	Starch (53.0 – 57.0)
Fibre (1.5 – 3.7)	Fibre (9.5)	Solids (17.3)	Sucrose (31.8)	Hemicellulose (6.3)	Fibre (9.9 – 11.8)
Sugars 0.0	Sugars (2.6)	Sucrose (16.5)	Invert Sugar (15.4)	Lignin (0.6)	Sugars (0.0)
Protein (1.7 – 3.8)	Protein (9.5)	Sugars (0.2)	Ash (13.8)	Sucrose (67.4)	Protein (12.5 – 15.1)
Oil (0.2–1.4)	Oil (4.3)	Impurities (0.3)	Others (20.1)	Glucose (3.7)	Oil (2.1–2.6)
Minerals/Ash (1.8 -2.5)	Minerals/Ash (1.4)			Ash (0.2)	Minerals/Ash (0.0)
Water (59.0–70.0)	Water (0.0)			Others (13.1)	Water (12.0)
Others (0.0)	Others (0.7)				Others (4.9-5.8)

Table-2: Composition of lignocellulosic biomass feedstocks[11].

Lignocellulosic biomass	Percentage(%) of total dry weight		
	Cellulose	Hemicellulose	Lignin
Wheat straw	33-40	20-25	15-20
Switch grass	30-50	10-40	5-20
Sugarcane bagasse	25-45	28-32	15-25
Softwood stems	45-50	25-35	25-35
Rice straw	29-35	23-26	17-19
Nut shells	25-30	25-30	30-40
Hardwood stems	40-50	24-40	18-25
Grasses	25-40	35-50	10-30
Corn stover	35-40	21-25	11-19
Corn cobs	45	35	15
Bamboo	49-50	18-20	23

Lignocellulosic biomass is an encouraging substrate for production of ethanol but could be challenging. The hydrolysis of lignocellulosic biomass led to the development of hexose sugar (from cellulose) and pentose sugar (from hemicellulose). Ethanol is produced mainly by the glucose fermentation discharged from cellulosic feedstock by microorganisms, mostly yeasts especially *Saccharomyces cerevisiae*[24]. *Saccharomyces cerevisiae* has been the mostly employed corporate microbe, which can produce ethanol at high concentration as 18% in the fermentation broth[25]. S. *cerevisiae* remains a moderately easy micro-organism to be handled since it's usually accepted as safe. Z. mobilis as a bacterium that is non-stained violet by Gram's method could likewise be employed in glucose fermentation to ethanol[26]. In the course of fermentation with S. *cerevisiae* and Z. *mobilis,* the biomass formed are acknowledged as safe for feed which makes those organisms appropriate for metabolic engineering to be applied in simultaneous fermentation of pentose and hexose sugars. Current reports recommended that under semi-aerobic conditions, some white rot fungi such as Iprex lacteus, Agaricus bisporus and Bjerkandera adusta are capable of producing ethanol from glucose[27]. Kluyveromyces marxianus is also used for fermentation of ethanol from empty palm fruit bunches[28]. Research are on-going in this area to discover effectual fermentative microbes for use in the concurrent fermentation of pentose and hexose sugars. *Saccharomyces cerevisiae* can easily ferment hexose sugars however may be unable to apply pentose sugars during its metabolism to generate ethanol. Hence, the simultaneous fermentation of pentose and hexose sugars is anticipated to enhance the yields of ethanol from lignocellulosics which could be feasible with the application of engineered and recombination of yeast strains in ethanol fermentation[29].

Production processes

Different feedstocks, especially lignocellulosic materials, are processed for production of ethanol via four major operations: (i) Pre-treatment process (which includes solid/liquid separation, alkaline treatment, drying and grinding) to liberate cellulose and hemicellulose; (ii) Acid or Enzymatic hydrolysis of cellulose and hemicellulose to produce fermentable sugars (glucose), (iii) Fermentation of reducing sugars, and (iv) Distillation of fermented solution to produce ethanol.

Pre-treatment

Pre-treatment process remains the first stage of ethanol production which encompasses delignification of lignocellulosic feedstocks for release of carbohydrate polymers (such as cellulose and hemicellulose) from lignin[4]. The aim of pre-treatment method is for alteration or removal of structural obstructions in lignocelluloses, in a sequence to enhance the degree of continual process of enzymatic hydrolysis and so increase the yields of fermentable sugars from cellulose and hemicelluloses[30]. Pre-treatment is a most-costly phase in the production of cellulosic ethanol, which accounts for about 33% of the entire processing costs (excluding feedstock) based on the design base-case of National Renewable Energy Laboratory (NREL)[31]. Physical pre-treatment is costly, requires large energy amounts, and employs only mechanical means to decrease the particle size of feedstock, hence increasing the surface area.

During the pre-treatment process, there is a breaking down of lignocellulose matrix which releases its three key components (cellulose, hemicellulose and lignin). Hemicellulose is partly hydrolysed into pentoses depending on the method of pre-treatment[32]. Simultaneously, through the pre-treatment, cellulose crystallinity degree can be reduced and lignocellulose structure porosity will increase to make the lignocellulosic feedstocks more vulnerable to enzyme hydrolysis[33]. A pre-treatment step is performed to release the percentage of cellulose from firmly knitted lignocelluloses structure. There are various categories of pre-treatment methods, which include: physical (mechanical commutation), chemical (acid or alkaline treatment), biological, and a combination of other methods (such as thermal treatment and microwave-assisted-alkaline treatment)[34]. Table-3 presents the advantages and disadvantages of different pre-treatment methods of lignocellulosic materials[35].

Table-3: Advantages and disadvantages of pre-treatment methods of lignocellulosic materials.

Pre-treatment methods	Processes	Advantages	Disadvantages
Physical pre-treatments	Milling and Grinding	Decrystallizationis intensive. Increased accessible surface area and porosity.	Energy Intensive.
Physicochemical and chemical pre-treatments	Ammonia Fibre Explosion (AFEX)	Increases available surface area. Removes lignin and hemicellulose to a level.	Not effective for biomass with high lignin content. Does not considerably solubilize hemicellulose compared to other pre-treatment processes.
	Dilute Acid	Does not produce inhibitors.	Equipment corrosion
	Sulphuric Acid	Mild condition.	Formation of toxic substances
	Hydrochloric Acid	High yields of xylose. Increase in surface area and porosity by eliminating hemicelluloses.	Relatively expensive
	Sodium Hydroxide	Ester is effectively removed. Increased surface area and porosity.	Expensive reagent. Recovery of Alkali
	Ammonia	Effective delignification.	Alkali recovery. Relatively expensive
	Lime	Removes lignin and acetyl effectively Inexpensive	Less effective because of poor solubility of lime
	Ozonolysis	Effectively remove lignin. Does not produce toxic residues. The reactions are carried out at room temperature and pressure	Requires large amount of ozone, making the process expensive
Biological pre-treatments	Fungi	Lignin and hemicellulose are degraded	Cellulose loss
	Actinomycetes	Mild environmental conditions	Hydrolysis rate is relatively slow

Hydrolysis

The hydrolysis process is frequently carried out with acid or enzymesal though it varies between sugar, starch and lignocellulose based substrates. The enzymes usually used for starch-based feedstocks are α-and β-amylase, pullulanase, isoamylase and glucoamylase, while cellulases and b-glucosidases are enzymes mainly for lignocellulosic-based feedstocks. Several reports suggested that the build-up of end products usually slows the action of enzymes, which finally results in inhibition of the process. As an example, endoglucanases and cellobiohydrolases led to the accumulation of cellobiose, thus affecting the yield of hydrolysis[36]. Likewise, the range of the substrate constituents (such as in food waste) occasionally call for accumulation of antimicrobial agents e.g. tetracycline or cycloheximide in the hydrolysis process, to keep away from being contaminated with microbes[37].

Acid hydrolysis: This method is achieved in two modes (dilute and concentrated) with diverse types of catalyst such as H_2SO_4, HCl, HNO_3, H_3PO_4, peracetic acid, etc.

Dilute acid hydrolysis: There are two types of dilute acid hydrolysis processes: (i) high temperature (180°C) during a short retention time (5 min) and (ii) low temperature (120°C) for long retention time (30-90 min)[38]. Dilute H_2SO_4 has been the most extensively used acid[30]. Dilute acid hydrolysis process is the most viable for industrial use. The benefit of the dilute acid hydrolysis over the concentrated type is low concentration of acid utilised with short retention time while its challenge is the high temperature during operation.

Concentrated acid hydrolysis: This method is done with low temperature of 40 °C and at high acid concentration of about 30-70% which led to high yields of sugar; but the apparatus becomes corrosive at high acid consumption[38]. Acid hydrolysate neutralization produces great amounts of gypsum[39]. The Arkenol Incorporation, a company of technology and project development from the United States, reported the process of concentrated acid hydrolysis that is made feasible economically and set for commercial operation. A full-scale cellulosic-to-ethanol project has also been developed by the Masada Resource Group in Northern America[40]. This method was not earlier considered as viable economically because it requires large amounts of acid. The improvement in technologies for recovery of acid has enhanced attention in accepting concentrated acid hydrolysis[41].

Acid hydrolysis is affected strongly by several factors which include temperature, solid-liquid ratio, acid type, reaction time and concentration[42].

During acid hydrolysis, the discharge of reducing sugars is determined by the type as well as structure of lignocellulosic material and the employed reactors in the process[43]. In order to accomplish maximum recovery of sugar and to decrease the inhibitors 'concentration released during acid hydrolysis, optimizing the aforementioned determinants is needed as this hydrolysate would be further utilized to perform fermentation[44].

Enzymatic hydrolysis: Enzymatic hydrolysis can be described as a process of breaking down cellulose into sugar (glucose) with the use of enzymes (catalysts). Trichoderma reesei is the most used enzyme and it produces *Cellulase* which hydrolyses cellulose[45]. The advantages of this type of hydrolysis include low energy consuming and eludes the application of poisonous substances or corrosive acids due to the moderately mild reaction conditions. Thus, cellulose hydrolysis altered by *Cellulase* has extensively been examined. However, during the hydrolysis process, high pre-treatment costs and *Cellulase* production, and substantial deactivation of enzyme happen. These economic difficulties have hindered enzyme hydrolysis of cellulose to glucose.

This type of hydrolysis is regarded as a main step in producing bioethanol from lignocellulosic materials. Using enzymes for hydrolysis is considered as the most feasible approach due to higher conversion yields, low energy requirements, minimal by-product formation, mild operating conditions, and environment-friendly processing[46,47]. Based on the enzyme employed, celluloses can be hydrolysed into glucose, and hemicelluloses could be hydrolysed to release glucose, xylose, galactose, arabinose and mannose.

Enzymatic hydrolysis is environmentally-friendly but needs an extended digestion time when likened to acid hydrolysis. Though it's likely to decrease the time of digestion by increasing the enzyme amount, but the cost of the enzymes will be increased. Furthermore, in situation where the substrate is saturated with enzyme, the digestion time may not be decreased even when an excess enzyme amount is added. Therefore, several researchers have made efforts to increase the particular enzyme activity and lessen the enzyme production costs. Table-4 shows the comparison of acid and enzymatic hydrolysis[40].

Fermentation

Fermentation is defined as the process of converting hydrolysed solutions (sugars) to ethanol by means of a range of micro-organisms, usually yeast, bacteria, or fungi under anaerobic (oxygen-free) environments. It is a metabolic way for microorganisms to acquire energy by way of degrading organic compounds with lactic acid, ethanol, butane, cellulose, carbon dioxide, nisin, as some of the by-products. Fermentation of ethanol is a facultative anaerobic process, since yeasts produce their energy in the absence of oxygen. The microorganisms of major importance in ethanol fermentation include *Saccharomyces cerevisiae* (which ferment mostly glucose, hexose, and pentose), *Pichia stipites* (which ferment xylose), *Schwanniomyces alluviums* (which hydrolyse starch), and *Kluyueromyces* yeast species (which ferment lactose)[48].

Table-4: Comparison of acid and enzymatic hydrolysis[38].

Factors	Acid hydrolysis	Enzymatic hydrolysis
Hydrolysis	High temperature at 100-240°C is used	Mild temperature condition of 40-50 °C is used
Yields	High yields of sugar is impossible	High sugar yields can be achieved
Inhibitors	There is development of inhibitors	No development of inhibitor
Inhibition of Product during hydrolysis	No	Yes
Catalyst Cost	Low	High
Hydrolysis Period	Requires little time	Requires long time

Yeasts are largely able to grow and efficiently produce ethanol at pH of 4.0-6.0 and at temperatures of 28-35°C. Primarily, yeast break down glucose to ethanol under anaerobic conditions by the Embden-Meyerhof pathway. Yeasts are extremely vulnerable to inhibition of ethanol. Ethanol concentration of about 1 to 2% (w/v) is adequate to slow down the development of microbes and at 10% (w/v) alcohol, the organism growth is nearly halted[49]. The advantages of *Saccharomyces cerevisiae* above other yeasts include greater efficiency achieved while producing ethanol, employ a range of hexoses and a greater ethanol compliant likened to other yeast strains[50].

S. cerevisiae has largely been known as safe and it remains as the most universally employed microbe in the fermentation industry[51]. The two main responsibilities of *Saccharomyces cerevisiae* are to produce alcoholic beverages and to grow bread dough. Production of alcohol occurs by conversion of sugar into energy, and at the same time, *S. cerevisiae* meets the need of its metabolic energy. In anaerobic conditions, yeast ferments glucose, while ethanol and CO_2 are by-products of the Embden-Meyehof (EM) route. Fermentation is performed in an anaerobic condition, but *Saccharomyces cerevisiae* needs lesser amounts of oxygen to combine fatty acid and sterols[52].

Fermentation of hydrolysed lignocelluloses encompasses the change of sugars (glucose) into ethanol that is mostly achieved by means of bacterium or yeast. The preferred micro-organism should have definite characters with respect to forbearance, that is, towards inhibitors, concentrations of glucose and other sugars and ethanol in the hydrolysis products and must as well tolerate greater temperatures then lesser pH with insignificant by-product formation[53].

Fermentation remains the main element wherever technology improvement shows significant role and is necessarily achievable. The usually engaged fermentation technologies are discussed as follows.

Separate hydrolysis and fermentation (SHF)

Separate hydrolysis and fermentation is a process that completely hydrolysed cellulose to glucose using *cellulase* under optimum conditions, particularly temperatures of about 50^{o}C that enable the enzyme hydrolysis. SHF similarly decrease the enzyme amount but cannot be allowed by microorganisms performing fermentation of ethanol at temperatures of about 35^{o}C. After complete cellulose hydrolysis, lignin is removed, which could be retrieved using a filter and treated as value-added by-products.

However, the glucose accumulation in the hydrolysis process substantially prevents b-glucosidase, which consecutively led to the cellobiose build-up that prevents the actions of exo-β-glucanase or cellobiohydrolase (CBH) and endo-β-glucanase (EG). Supplementing β-glucosidase might be a solution to this problem provided the enzyme costis affordable, for instance, β-glucosidase from *Aspergillus niger*[54]. Another issue with SHF process is microbe contamination throughout the cellulose hydrolysis and the movement of hydrolysis product via pipelines, which can worsen during fermentation of ethanol and compromise the yield of ethanol, as the greater quantity of medium for ethanol fermentation is not ever treated in the industry because of the consumption of energy and sugar loss connected with the operation.

Simultaneous saccharification and fermentation (SSF)

Simultaneous saccharification and fermentation is a continuous process which encompasses fresh sterile media fed into a reactor. It is also referred to as chemostat, continuous-flow, or stirred-tank fermentation. Aside the feeding of the reactor with fresh nutrients, the effluent is discharged from the reactor and the reactor volume is always constant. The feeding and removal rates are also equivalent. To avoid removing the cells from the reactor, the growth rate of microbe is taken as a rate of removing cells[55].

Simultaneous saccharification and fermentation (SSF) is assumed to be the best process for enzymatic conversion of cellulose to ethanol, according to Schell and Walter[56]. The simultaneous saccharification and fermentation process synthesizes enzyme hydrolysis of cellulose with co-fermentation of its major resulting sugar (glucose) to ethanol[57]. In SSF, enzyme hydrolysis of cellulose and fermentation of glucose to ethanol by yeast continue concurrently inside a vessel. Likened with saccharification without yeast, SSF using Trichoderma cellulose and saccharomyces cerevisiae improved the rate of cellulose hydrolysis by 13–30%[58]. The optimal temperature for SSF process was 35^{o}C. The condition for βG in SSF was less than for saccharification. This is a very encouraging way of ethanol production because of its capacity to enhance rates and yields of hydrolysis and product concentration compared to SHF.

Simultaneous saccharification and co-fermentation (SSCF)

In SSCF, the lignocellulose biomass which has been pre-treated is neutralized and opened directly to diversemicro-organisms and enzymes which are able and efficient to hydrolyse cellulose and hemicelluloseto fermentable sugars likewise ferment hexoses and pentoses in any phase to ethanol[35]. A major problem of this method is that the pentose using organisms as well have preference for hexoses as substrate. Thus, if combined with an organism such as *S. cerevisiae*, there is a struggle amid them, what generally led to lesser yields of ethanol. In order to surmount this effect, consecutive fermentation of pentoses and hexoses has been recommended, where pentose fermenting organisms are added to the substrate after the hexose fermentation is done, but in the similar equipment. Yet, there are still low ethanol yields of sequential fermentation[35].

In SSCF process, glucose and xylose are concurrently fermented in the same container. Strains of *Saccharomyces cerevisiae* and *Zymomonas mobilis* are genetically engineered to simultaneously ferment both glucose and xylose[59,60]. The incorporated system produced more than 30g/L of ethanol and accomplished 54% conversion of all possibly obtainable sugars in the biomass (total sugars) entering SSCF[35]. This technology is greater than SSF technology in regard to cost efficiency, improved yields and little time[61].

Consolidated bio-processing (CBP)

Consolidated bio-processing (CBP) is a most encouraging and possible approach which comprises production of enzymes, hydrolysis and fermentation into a certain system, for effective ethanol production from lignocellulosic materials[38]. Here is a condition of extremely engineered microbe which can hydrolyse biomass with the production of enzymes alone and produces high ethanol titre[62]. CBP turn out to be practicable when an engineered microbe or microbial conglomerate might be fully formed in a particular bioreactor ethanol, combined with the enzymes made by a lone assembly of microorganism[63,40]. CBP is gaining grounds as it favorable leaps forward to have low cost bioethanol, however its feasibility extremely depends on whether an appropriate microorganism could be established naturally or made by engineering approaches in the laboratory[64]. As *Saccharomyces cerevisiae*, in non-capable of using pentoses and because of the deficiency of appropriate enzymes for using the lignocellulosic feed stocks, makes the organisms inappropriate for CBP applications[65]. The advantages and disadvantages of different fermentation methods are given in Table-5.

Table-5: Advantages and disadvantages of various fermentation methods[38].

Process	Advantages	Disadvantages	Ref.
Separate hydrolysis and fermentation (SHF)	Both hydrolysis and fermentation are carried out independently at optimum temperatures.	The process is expensive and time-consuming while hydrolysis and fermentation are done independently.	66
Simultaneous saccharification and fermentation (SSF)	Less equipment is used, reduction of investment cost, simplified operation, no inhibition of end product by glucose hence the increase in the amount of hydrolysis and ethanol yield.	The reaction time is prolonged, which produces low yield of fermentable sugar leading to lesser yield of ethanol.	61
Simultaneous saccharification and co-fermentation (SSCF)	Low residence time, reduced in use of reactors, decreased capital cost, high bioethanol productivity, the feedback inhibition problem is resolved by constant removal of saccharification end products	High enzyme loading, variance of optimal temperature between hydrolysis and fermentation micro-organisms	66
Consolidated bio-processing (CBP)	Reducing capital investment, elimination of utilities related with enzyme production, using vessels for saccharification and fermentation is reduced, simplified operation, reduction of contamination risk by reducing the levels of glucose while making ethanol, the product inhibition by cellulose is enhanced for hydrolysis development	Lack of appropriate thermophilic microbes	61

Distillation

Distillation process is the final stage of ethanol production, which involves the separation of more volatile parts of the substance from less volatile ones by heating and condensing. The product from fermentation contains ethanol, water and cell mass. The maximum ethanol concentration allowed by the microorganisms is about 10% at 30°C, and can be up to 37°C to maximise cellulose activity[67,31].

On the part of processing, slurries become challenging to be handled when having over 15% solids, which also equivalent to 5% ethanol[31]. Ethanol is retrieved in a distillation chamber, where greater part of the water is left with the solids.

The product (with 37% ethanol) is at that time concentrated to a proportion just below the azeotrope (95%)[67]. In the end, 99.9% of the ethanol in the fermented extract could be recollected in the dry product by reprocessing between distillation and dehydration[67].

Using membranes to recover ethanol by pervaporation (removal of ethanol by vacuum applied at the membrane permeate side) is another procedure which conserves energy by eliminating energy-expensive distillation. It is probable to concentrate ethanol from 80 to 99.5% by pervaporation[68]. It could also lessen yeast ethanol (and inhibitor) problems of toxicity provided it is applied during fermentation.

Processing ethanol production from livestock manure with established findings

The ethanol production processing from livestock manure is described in Figure-1[1,4]. The raw manure samples are washed to remove the sand through sedimentation process where solid and liquid are separated. The liquid contents are discharged into the environment while the solid fractions are pre-treated with alkaline (KOH), then dried and milled before hydrolysis. The milled manures are then hydrolysed using acid (H_2SO_4) or enzyme (Cellulose).

The hydrolysis process breakdown the cellulose part of the manure into sugar solution (glucose) for fermentation. The solution is at that point fermented with yeast (*Saccharomyces Cerevisiae*) and eventually distilled to extract ethanol. Researchers have revealed also that pentose and glucose sugars can be retrieved at sufficient points (96% and 40-52% respectively) from raw dairy manure, by means of dilute acid pre-treatment followed by enzymatic hydrolysis[69,70].

According to a study using different animal manures, poultry manure produced higher yields of ethanol[4]. The alkaline treatment method was effective in improving enzymatic hydrolysis to glucose[4,71]. Physical pre-treatments which include drying and grinding as well impacted greatly on the enzymatic hydrolysis. The glucose yields for poultry manure by means of several pre-treatments are shown in Figure-2.

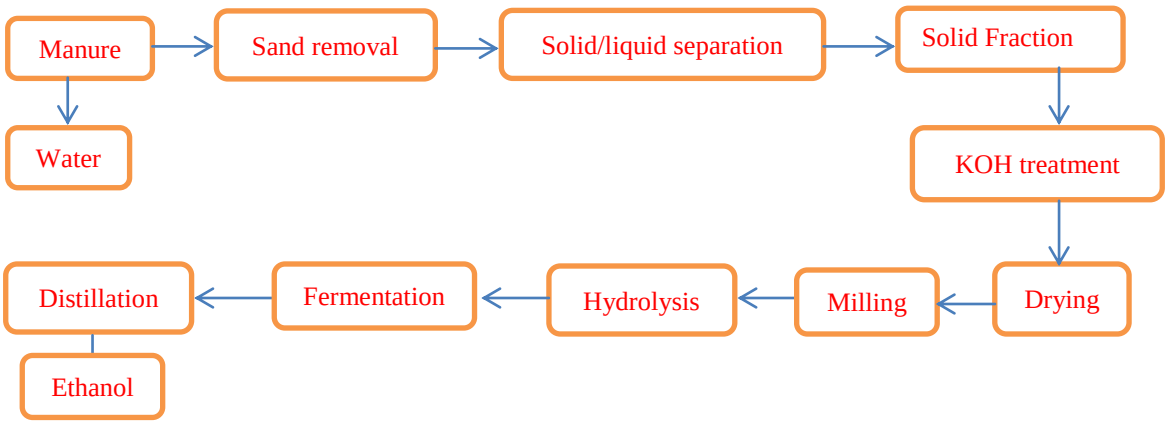

Figure-1: Flow diagram for process of ethanol production from manure.

It was established further that at 40°C of enzyme hydrolysis, the glucose yield for poultry manure showed 7.1, which increased to 10.2 when alkaline (KOH) pre-treatment was used. The results indicated significant differences to that noticed with crop residues, where a significant increase in the yield of glucose was noticed due to alkaline (KOH) treatment.

The results therefore suggested that in the poultry manure, most of the cellulose were not as simply removed from other organic matters. While the incorporated structure of cellulosic material can be disintegrated during the KOH treatment, the broken structure might still not give sufficient entry for the enzymes to act on the cellulosemolecules[4].

The glucose yield increased to 13.9 when the KOH-treated manure was dried before enzyme hydrolysis. These results revealed that drying the KOH-treated manure improved the glucose yield from enzyme hydrolysis. It can be concluded that some of the organic matter could be degraded at high temperature (up to 70°C), hence more cellulose could be discharged during the process of drying; or that the physical structure of the feedstock turned to be more reachable to the enzyme because of the drying. The maximum glucose yield of 27.6 was reported for milled KOH-treated poultry manure. This therefore suggested a two-fold increase in yield likened to that of un-milled manure. These results recommended that by milling the poultry manure, it would enhance the separation of the substances which covered the cellulose, hence opening up the cellulose surface. Also, reducing the substrate particle also provided a more surface area for reaction. Hence, the substrate turns out to be so greatly reachable to the enzyme. Consequently, the glucose yield increased considerably and eventually increased the amount of ethanol produced.

Environmental impacts of ethanol

Provided worldwide energy demand grow continuously, prices of oil are unlikely to reduce. Subsequently, there would be an increase in the demand for a renewable and environment-friendly fuel.

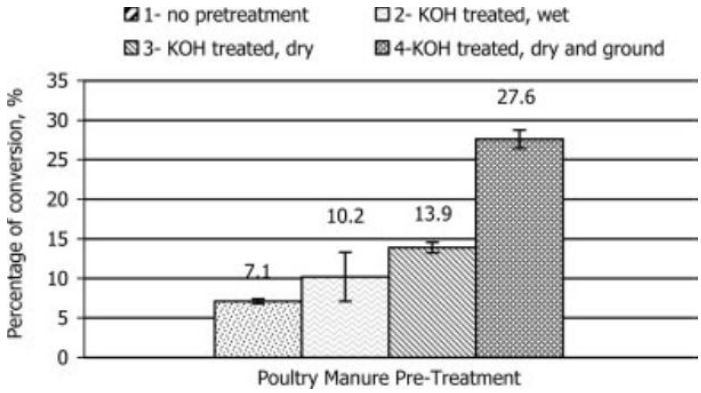

Figure-2: Glucose yields from poultry manure using various pre-treatment methods[4].

Over the years, human activities have triggered a vivid increase in the emission of several greenhouse gases such as CO_2 which has led to alterations in the equilibrium of the earth's atmosphere[72]. Fuel ethanol is proposed as a sustainable fuel which can be made from renewable resources, which can maintain or even decrease the level of greenhouse gases. The net emissions of CO_2 are stated to be close to zero, since the CO_2 discharged during production of ethanol and combustion is evoked by the raw materials (such as crops and plants) producing ethanol. Ethanol blended with gasoline increases octane and delivers oxygen to encourage more full combustion. Adding ethanol or derivative such as methyl tertiary butyl ether (MTBE) to gasoline as oxygenate lessens tailpipe emissions of Carbon monoxide (CO) and unburned hydrocarbons, which can lead to enhancing the urban air quality. Unlike MTBE, which is unreadily decomposable as well referred to as a groundwater pollutant, ethanol is a water-soluble and biodegradable compound and so is comparatively harmless to the environment, ground water and soil[73,74]. However, due to addition of solar energy, other energy inputs (frequently in the form of fossil fuel) are needed in the production and promotion of biofuel e.g. ethanol while the whole process is unlikely to be absolutely carbon-neutral[75].

Conclusion

Having reviewed the various technologies for production of ethanol from animal manure and other available feedstocks, the following conclusions are hereby made: i. Lignocellulosic biomass has been projected to be a key resource for economically promising bioethanol production. Although theoretical ethanol produces from sugar and starch are greater than the ones from lignocellulose, these orthodox sources are not sufficient for global bioethanol production. In that aspect, agricultural wastes including animal manures are renewable, abundantly available and less costly in nature. ii. The ethanol production using various conversion technologies and different renewable non-food feedstocks shows the emergence of sustainable energy future. iii. As regards pre-treatment process, the difficulties are processing of biomass, appropriate and cost effective technology to release cellulose and hemicellulose from their complex with lignin. iv. Concerning hydrolysis process, the problem is attaining an effective process for depolymerisation of cellulose and hemicellulose to produce fermentable sugars with high concentration. Thus, enzymatic hydrolysis might be the most effective alternate process for hydrolysis of complex polymer. v. With respect to fermentation, the challenges involved are co-fermentation of xylose and glucose, and the use of recombinant microbial strains. vi. As regards distillation process, the challenge will be designing a cost-effective distiller and condenser for separation of more volatile substances from less volatile ones. vii. Hence, in order to resolve the technology blocks of the bio-conversion process, innovative science and efficient technology need to be applied, so that production of ethanol from these agricultural wastes may be developed and improved effectively in the near future. Ultimately, the reduction in the cost of ethanol production enhances their likelihood to become a sustainable substitute to fossil fuels.

References

1. Woldesenbet, A. G., Shiferaw, G., & Chandravanshi, B. S. (2013). Bio-ethanol production from poultry manure at Bonga Poultry Farm in Ethiopia. *African Journal of Environmental Science and Technology*, 7(6), 435-440.

2. Attygalle, A. (2008). Instrumental analysis. Institute of Technology, Diaz Publication, London.

3. Scott, F., Quintero, J., Morales, M., Conejeros, R., Cardona, C. and Aroca, G. (2013). Process design and sustainability in the production of bioethanol from lignocellulosic materials. *Bio. Technol.*, 16(3), 1-7.

4. Champagne, P. (2008). Bioethanol from agricultural waste residues. *Environmental progress*, 27(1), 51-57.

5. Fernandez-Lopez, M., Puig-Gamero, M., Lopez-Gonzalez, D., Avalos-Ramirez, A., Valverde, J., and Sanchez-Silva, L. (2015). Life cycle assessment of swine and dairy manure: Pyrolysis and combustion processes. *Bioresource Technology*, 182, 184-192. DOI:10.1016/j.biortech.2015.01.140.

6. Nasir, I.M., Mohd Ghazi, T.I., and Omar, R. (2012). Anaerobic digestion technology in livestock manure treatment for biogas production. *Engineering in Life Sciences*, 12(3), 258-269. DOI: 10.1002/elsc.201100150.

7. Yue, Z.B., Teater, C., Liu, Y., MacLellan, J., and Liao, W. (2010). A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with bio-refining. *Biotechnology and Bioengineering*, 105(6), 1031-1039. DOI:10.1002/bit.22627

8. Mojović, L., Nikolić, S., Rakin, M., & Vukasinović, M. (2006). Production of bioethanol from corn meal hydrolyzates. *Fuel*, 85(12-13), 1750-1755. http://dx.doi.org/10.1016/j.fuel.2006.01.018

9. Almodares, A. and Hadi, M.R. (2009). Production of bioethanol from Sweet Sorghum: A review. *African Journal of Agricultural Research*, 4, 772-780.

10. Vohra, M., Manwar, J., Manmode, R., Padgilwar, S. and Patil, S. (2014). Bioethanol production: Feedstock and current technologies. *Journal of Environmental Chemical Engineering*, 2, 573-584. http://dx.doi.org/10.1016/j.jece.2013.10.013

11. Muktham, R., Bhargava, S.K., Bankupalli, S. and Ball, A.S. (2016). A review on 1st and 2nd generation bioethanol production- recent progress. *Journal of Sustainable Bioenergy Systems*, 6, 72-92.

12. Bai, F., Anderson, W. and Moo-Young, M. (2008). Ethanol fermentation technologies from sugar and starch feedstocks. *Biotechnol. Adv.*, 26(1), 89105.

13. Ogbonna, J.C., Mashima, H. and Tanaka, H. (2001). Scale up of fuel ethanol production from Sugar beet juice using Loofa sponge immobilized bioreactor. *Bioresource Technology*, 76, 1-8. http://dx.doi.org/10.1016/S0960-8524(00)00084-5

14. Berguninger, W.F., Piyachomkwan, K. and Sriroth, K. (2008). Tapioca/Cassava starch: Production and use. In: Be-Miller, J. and Whistler, R., Eds., Starch Chemistry and Technology, 3rd Edition, Academic Press, New York, 544.

15. Hashizume, T., Higa, S., Sasaki, Y., Yamazaki, H., Iwamura, H. and Matsuda, H. (1966). Constituents of cane molasses. *Agricultural and Biological Chemistry*, 30, 319-329. http://dx.doi.org/10.1080/00021369.1966.10858603

16. Billa, E., Koullas, D.P., Monties, B. and Koukios, E.G. (1997). Structure and composition of Sweet Sorghum stalk components. *Industrial Crops and Products*, 6, 297-302. http://dx.doi.org/10.1016/S0926-6690(97)00031-9

17. Nigam, P. and Singh, D. (1995). Enzyme and microbial systems involved in starch processing. *Enzyme Microb. Technol.*, 17(9), 770-778.

18. Balan, V., Chiaramonti, D. and Kumar, S. (2013). Review of US and EU initiatives toward development, demonstration and commercialization of lignocellulosic biofuels. *Biofuels, Bioproducts and Biorefining*, 7, 732-759. http://dx.doi.org/10.1002/bbb.1436

19. Naik, S., Goud, V.V., Rout, P.K., and Dalai, A.K. (2010). Production of first and second generation biofuels: A comprehensive review. *Renew. Sustainable Energy Rev*., 14(2), 578-597.

20. Verpy, T., & Petersen, G. (2004). Top Value Added Chemicals From Biomass, National Renevable Energy Labotatory. DOE/GO-102004-1992.

21. Kumar, P., Barrett, D.M., Delwiche, M.J. and Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuels production. *Industrial and Engineering Chemistry Research*, 48, 3713-3729. http://dx.doi.org/10.1021/ie801542g

22. Menon, V. and Rao, M. (2012). Trends in bioconversion of lignocelluloses: Biofuel, platform chemicals & biorefinery concept. *Progress in Energy and Combustion Science*, 38, 522-550. http://dx.doi.org/10.1016/j.pecs. 2012.02.002

23. Cherubini, F. (2010). The biorefinery concept: Using biomass instead of Oil for producing energy and chemicals. *Energy Conversion and Management*, 51, 1412-1421. http://dx.doi.org/10.1016/j.enconman.2010.01. 015

24. Demirbas, A. (2005). Bioethanol from cellulosic materials: A renewable motor fuel from biomass. *Energy Sources*, 27, 327-337. http://dx.doi.org/10.1080/00908310 390266643

25. Lin, Y. and Tanaka, S. (2006). Ethanol fermentation from biomass resources: Current state and prospects. *Applied Microbiology and Biotechnology*, 69, 627-642. http://dx.doi.org/10.1007/s00253-005-0229-x

26. Dien, B.S., Cotta, M.A. and Jeffries, T.W. (2003). Bacteria engineered for fuel ethanol production: Current status. *Applied Microbiology and Biotechnology*, 63(3), 258-266. http://dx.doi.org/10.1007/s00253-003-1444-y

27. Saha, B.C., Qureshi, N., Kennedy, G.J. and Cotta, M.A. (2016). Biological pretreatment of Corn stover with white-rot fungus for improved enzymatic hydrolysis. *International Biodeterioration and Biodegradation*, 109, 29-35. http://dx.doi.org/10.1016/j.ibiod.2015.12.020

28. Jung, Y.R., Park, J.M., Heo, S.Y., Hong, W.K., Lee, S.M., Oh, B.R., Park, S.M., Seo, J.W. and Kim, C.H. (2015). Cellulolytic enzymes produced by a newly isolated soil fungus *Pencillium sp*. TG2 with potential for use in cellulosic ethanol production. *Renewable Energy*, 76, 66-71. http://dx.doi.org/10.1016/j.renene.2014.10.064

29. Ha, S.J., Galazka, J.M., Kim, S.R., Choi, J.H., Yang, X., Seo, J.H., Glass, N.L., Cate, J.H.D. and Jin, Y.S. (2011). Engineered Saccharomyces cerevisiae capable of simultaneous Cellobiose and Xylose fermentation. Proceedings of the National Academy of Sciences of the United States of America, 108, 505-509. http://dx.doi.org/10.1073/pnas.1010456108

30. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource technology*, 96(6), 673-686.

31. Lynd, L.R. (1996). Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment and policy. *Annual Review of Energy and the Environment*, 21, 403-65, 1996.

32. Gutierrez, L.F., Sanchez, O.J. and Cardona, C.A. (2009). Process integration possibilities for biodiesel production from Palm Oil using ethanol obtained from lignocellulosic residues of Oil Palm industry. *Bioresource Technology*, 100(3), 1227-1237.

33. Mooney, C.A., Mansfield, S.D., Beatson, R.P., and Saddler, J.N. (1999). The effect of fibre characteristic on hydrolysis and cellulose accessibility to softwood substrates. *Enzyme and Microbial Technology*, 25 (8-9), pp. 644-650.

34. Mood, S.H., Golfeshana, A.H., Tabatabaeib, M., Jouzanib, G.S., Najafic, G.H., Gholamib, M., and Ardjmand, M. (2013). Lignocellulosic biomass to bioethanol, a comprehensive review with a focus to pre-treatment. *Renewable and Sustainable Energy Reviews*, 27, 77-93.

35. El-Naggar, N.E., Deraz, S. and Khalil, A. (2014). Bioethanol production from lignocellulosic feedstocks based on enzymatic hydrolysis: Current status and recent developments. *Biotechnology*, 13(1), 1-21.

36. Li, A., Antizar-Ladislao, B. and Khraishah, M. (2007). Bioconversion of municipal solid waste to glucose for bioethanol production. *Bioprocess and Bio-systems Engineering;* 30(3), 189 – 196.

37. Yan, S., Li, J., Chen, X., Wu, J., Wang, P. and Ye, J. (2011). Enzymatical hydrolysis of food waste and ethanol production from the hydrolysate. *Renewable Energy*, 36 (4), 1259 - 1265.

38. Jahnavi Gentela, Govumoni Sai Prashanthi, Koti Sravanthi and Linga Venkateswar Rao (2017). Status of availability of lignocellulosic feedstocks in India: Biotechnological strategies involved in the production of bioethanol. *Renewable and Sustainable Energy Reviews*, 73(2017), 798 - 820.

39. Taherzadeh, M.J. and Karimi, K. (2008). Pre-treatment of lignocellulosic waste to improve ethanol and biogas production: A review. *International Journal of Molecular Sciences*, 9 (9), 1621-1651.

40. Taherzadeh, M. J. & Karimi, K. (2007). Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review. *Bio Resources*, 2(3), 472-499.

41. Moe, S. T., Janga, K. K., Hertzberg, T., Hägg, M. B., Øyaas, K. & Dyrset, N. (2012). Saccharification of lignocellulosic biomass for biofuel and biorefinery applications–a renaissance for the concentrated acid hydrolysis?. *Energy Procedia*, 20, 50-58.

42. Zhuang, J., Liu, Y., Wu, Z., Sun, Y. and Lin, L. (2009). Hydrolysis of wheat straw hemicellulose and detoxification of the hydrolysate for xylitol production. *Bioresources,* 4, 674–86.

43. Lenihan, P., Orozco, A., O'Neill, E., Ahmad, M.N.M., Rooney, D.W. and Walker, G.M. (2010). Dilute acid hydrolysis of lignocellulosic biomass. *Chem. Eng. J.* 156, 395–403.

44. Gírio, F.M., Fonseca, C., Carvalheiro, F., Duarte, L.C., Marques, S., and Bogel-Łukasik, R. (2010). Hemicelluloses for fuel ethanol: A review. *Bioresour. Technol.,* 101, 4775–800.

45. Persson, I., Tjemeld, F., and Hagerdal, B.H. (1991). Fungal cellulolitic enzyme production: An overview. *Proc. Biochem.,* 26, 65-74.

46. Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V. (2005b). Dilute acid pre-treatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochemistry*, 40(12), 3693-3700.

47. Wingren, A., Galbe, M., Roslander, C., Rudolf, A., and Zacchi, G. (2005). Effect of reduction in yeast and senzyme concentrations in a simultaneous-saccharification-and-fermentation-based bioethanol process: Technical and economic evaluation. *Applied Biochemistry and Biotechnology*, 122-124, 485-499.

48. Waites, M.J., Morgan, N.L., Rockey, J.S., and Higton, G. (2011). Industrial microbiology: An introduction. *Osney Mead, Oxford: Blackwell Science*.

49. Osunkoya, O. A. and Okwudinka, N. J. (2011). Utilization of sugar refinery waste (molasses) for ethanol production using *Saccharomyces Cervicae*. *American Journal of Scientific and Industrial Research*, 2(4), 694-706.

50. Classen, P.A.M., Van Lier, J.B., López, A.M., Van Niel, E.W. J., Sijtsma, L., Stams, A.J.M., De Vries, S.S., and. Weusthuis, R.A. (1999). Utilization of biomass for the supply of energy carriers. *Applied Microbiology and Biotechnology*, 52 (6), 741-755.

51. Kunz, M. (2008). Bioethanol: Experiences from running plants, optimization and prospects. *Biocatalysis and Biotransformation*, 26(1-2), 128-132.

52. Sanchez, O.J. and Cardona, C.A. (2008). Trends in biotechnological production of fuel ethanol from different feedstock. *Bioresource Technology*, 99(13), 5270-5295.

53. Picataggio, S. and Zhang, M. (1996). Microorganism development for bioethanol production from hydrolysates. In: Wyman CE, editor. Handbook on bioethanol: Production and utilization. Washington, DC: Taylor and Francis; 163–78.

54. Wang, C., Wu, G., Chen, C., & Chen, S. (2012). High production of β-glucosidase by Aspergillus niger on corncob. *Applied Biochemistry and Biotechnology*, 168(1), 58-67.

55. Shuler, M.L., and Kargi, F. (2008). Bioprocessing engineering basic concepts. Ed. Prentice Hall International Series, Castleton, New York, 2nd Edition, p. 551.

56. Schell, D. J., Torget, R., Power, A., Walter, P. J., Grohmann, K., & Hinman, N. D. (1991). A technical and economic analysis of acid-catalyzed steam explosion and dilute sulfuric acid pretreatments using wheat straw or aspen wood chips. *Applied biochemistry and biotechnology*, 28(1), 87-97.

57. Takagi, H., Iwamoto, F. and Nakamori, S. (1997). Isolation of freeze-tolerant laboratory strains of saccharomyces cerevisiae from proline-analogue-resistant mutants. *Appl. Microbiol. Biotechnol.,* 47(4), 405-11.

58. Jesper Norgard (2005). Ethanol production from biomass, optimization of simultaneous saccharification and fermentation with respect to stirring and heating. pp. 1-5.

59. Dien, B.S., Cotta, M.A. and Jeffries, T.W. (2003). Bacteria engineered for fuel ethanol production: Current status. *Applied Microbiology and Biotechnology*, 63(3), 258-266. http://dx.doi.org/10.1007/s00253-003-1444-y

60. Hahn-Hagerdal, B., Karhimaa, K., Fonseca, C., Spencer-Martins, I. and Gorwa-Grauslund, M. S. (2007). Toward industrial pentose-fermenting yeast strains. *Appl. Microbiol. Biotechnol*., 74, 937–953.

61. Lynd, L.R., Zyl, W.H.v, McBride, J.E., and Laser, M. (2005). Consolidated bioprocessing of cellulosic biomass: An update. *Curr. Opin. Biotechnol*, 16(5), 577–583.

62. Hasunuma, T. and Kondo, A. (2012). Consolidated bio-processing and simultaneous saccharification and fermentation of lignocellulose to ethanol with thermotolerant yeast strains. *Process Biochem.*, 47(9), 1287-94.

63. Parisutham, V., Tae, H.K., and Sung, K.L. (2014). Feasibilities of consolidated bioprocessing microbes: from pre-treatment to biofuel production. *Bioresour. Technol.*, 161, 431 – 40.

64. Xu, Q., Singh, A., and Himmel, M.E. (2009). Perspectives and new directions for the production of bioethanol using consolidated bioprocessing of lignocellulose. *Curr Opin Biotechnol.*, 20, 364–71.

65. Dashtban, M., Schraft, H., and Qin, W. (2009). Fungal bioconversion of lignocellulosic residues; opportunities and perspectives. *Int. J. Biol. Sci.*, 5, 578–95.

66. Choudhary, J., Singh, S., & Nain, L. (2016). Thermotolerant fermenting yeasts for simultaneous saccharification fermentation of lignocellulosic biomass. *Electronic Journal of Biotechnology*, 21, 82-92.

67. Hamelinck, C.N., Hooijdonk, G.V., and Faaij. A. (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short, middle and long-term. *Biomass and Bioenergy*, 28, 384 -410.

68. Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H., & Galvez, A. (1999). Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis current and futuristic scenarios (No. NREL/TP-580-26157). National Renewable Energy Lab., Golden, CO (US).

69. Parisi, F. (1986). Bioconversion and separation. In: European workshop on bioethanol. Commission of the European Communities: Brussels, 1986; 59-69.

70. Davison, B., Evans, B., Finkelstein, M., McMillan, J., Liao, W. and Wen, Z. (2005). Effects of hemicellulose and lignin on enzymatic hydrolysis of cellulose from dairy manure. in: 26th Symposium on biotechnology for fuels and chemicals. *Humana Press*, pp. 1017–1030.

71. Wen, Z., Liao, W. and Chen, S. (2004). Hydrolysis of animal manure lignocellulosics for reducing sugar production. *Bioresource Technology*, 91(1), 31–39.

72. Li, C. (2004). Enzymatic hydrolysis of cellulose from various waste sources and their feasibility as feedstocks for ethanol production. Doctoral dissertation, Carleton University.

73. Galbe, M. and Zacchi, G. (2002). A review of the production of ethanol from softwood. *Applied Microbiology and Biotechnology*, 59; 618 – 628.

74. Isenberg, G. (1999). Assessment of automotive fuels. *Journal of Power Sources*, 84(2), 214-217.

75. Wyman, C.E. (1999). Biomass ethanol: Technical progress, opportunities, and commercial challenges. *Annual Review of Energy and the Environment*, 24, 189-226.

76. Granda, Cesar B., Holtzapple, Mark T., and Zhu, Li (2007). Sustainable liquid biofuels and their environmental impact. *Environmental Progress*, 26(3), 233-250.