

Simultaneous Saccharification and Fermentation (SSF), An Efficient Process for Bio-Ethanol Production: An Overview

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Excessive exploitation of conventionally used fossil fuel has been the major root cause of depletion of its reserves. Additionally, environmental concern, energy security, short supply with an increasing demand for fuel lead to adoption of sustainable energy resources such as solar, wind, biofuel, etc. Among all, ethanol was proved as a promising biofuel with various advantages. Sugarcane and corn which are considered as conventional raw material for ethanol production, hardly meets the current global demand for biofuel. Search for most promising feed stock for ethanol production, pioneered the use of lignocellulosic biomass and starch based materials. But low ethanol yield of lignocellulosic biomass without technological breakthrough forced researchers to opt for the starchy based routes. In recent years, only microbe based simultaneous saccharification and fermentation (SSF) has been evolved successfully as a starch based bio-ethanol production process while overcoming the problems associated with using harmful chemical and expensive enzymes. The principal advantage of microbial SSF process is starch hydrolysis and sugar fermentation can be processed in a single vessel while minimizing the substrate inhibition effects and overall reaction time. This review discusses the multiple aspects of Simultaneous Saccharification and Fermentation (SSF) process in the context of existing ethanol production routes.

Key word: Saccharification, Fermentation, Ethanol, Starch, Glucose.

In the recent years, demand of energy has increased drastically due to transportation, heating and industrial processing¹. The conventional source of energy alone is insufficient to meet the growing energy demand. Dependence and unplanned exploitation of non-renewable fossil fuel leads to depletion of its reserve². Hossain et al.³ and Prasad et al.⁴ reported that global energy crisis, political crisis, depletion of fossil reserve lead to adoption of ethanol as viable, economical, efficient, safer, eco-friendly, and renewable alternate to conventionally used fossil fuel. In addition to fuel grade ethanol, ethanol has broad

applications in beverage, pharmaceutical, cosmetic, chemical and other industries. Worldwide production of ethanol has been estimated around 23,429 Million Gallons in year 2013⁵. Fuel accounts for 73% of produced ethanol, while beverage and industrial ethanol constitute 17 and 10%, respectively⁶. Ethanol is used as prime renewable biofuel in the transport sector in Brazil, US and some European countries and such necessarily is expected to increase rapidly¹. As a fuel, ethanol combustion emits low CO, SO₂ and unburned hydrocarbon compared to gasoline. It was also reported that ethanol is an octane enhancer with is rich in octane number of 120 as compared to 87- 98 in case of gasoline. Another significance of ethanol is its low green house gas emission. As ethanol burns, CO₂ released is photo-synthetically

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assimilated by growing feed stock plant for carbohydrate production. Due to these advantages, ethanol is blended in gasoline in a range of 5-20 %^{3,7}.

Various agricultural based raw materials like sugar crops, starch-containing plants and lignocellulosic biomass can be utilized for ethanol fermentation. In order to select the cost effective process, lignocellulosic biomass has been the primary choice for ethanol fermentation. But lignocellulosic biomass has to undergo the most complicated pre-treatment process prior to fermentation which includes removal of lignin followed by the hydrolysis of cellulose. However, research effort to reduce the production cost of bio-ethanol was successful when starchy raw materials were introduced in simultaneous saccharification and fermentation (SSF) process with improved starch hydrolysis efficiency. The SSF process was first introduced by Gulf Oil Company, US and the University of Arkansas. Bio-ethanol production using the same process has been successfully implemented by different researchers⁸⁻¹⁷ using different starch based material. Among all the researchers, Purohit and Mishra⁷, Srichuwong *et al.*¹³ implemented potato based starchy material while Li *et al.*¹⁶ and Arasaratnam *et al.*¹⁷ used rice based starchy material for the production of ethanol. Olukotun *et al.*¹⁰, Itelima *et al.*¹⁵ developed the overall cost effective microbial based saccharification and fermentation process in SSF by using *Aspergillus niger* and *Saccharomyces cerevisiae* while reducing the dependency of direct enzymatic use for first stage saccharification. Among all, Lu *et al.*¹¹ and Moon *et al.*⁹ adopted fed batch and continuous fermentation approach to improve the productivity of ethanol in SSF. Hence it is transparent that microbe based SSF process using food waste starch can be evolved with a novel and promising concept for cost effective, environment friendly, large scale industrial production process for ethanol which has not been largely implemented.

Potential Feed stocks used for bio-ethanol production

Bio-ethanol is mainly produced by fermentation of various raw materials. The commonly used feed stocks for ethanol production are sugar crops (sugar cane molasses, sugar beet), starchy crops (corn, wheat, potato and Cassava)

and lignocellulosic biomass (soft wood). Sugarcane, sugar beets and corn are most feasible for ethanol production and have been used extensively though these are valuable food sources^{8,9}. As a by product of sugar industries, molasses contain high amount of impurities which necessitate a long pre-treatment steps. In order to select the cost effective process, lignocellulosic biomass is another option for ethanol fermentation. Important feed stocks for bio-ethanol production have been represented in Table 1. But lignocellulosic biomass (grasses, soft woods, bamboo, forest residue and agricultural residues; straw, corn stover, rice husk, corn cobs, corn stover, etc.) has to undergo the most complicated time taking pre-treatment processes prior to fermentation accompanied by removal of lignin followed and hydrolysis of cellulose. The hydrolysis of cellulose is far more difficult than the saccharification of starch. To avoid such complications in the process and bring down the overall production cost of bio-ethanol, starchy based raw materials like corn starch, wheat starch and potato starch were introduced with improved and efficient hydrolysis.

Starch is a polysaccharide, largely present in plants. It is found in the leaves, seeds, roots and fibres as food reserve. Native starch measures small globules 1 to 100 μm . It is composed of mixture of two structurally different polyglucans – amorphous amylose (20-30%) and crystalline amylopectin (75-80 %) ¹⁸. Amylose (shown in **Figure 1**) is linear chain of D-glucose units bonded by \pm -1, 4-linkages with an average degree of polymerization (DP) up to 6,000, making its molecular mass of 105 to 106 g/mol. Amylopectin (shown in **Figure 2**) is highly branched polymer with average degree of polymerisation of 2 million and consists of short α -1, 4-oligomers linked by α -1, 6-bonds making its molecular mass of 107 to 109 g/mol ¹². To make SSF based ethanol production process economically feasible attention has been given more on starchy based waste materials which are largely available in the environment in different forms.

Food wastes for cheaper bio-ethanol production

According to estimates of UN's Food and Agriculture Organization (FAO) ¹⁹, one-third of food is wasted every year. The amount of food products lost or wasted accounts 1.6 Gtonnes of primary

products and 1.3 Gtonnes of edible part of 6 Gtonnes of the agricultural production (for food and non-food uses). Food wastes damages environment and causes economic loss of US \$ 750 billion annually to food producers. Wasted edible starchy foods are generally potatoes, bread, cereals, rice and pasta. In India, about 40 per cent fresh fruit and vegetables costing \$8.3bn annually perish before reaching consumers. Each year, 21m metric tonnes of wheat rots in India due to improper storage facilities in government regulated Food Corporation ²⁰. Instead of wasting one-third food produced when 870 million people go hungry every day, the most innovative way to recover is use of these food wastes as a feedstock in ethanol production. By chemical and biological methods, food waste can be hydrolysed to glucose, free amino nitrogen and phosphate, which can be utilised as nutrients by many microorganisms production of wide range of diversified products like ethanol. This would be an innovative waste

management and secures both energy and fuel crisis.

Starchy potato waste could be potential feedstock for production of bio-ethanol. In India, 5 to 20% of potato crops (starch content in a range of 11.2% to over 19.3%) were wasted as by products from potato cultivation and due to poor storage facility ²⁰⁻²². Yamada *et al.* ²³ also reported that 18% of the starchy potatoes are wasted in potato industry. Arumugam and Manikandan²⁴ obtained high ethanol yield from dilute acid pre-treated waste mango and banana by enzymatic saccharification. Similarly, modified kitchen garbage having high sugar content, low cost, requiring short fermentation time could be used to produce ethanol in an optimised fermentation process²⁵. High starch content rice, barley, corn and wheat are also wasted in huge amount which could be used for cheaper ethanol production.

Conventional roots of bio-ethanol production

In early days, ethanol was used to

Table 1: Important feed stocks for bio-ethanol production

First generation (1G) feed stocks		Second generation (2G) feed stocks
Sugar crops	starchy crops	Lignocellulosic biomass
Sugar cane	Corn	Softwood
Sugar beet	Cassava	Switch grass, Reed canary grass
Molasses	Potato	Agricultural and Forest residues
Sweet sorghum	Barley	Leaves and Bagasse of sweet sorghum
Different Fruits	Rice	Spruce

Table 2: Enzymes and related Microorganisms involved in the processes

Process	Enzyme Involved	Microorganisms producing the enzyme
Liquefaction	α -amylase	<i>Aspergillus niger</i> , <i>Aspergillus oryzae</i>
Saccharification	Glucoamylase	<i>Aspergillus niger</i> , <i>Aspergillus oryzae</i>
Fermentation	Invertase, Zymase	<i>Saccharomyces cerevisiae</i>

Table 3: Microorganisms producing amylases and glucoamylase

Microorganisms	α -amylase/ amylase producers	Glucoamylase
Bacteria	<i>Bacillus amyloliquefaciens</i> <i>Bacillus licheniformis</i>	Bacillus species <i>Bacillus subtilis</i> <i>Bacillus megaterium</i> <i>E. Coli</i>
Fungi	<i>Aspergillus oryzae</i> <i>Aspergillus niger</i> <i>Aspergillus awamori</i>	<i>Aspergillus niger</i> <i>Aspergillus oryzae</i>

produce by anaerobic yeast fermentation of simple sugars. In such fermentation, natural yeast had been grown on fruits to produce wines. Normally fermented beer is made by using amylases of germinating grain to hydrolyse the grain starches to fermentable sugars¹². As depicted in **Figure 3**, Ethanol was also produced chemically by hydration of ethylene (IUPAC name: ethene) using phosphoric acid embedded on to porous silica gel as catalyst at 300 °C and 60-70 atm pressure²⁶.

Ethanol is produced in large scale by fermentation of different feed stocks like sugar crops, starchy materials or lignocellulosic biomass using various technologies with action of anaerobic and ethanologenic *Saccharomyces cerevisiae* like microorganisms^{27, 28}. The overall process of fermentative ethanol production involves: (1) preparing of solution of fermentable

sugars, (2) fermentation of sugars into ethanol at 25-30 °C for 6-72 hours depending on nature of substrate, cell density, physiological activity and yeast species and (3) separation and purification of ethanol. Based on various sources for bio-ethanol production, different processes are employed for obtaining fermentable sugars. Usually, for starch and lignocellulosic ethanol productions necessitate milling, liquefaction and saccharification processes to prepare fermentable sugars unlike direct fermentation of sugar juice in case of sucrose based feed stocks²⁹. Different amylases (α -amylase, β -amylase and glucoamylase) are utilised for obtaining fermentable sugars (Dextrose or D-glucose) from starch instead of energy consuming acid hydrolysis although acid hydrolysis has been implemented. Similarly,

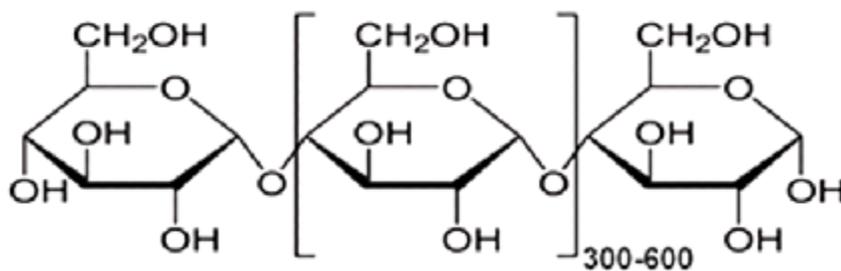


Fig. 1. Structure of amylose

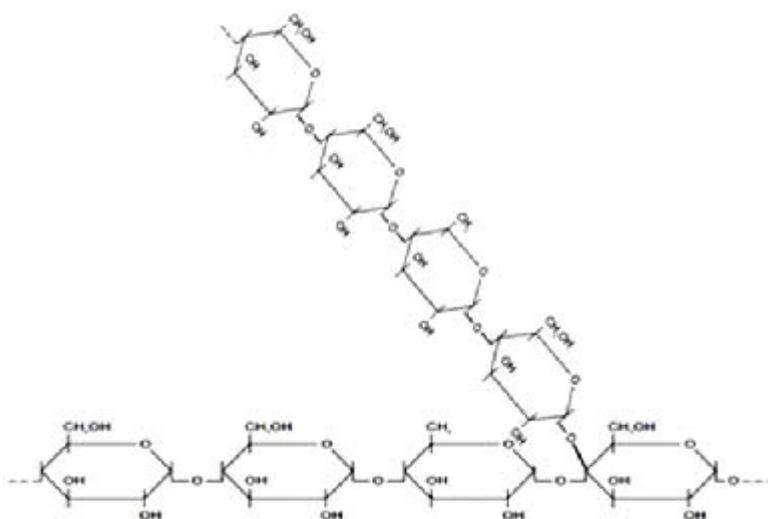


Fig. 2. Structure of amylopectin

cellulases are used for saccharification of cellulosic biomass where as xylanases are utilised for generating fraction of glucose from hemicelluloses to achieve comparatively higher yield of ethanol from complex hemicelluloses and lignocellulosic biomass¹².

Some time sugar crop based ethanol production is advantageous for its low cost of production. The short supply during non-seasonal months is main problem associated with sucrose based feedstock. Generally, sugar cane based ethanol production involves crushing of stalks after initial process after harvest by specialised rollers to extract sugar juice. Calcium hydroxide is added to the juice extract to precipitate the fiber and sludge and the mixture is filtered. The filtrate solution is evaporated to crystallise sugar. The non crystallised sugar mixture is called blackstrap molasses which is used for ethanol production by fermentation using *Saccharomyces cerevisiae* at 33-35 °C. Ethanol can be directly produced from processed sugar juice. Similarly, sugar beets and sweet sorghum are also used for ethanol production²⁹. Two different pretreatment processes are normally employed for production of ethanol from

starch biomass like corn namely: (i) dry grinding and (i) wet milling³⁰. In dry grinding, corn is processed in sequence of steps viz. (i) milling or mechanical grinding by hammer mills into fine powder (ii) liquefaction i.e. gelatinisation of corn starch by cooking of powdered corn and water slurry at 85 °C and subsequent heating at 150 °C for an hour on addition of α -amylase and adding more α -amylase to the cooled intermediate at 85 °C for 1 hour (iii) saccharification or enzymatic hydrolysis of corn starch to dextrose at room temperature by gluco-amylase (iv) fermentation of sugars into ethanol for 40-50 hours operated in

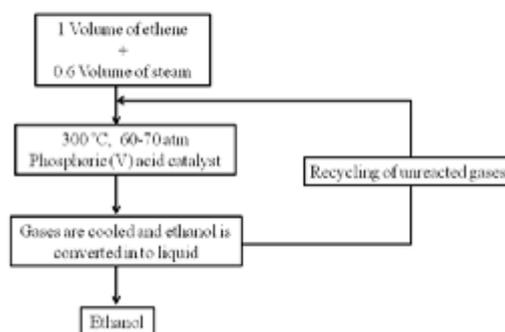


Fig. 3. Chemical synthesis pathway of ethanol

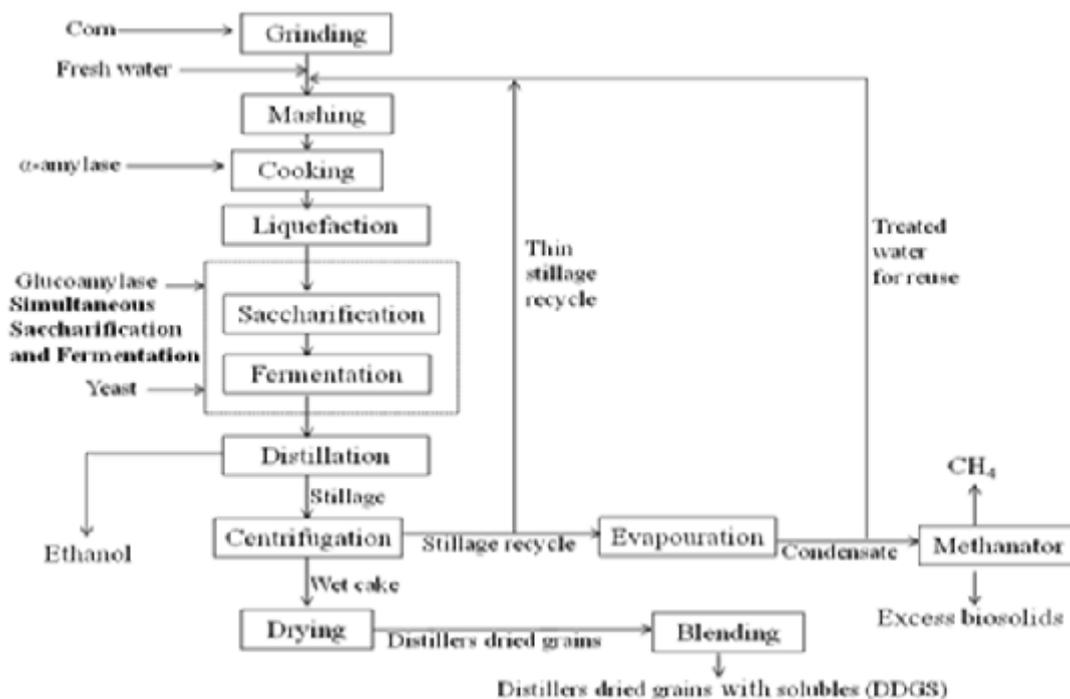


Fig. 4. Diagrammatic representation of corn dry milling process

either batch or continuous process, and (v) distillation and recovery ethanol²⁹. A typical dry grinding plant has incorporation of simultaneous saccharification and fermentation (SSF) to lower contamination and cost³¹. Flow sheet of corn dry milling process has been represented in **Figure 4**. By-products of the process like distillers dried grains with solubles (DDGS) containing protein, oil and fibre, Carbon dioxide and others products are used as animal feeds. In wet milling process of corn, initially shelled corns are supplied into mechanical cleaners in wet mill to remove undesirable parts like stones, meal, pieces of cobs, husk and sticks. The detail of the process has been represented in **Figure 5**. The clean corns are

passed to steep tank where these are soaked in dilute sulphuric acid at 52 °C for 24-48 hours. This step soften corn kernel and release starch with removal of soluble components. On passing and processing of the intermediate in series of tanks, parts of corn kernels like germ, gluten, and fiber are separated from starchy materials. Starch is mainly separated by centrifugation. The separation generates primary products like starch and starch derived primary products (such as high fructose content corn syrup and ethanol), corn oil, and corn gluten. These by products, hull and steep liquor are sold as animal feed. The processed corn starch is liquefied at pH 5.8-6.2 by α -amylase. About 20-100 ppm of Calcium is often used to stabilise α -

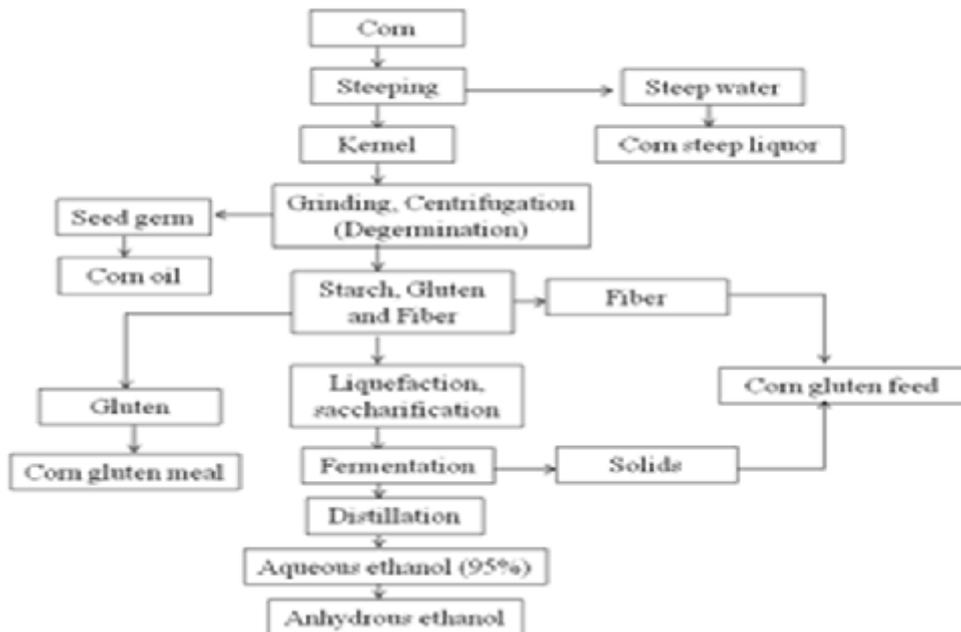


Fig. 5. Diagrammatic representation of corn wet milling process

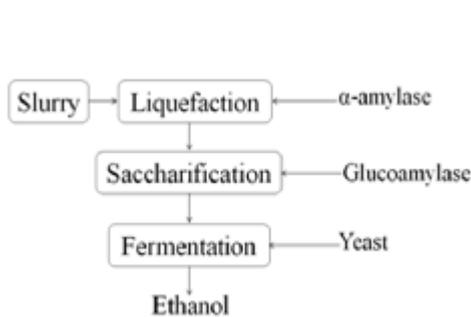


Fig. 6. Diagrammatic representation of SHF Process

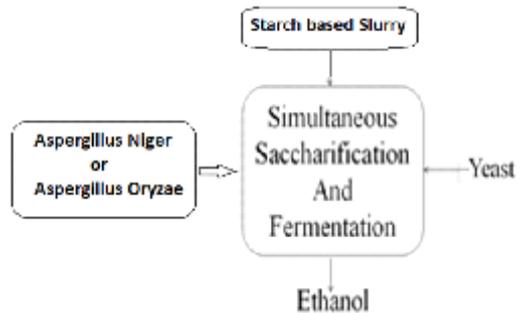


Fig. 7. Diagrammatic representation of microbial SSF process

amylase. Liquefaction is followed by saccharification at pH of 4.5 and 65°C using glucoamylase. The enzymatically produced sugars are then fermented in continuous process by *S. Cerevisiae* for 20-60 hours²⁹. The wet milling process is relatively more flexible than dry milling process of corn starch¹². The comparative disadvantages of conventional processes of ethanol production are those are non environment friendly, heat involving processes or costly process due to involvement of enzymes.

Cellulosic bio-ethanol production and drawbacks

Cellulosic ethanol produced from second generation feedstock could be an alternative to the ethanol produced from energy crops. But, conversion of non-edible lignocellulosic biomass constituted by cellulose (40-50%), hemicelluloses (20-30%), and lignin (20-30%) into ethanol is more complex, expensive and yield relatively less ethanol compared to starch hydrolysis and fermentation¹². Lignocellulosic ethanol is produced either by²⁹;

- (i) Biochemical conversion/sugar platform and
- (ii) Thermochemical conversion/syngas platform (not commercialised): Involves gasification of lignocellulosic biomass and catalytic or microbial conversion of syngas into ethanol.

Lignocellulosic ethanol production involves intense pre-treatment by physical, biological, chemical or combination of these followed by saccharification into pentose sugars (like xylose, rhamnose and arabinose) and hexose sugars (like glucose, mannose and galactose) and fermentation of sugars obtained¹². Among the various pre-treatment methods like use of dilute acid of H₂SO₄ or HCl, alkaline (like NaOH or CaOH), liquid ammonia (ammonia fiber explosion), SO₂, CO₂, Sulfite (Sodium bisulfite, Calcium bisulfite or Magnesium bisulfite) and steam explosion, use of cheaper acids like H₂SO₄ in large quantity in an efficient acid recycling system achieves economic operation³². But, steam explosion is an economical, efficient, eco-friendly and offers complete sugar recovery of all pre-treatment processes³³.

Enzymatic hydrolysis (EH) is also an extensively studied pre-treatment. It employs different fungal cellulolytic enzymes for conversion of cellulose into glucose namely;^{12,29}

1. Endo-1, 4-β- glucanases (EC 3.2.1.4), hydrolyse soluble and insoluble 1, 4-β-

glucan substrates.

2. Exo-1, 4-β-D-glucanases hydrolyze D-cellobiose.
3. β-D-glucosidases (EC 3.2.1.21) which catalyse cellobiose and soluble cellodextrins to release D-glucose units.

All these biodegradable and synergistic enzymes efficiently decrystallise and hydrolyse cellulose to achieve high yields under mild conditions with low supply of catalyst^{29, 32}. However, enzyme hydrolysis yield is affected by thermostability of enzyme, effect of pH and temperature of system, nature of substrate and enzyme, substrate concentration, etc. This problem was minimized when saccharification and fermentation processes are employed simultaneously using co-culture of *Aspergillus niger* or *Aspergillus oryzae* and yeast^{10, 15}. Moreover, simultaneous saccharification and fermentation starch is cheaper, efficient and higher ethanol yielding process compared to cellulosic ethanol production.

SSF process, current trend for bio-ethanol production

Most adopted process for bio-ethanol production now is Separate hydrolysis and fermentation (SHF) and Simultaneous saccharification and fermentation (SSF). Separate hydrolysis and fermentation (SHF) process has been fairly implemented for ethanol production and is basically starch based ethanol production process. In this process, starch is initially catalysed by the action of amylolytic enzymes viz. α-amylase (for liquefaction) and glucoamylase (for saccharification). The process has been described in **Figure 6**. The process can be accomplished by fermentation in separate vessels. Major disadvantage with this process is inhibition of enzyme activity due to accumulation of hydrolysed sugar. It is also an expensive and time consuming process^{12, 29}. Only microbe based Simultaneous saccharification and fermentation (SSF) process was innovated by Gulf Oil Company, US and Arkansas University⁷ which solves the problems associated with separate hydrolysis and fermentation (SHF) process. In microbe based SSF process, both saccharification and fermentation are achieved simultaneously in a single vessel at optimised enzyme activity with least accumulation of sugars^{34, 35}. To make the process less time

consuming, two organisms with synergistic relationships are co-cultured together in the same vessel. The process has been diagrammatically represented in **Figure 7**. This process assures less contamination by microorganism as ethanol is produced in the single tank³⁶. Many reports on bio-ethanol production stating that SSF is superior in terms of ethanol yield and productivity than bio-ethanol produced by SHF process¹². Simultaneous Saccharification and Co-fermentation (SSCF) is another alternate process to SSF which allows pentose fermentation. In SSCF configuration, microorganisms used for fermentation should have similar operating pH and temperature. Successful co-culturing of *C. shehatae* and *S. Cerevisiae* by SSCF process was successfully reported by Cardona and Sanchez³⁷.

Consolidated bioprocessing (CBP) is also known as direct microbial conversion (DMC) and it integrates maximum biotransformation of biomass into ethanol in a single reactor by a single microorganism community³⁸. It promises use of one microbial community in maximum production of cellulases and fermentation, i.e., cellulase production, cellulose hydrolysis, and fermentation are accomplished in one step. This process saves operation expenditures needed for enzyme production within the process^{36,39}. Thermophilic cellulolytic anaerobic bacteria like *Thermoanaerobacter ethanolicus*, *Clostridium thermohydrosulfuricum*, *Thermoanaerobacter mathranii*, *Thermoanaerobium brockii*, *Clostridium thermosaccharolyticum* strain, etc have been explored for bioethanol production by consolidated bioprocessing (CBP). These anaerobic bacteria are superior than conventionally used yeasts for bioethanol production for their ability to direct conversion of various cheaper biomass feedstocks for bioethanol production at extreme temperature. Low bioethanol tolerance of thermophilic cellulolytic anaerobic bacteria (<2%, v/v) is a major problem for their bioethanol production^{35, 39}. Cell surface modification by genetic engineering of the yeast *K. marxianus* has been explored for the production of cellulolytic enzymes on the cell surface. Recombinant *K. marxianus* strain produce both endoglucanase and β -glucosidase on the cell surface survive at 48 °C and produce ethanol from cellulosic material β -glucan with a yield of 0.47 g

ethanol from one gram of carbohydrate²⁹. Ethanol production from syngas is emerging technology in which syngas is produced from various biomass including lignin through a process called gasification. Gasification is a thermochemical conversion process where biomass comprising mainly carbonaceous materials like oil or coal is reacted with oxygen, air or steam to produce syngas (also called producer gas). The syngas is composed of mixture of gases like CO, H₂, CO₂, CH₄ and N₂ in various proportions³⁷. The clean syngas is used for metal-catalytic or bio-catalytic methods of production of ethanol and other biofuels like methanol, hydrogen. Rhodium, Cobalt, Molybdenum are some components of catalysts used. Syngas catalytic conversion is energy intensive process requiring catalytic conversion under high pressure and temperature²⁹.

Microorganism involved in SSF process

Numerous bacteria and fungi which can produce α -amylases and glucoamylase are used for bio-ethanol production by SSF process. In general, \pm -amylase is produced by most of *Bacillus* species like *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus megaterium*. Amylases are also produced by various fungus species like *Aspergillus oryzae* and *Aspergillus niger*. Details of microorganisms participated in SSF processes are presented in **Table 3**. It has been reported that *Aspergillus* amylase produces more degraded sugar than *Bacillus* amylase. In the similar way, glucoamylase can be produced by bacterial *Rhizopus* species, *Endomyces* species and few *Bacillus* species. Fungal resources for the same are *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus saitari* and *Aspergillus awamori*⁴⁰. For fermentation process of ethanol production, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are most promising microorganisms.

Separation of Bio-ethanol after SSF process

Ethanol (or bioethanol) is mainly produced by fermentation using yeast in industries. After SSF, ethanol can be produced along with different by-products like esters, organic acids, or higher alcohols as starch derivative, and lignin derivative like cyclic and heterocyclic compounds. These by-products need to be removed to obtain pure ethanol. A typical method of separation and purification of these by products is distillation.

Distillation method of purification requires much energy for recover and dehydrate ethanol. Distillation utilises the differences of volatilities of components in a mixture. The fundamental principle of distillation is that by heating a mixture, more volatile components mainly ethanol with low boiling point vaporises to form vapour phase. Condensation of the vapour phase is obtained in liquid phase as distillate in a separate chamber. The distillate is mostly an azeotropic mixture composed of 95.5% alcohol and 4.5% water. As the distillate usually carries impurities with similar boiling points to ethanol, it is limitation of this separation technique. Moreover it is expensive, energy intensive separation^{29, 41}.

Some of proposed alternative separation and purification of ethanol to distillation are (i) non-heating fractional distillation by ultrasonic irradiation, (ii) oxidation of impurities by ozone and (iii) adsorption of impurities by activated carbon or zeolite. The purification technique for ethanol is less studied area and search for alternative methods to replace distillation are still under progress. It is expected that typical water purification techniques such as adsorption, ozonation, and gas stripping could be used to purify ethanol⁴¹.

Ozonation

Ozone (O₃) has strong oxidation potential which makes it capable of decomposing various compounds. Decomposition of compound increases volatility, biodegradability, and decrease toxicity. As ethanol oxidation does not occur under the atmospheric condition, ozone can be used to remove impurities with least damage on ethanol⁴¹. Major problem associated with ozonation is generation of ozonolysis by-products and lodging of non oxidisable compounds. These compounds should be removed after ozonation by post-ozonation treatments.

Adsorption

Adsorption utilises large surface area of adsorbent to absorb compounds depending on their physical and chemical properties. Usually, bigger particles and compounds having similar polarity to the adsorbent surface are adsorbed more. For purification of ethanol, non-polar surface with varying pore distribution are favourable as ethanol is polar compound mixed with varying particles of impurities. From water treatment, activated carbon and activated alumina are the

most expectable adsorbents⁴¹.

Gas stripping

Separation by gas stripping technique utilise the differences of volatilities among compounds. The separation efficiency is simply governed by Henry's law constant.

$$H = \frac{P_{\text{vap}}}{C_{\text{sat}}}$$

Where, H = Henry's constant (moles/L atm)

P_{vap} = Partial pressure of a pure compound (atm), and

C_{sat} = Saturation concentration of the pure compound in the liquid phase (moles/L or mg/L)

Henry's law constant varies depending on the vapour and liquid phases. As the compounds with lower boiling points are stripped more easily, major impurity in ethanol like acetaldehyde is stripped more easily⁴¹.

Quantitative estimation of Bio-ethanol

There is different analysis techniques used to determine quality of ethanol and optimise steps in production. Some of common analytical methods are used earlier for quality control of alcoholic beverages use distillation and picnometry, electronic densimetry, colorimetry, photometry, by fluorescent chemical sensor. At present, advanced analytical techniques are used. These can be either (i) Chemical analyses for identification and quantification of components of ethanol or (ii) sensory analyses. Chemical analyses includes Infrared spectroscopy (IR), Near-infrared (NIR) spectrometry, high performance liquid chromatography (HPLC), Gas chromatography (GC) or mostly Gas chromatography with flame ionisation detector (GC-FID), and mass spectrometry (MS). The typical sensory analyses technique is olfactometry. Olfactometry coupled with GC is used for better flavour analysis of alcoholic beverages⁴¹⁻⁴³.

Gas chromatography (GC) has been used for ethanol analyses as impurities in ethanol and ethanol are basically volatile. Ethanol sample is injected into heated injection port where ethanol is vaporises. The sample vapour passed through column packed with adsorbent or absorbent. Within the column, components in sample are separated based on physical and chemical property. The concentration of each component is measured at the end of column by a detector.

Usually, flame ionisation detector is used to analyse ethanol. Results are interpreted from a chromatogram generated by data system attached to the apparatus⁴⁴. Gas chromatography-mass spectrometry (GC-MS) is used for fast ethanol analysis with its simultaneous separation and identification capacities.

High performance liquid chromatography (HPLC) uses liquid as the mobile phase unlike gas in case of Gas chromatography (GC). Though non-volatile compounds or heat sensitive compounds can be analyzed by HPLC, it is more expensive and less sensitive compared to GC. The ethanol analysis with HPLC has been studied extensively⁴⁵.

Infrared spectroscopy (IR) utilises infrared adsorption characteristics of different compounds. Varying wavelengths of infrared are passed through the liquid sample, and the absorbability of infrared by components of compound at different infrared wavelengths is measured. Results are interpreted from IR spectra generated by data system coupled with the apparatus. IR does not have as high resolution like GC or HPLC, but it is cheap with simple and quick analysis technique⁴⁶. This technique is utilised more for quality assurance and analytical purposes.

Olfactometry is a typical sensory analysis mostly integrated with GC. In GC-Olfactometry (GCO) system, a Gas chromatography column is connected to a separator where samples are directed into two paths, one of olfactometry and other of detector such as FID, PID, and MS. Olfactometry is a simple system with an open-end column, and a panelist that sniffs analytes coming from the column. The odour characteristics and intensity of the analytes are measured by panelist that corresponds with a peak in chromatogram. Olfactometry provides flavour data rather and is used for analysis of alcoholic beverage. GC is advantageous for its high resolution of analysis where as HPLC is useful for heat sensitive sample. IR is usually used for routine analysis for quality assurance. The olfactometry technique is useful for flavour analysis of alcoholic beverages⁴¹.

Advances and development through bio-ethanol

Ethanol is widely used as (i) sole solvent in production of perfumes and varnishes, (ii) preservative for biological specimens, (iii) agent in the preparation of flavourings, (iv) ingredient in

numerous medicines and drugs, disinfectant and in tinctures and (v) fuel or gasoline additive. Many U.S. automobiles manufactured since 1998 were equipped with either gasoline or E85 engine⁴⁷.

In 2013, US alone produced 13,300 million gallons while the second largest producer, Brazil, produced about 6,267 million gallons of ethanol. China has invested much in the production of ethanol and is now producing over 696 million gallons, becoming Asia's largest ethanol producer^{48,49}. Worldwide bioethanol production is expected to increase up to 100 x10⁹ litres in 2015⁵⁰. In the United States, dry milling and wet milling are the two primary processes used for production of ethanol. The U.S. ethanol industry is not only helping to meet demands for energy, it also helps to meet the growing food and feed needs of the world. In 2013 alone, US exported 9.7 million metric tons of distillers grains to China, Mexico, Canada, Vietnam and South Korea. The country also exported approximately 1.9 million metric tons of corn gluten feed and corn gluten meal to Ireland, Israel, Turkey, Morocco, Indonesia, Egypt, Chile, Colombia and Mexico⁵¹.

In Brazil, Ethanol Interministerial Council (CIMA) has increased the percentage of ethanol blended to gasoline from 20 to 25 percent in May 2013⁵². Likewise, most of countries are strategising the use of bioethanol produced from various feedstocks and mandated use of varying percentages of ethanol blended to gasoline. Japanese government proposed to use E-3 where as Indonesia and Philippines mandated use of E-10 and E-5 respectively¹². In France, 'Superethanol E85' was authorised to use as transport fuel. Similarly, In Sweden ethanol production from softwood was operated in mid 2004^{1,12}. India with initial blending of 5% practiced by the Oil Marketing Companies (OMCs) and use of E-10 mandating since October, 2008 in few states and four Union territories has proposed to blend 20% ethanol by 2017. It is thus expected a drastic increase in demand of fuel grade ethanol for which the sugar industries are allowed direct production of ethanol from sugarcane without creating problem in production of sugar. OMCs still faces short supply of fuel grade ethanol from sugar distilleries, which in turn demands need of alternate feed stock for ethanol production⁵³. Large amount of starchy

food wastes of potato, rice, wheat etc could be used as alternative sources for bioethanol production⁵⁴. Simultaneous Saccharification and Fermentation (SSF) of these sustainable starch rich wastes could efficiently produce bio-ethanol with least impact on environment.

Socio-economic aspects

In recent advances and development through bioethanol, studies were aimed to evaluate the social and economic aspects of ethanol production by many international organizations. Generally, bioethanol production cost is given by total annual costs of a system divided by amount of fuel produced. The total annual cost comprises of annual capital investments, operating and maintenance expenses, cost of biomass feedstock and electricity charges⁵⁵. The expenses for production are liable to decrease through time due to different reasons like installation cost, price of feedstock used and availability of feedstock, process improvement, etc which in turn affect the final price of fuel in the market⁵⁶.

The expansion of bioethanol industry and a like biofuels industries have been major socio-economic impact especially in developing countries. Generally, in most of developing countries bioethanol industry has been the major or only source of income to many farmers and increase job opportunities for both skilled workers, reduce regional income difference, reduce poverty and develop country's economy. Among developing countries, the Brazilian fuel-ethanol program (Pro-alcool) started in 1975, overseeing the reduction in regional income differences, and an increase in job opportunities for both skilled and unskilled workers, the sugarcane agro-industry has generated more than 700,000 jobs in the country⁵⁷.

Biofuel production leads to soaring of prices of agricultural products which has become major concern for governmental agencies and many nations. There is emphasis about careful monitoring of inflation of agricultural commodities caused by expansion of biofuels business⁵⁸. So, major efforts have been directed towards better use of non-edible, diverse agricultural products for bioethanol production⁵⁹. Large amounts of renewable agro-industrial wastes generated during industrial processing of agricultural products which

can be for economical production of biofuels⁵⁰. In US, one-third of each bushel of grain input for ethanol process is usually supplied to the animal feed market in the form of distiller's grains, corn gluten meal and corn gluten feed. These nutrient rich co-products are used to feed beef cattle, cows, poultry, swine, and fish. In 2013-14 marketing year, about 39.2 million metric tons of high-quality feed having estimated market value of U.S \$7.2 billion was produced by the US ethanol industry, making the industry one of the largest feed producing sector in the US. There is no doubt that ethanol industry is well positioned to meet the increasing demand of renewable fuels in the near future as well as to help satisfy growing global demand for food and feed⁶⁰.

CONCLUSION

The review clearly describes the concept and potential of cost effective microbial SSF process for starch based bio-ethanol production in the background of the feasibility of other processes. The process so-called Simultaneous Saccharification and Fermentation (SSF) was incited with expensive enzymatic hydrolysis with simultaneous fermentation and was virtually similar as for the separate process. The process has been modified by combining two separate processes in one vessel in order to reduce the time and increase the efficiency of the overall process. The presence of yeast or bacteria along with enzymes or enzyme containing microorganism minimizes substrate inhibition effects by reducing the sugar accumulation in the vessel. The presence of ethanol in the broth makes the mixture less susceptible to unwanted microorganisms contamination and hence helped in increasing the overall ethanol yield and productivity using the SSF process. Such developed process with improved hydrolysis-fermentation efficiency could help in significant reduction of ethanol production costs. Such technological advancement towards green and clean bio-ethanol production will definitely contribute in reducing the fossil fuels dependency for future energy needs and hence eliminating the chances of air pollution caused due to combustion of petroleum based derivatives.

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