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An Innovative Approach towards Economic Bio-ethanol Production from Starchy and Ligno-Cellulosic Biomass through Simultaneous Saccharification and Fermentation (SSF)

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ABSTRACT

Keywords

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For the last few decades, fossil fuels played most dominant role as an important energy resource in the enormous energy demanding scenario throughout the world. However, as conventional fossil fuel resources are limited, excessive exploitation of such fossil fuel comes up with major global problem of energy resource scarcity. In such scenario, alternative and renewable sources of energy such as solar, wind, biofuel gained huge attention from governments of many countries across the world. Among these alternate energy sources, bio-ethanol was proved to be a most promising energy source with various added advantages. Conventional indigenous raw material for bio-ethanol production includes sugarcane, molasses and corn based materials which became highly popular for bio-ethanol production although amount of produced bio-ethanol can hardly meet the current global demand for biofuel. Extensive search for most promising feed stock for ethanol production pioneered the use of lignocellulosic biomasses and starch based materials. For successful conversion of starch and lignocellulosic material to bio-ethanol, microbial single stage simultaneous saccharification and fermentation (SSF) approach has been successfully evolved in recent years with outstanding potential to meet global need bio-ethanol.

Introduction

To facilitate transportation, heating and industrial processing, energy demand has been increased drastically in recent years. For the last few decades, basically fossil fuels and its derived products were utilized randomly as energy resource for transportation and industrial purposes. Unlike fossil fuels, ethanol is considered as a renewable energy source, produced from the fermentation of sugars and can be

utilized as the replacement of gasoline in different countries of the world (Sharma *et al.*, 2007). Most commonly used feedstock for ethanol production are mainly agricultural based raw materials associated with sugar crops (sugar cane), starch-containing plants (corn, wheat, potato) and lignocellulosic biomass. Sugarcane, sugar beets and molasses are also feasible for ethanol fermentation and have been used

extensively (Raposo *et al.*, 2009). Being an industrial waste, molasses contain high amount of impurity which necessitates a long pretreatment stages. In order to make most efficient, simple and cost effective process, starch based agricultural residues and lignocellulosic biomass gained huge attention and interest for bio-ethanol production. Extensive research is being conducted on liquid bio-fuel production from starchy waste biomass such as waste potato, potato peel waste, orange peel waste, banana peel waste and lingo-cellulosic substances such wood chips, leaf litter biomass wastes etc. Agricultural wastes are cost effective, renewable and abundant. In order to select the cost effective process, lignocellulosic biomass has been the primary choice for ethanol fermentation. But lignocellulosic biomass has to undergo time consuming and effective pre-treatment process prior to fermentation which includes removal of lignin, hemi-celluloses followed by the hydrolysis of cellulose. However, research effort to reduce the production cost, complexity of the process and time of bio-ethanol production was successful when starchy based raw materials were introduced in simultaneous saccharification and fermentation (SSF) process with improved starch hydrolysis efficiency. The SSF process was first developed by Gulf Oil Company, US and the University of Arkansas. Bio-ethanol production using the same process has been successfully implemented by different researchers like (Powabowska *et al.*, 2014, Arasaratnam *et al.*, 2012) using different starch based material. Among all, researchers like Powabowska *et al.* (2014) implemented potato and rice based starchy material for production of bio-ethanol whereas lignocellulosic materials were utilized by researchers like Ballesteros *et al.* (2004). Most of the researchers adopted expensive and time consuming commercial enzymatic

approach for initial conversion of polysaccharide to simple sugars in SSF process. To improve hydrolysis efficiency, reduce the production time and cost of the process, microbe based SSF process has been developed. Some specific research effort (Itelima *et al.*, 2013) was successfully directed towards such improved microbial single stage, single vessel SSF process. For lignocellulosic materials, proper pretreatments to remove lignin and hemi-cellulosic material can significantly improve overall efficiency of the process. The biggest advantage of such SSF process is it prevents the accumulation of simple sugars like glucose, normally generated by first stage hydrolysis process and prevents the process from substrate inhibition effects. Hence it became transparent that microbe based SSF process using lignocellulosic and starch based waste material can be evolved with a novel and promising approach towards cost effective, environment friendly, large scale industrial process for bio-ethanol production.

Biological Process for Bio-ethanol Production

The term bio-fuel is attributed to any alternative fuel that derives from organic material, such as energy crops (corn, wheat, sugar cane, sugar beet, cassava, among others). Bio-ethanol is a fuel which is produced from the plant sources like corn, maize (USA), sugarcane (Brazil), sugar beet (Europe) (Dey *et al.*, 2015). Higher content of bio-ethanol production is mainly done from sugarcane. Crop residues like rice straw, rice husk, corn Stover, corn cobs or waste biomass like potato peel waste, orange peel waste, banana peel waste, food waste, livestock waste, paper waste, construction-derived wood residues are largely being used for the production of bio-ethanol. Present state for the bioconversion of

ethanol is based on three different types of carbohydrates like cellulosic, starchy, and sugary. Different carbohydrate sources which are normally utilized for bio-ethanol production have been represented in Table 1.

The use of waste product like agriculture waste including rice straw, manure, bagasse and household cooked food waste including rice water (Pitch), potato, corns, etc has been the prime object of current trends of microbial bio-ethanol production. The overview of the bio-ethanol production process based on three different sources has been represented in Figure 1.

Basically Bio-ethanol production process is considered as biological process as the main and final stage of ethanol conversion from sugars is based on microbial fermentation technique although initial pretreatment process may be based on chemical process or biological process. Chemical based initial pretreatment techniques are non environment-friendly techniques as it comprises the use of harsh acids and alkalis which leads to the generation of heat and unwanted chemical byproducts. In this respect, biological pretreatment process is much more advantageous and reaction specific. In biological process, enzymatic process for initial conversion simple sugars from Polysaccharides based material is highly competent although the process is extremely expensive and time taking. The overall process from initial pretreatment to final fermentation can be efficiently configured using only microbes. This microbial based hybrid simultaneous saccharification and fermentation process is less expensive, time taking and space consuming. The advantages and features of such newly developed process have been highlighted in this review article. The detail classification of the process has been represented in Figure 2.

Starch and Cellulose Hydrolysis Based Bio-ethanol Production

Carbohydrate which is the major contributor of energy, normally derived from plants and animal. Among all Carbohydrate, Starch and cellulose are large molecular weight polysaccharides which are constructed by joining of large units of monosaccharide units by glucosidic bond. Starch is largely present in plants and insoluble in cold water but can be hydrolyzed by using hot water. It generally found in the leaves, seeds, roots and fibres as food reserve. Composition of starch is basically a mixture of two structurally different polyglucans – amorphous amylose (20-30%) and crystalline amylopectin (75-80 %) molecules. The details regarding its structure and formulation have been represented by Das *et al.* (2007). For bio-ethanol production, starchy potato waste is considered as potential feedstock. 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 (Limatainen *et al.*, 2004, Adarsha *et al.*, 2010), could be used for cheaper production of bio-ethanol. Just like starch, cellulose is a polysaccharide which consist of a linear chain of several hundreds of $\beta(1\rightarrow4)$ linked D-glucose units. It has no branching in chain. The cellulose content of cotton fiber (90%) and that of wood is 40–50% (Yin, 2010). Corn stover, *Panicum virgatum* (switch grass), *Miscanthus* grass species, wood chips, sugar cane bagasse, wheat and rice straw are most popular cellulosic materials for ethanol production. Production of ethanol from lingo-cellulosic materials requires initial pretreatment process to remove lignin and hemi-cellulosic material to achieve only cellulose based material. Next processing stages are required to make simple sugar monomer units from cellulosic

material to facilitate microorganisms to produce ethanol by fermentation. Details regarding every aspects of lingo-cellulosic

ethanol production have been represented in Cheng and Sun, 2002.

Table.1 Different Sources of Ligno-Cellulosic, Starch, Sugary Biomass for Production of Bio-ethanol

Ligno-Cellulosic	Starchy	Sugary
Rice straw, Corn stalks, Corn cobs, Bagasse, Cane filter cake, Kenaf stalk, Animal manure, Pineapple pulp, Wheat brain.	Cassava meal, Cassava effulent, Potato, Rice, Corn, peel waste	Molasses, Corn steep liquor, Pulp water liquor, Coconut water.

Fig.1 Overview of the Bio-Ethanol Production Process Based on Three Different Sources

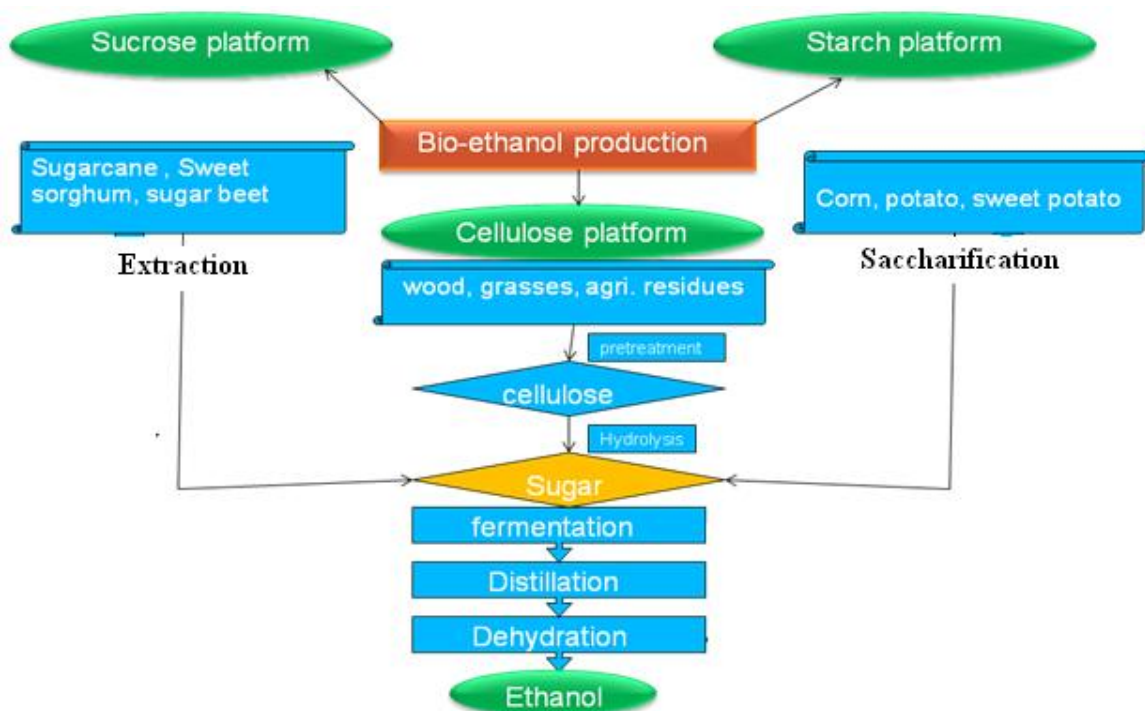


Fig.2 Classification of Overall Bio-Ethanol Production Process

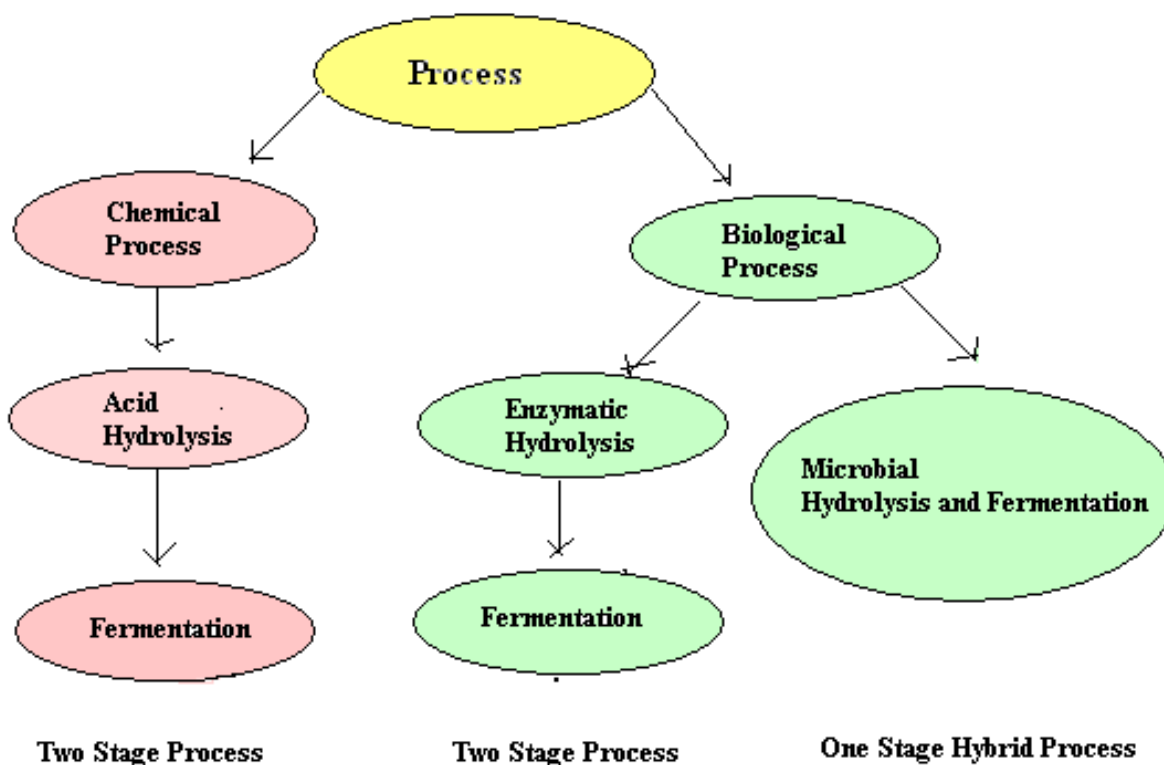


Fig.3 Schematic Representation of SHF Process

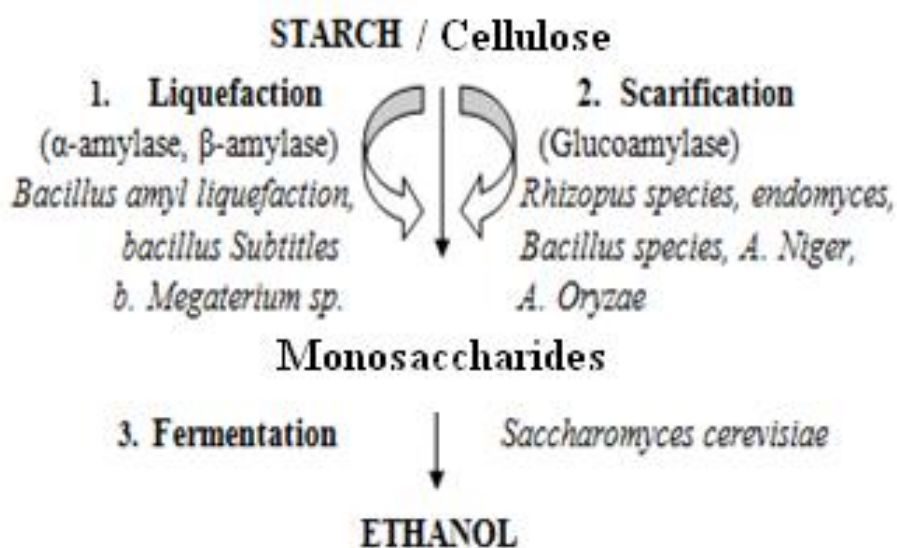
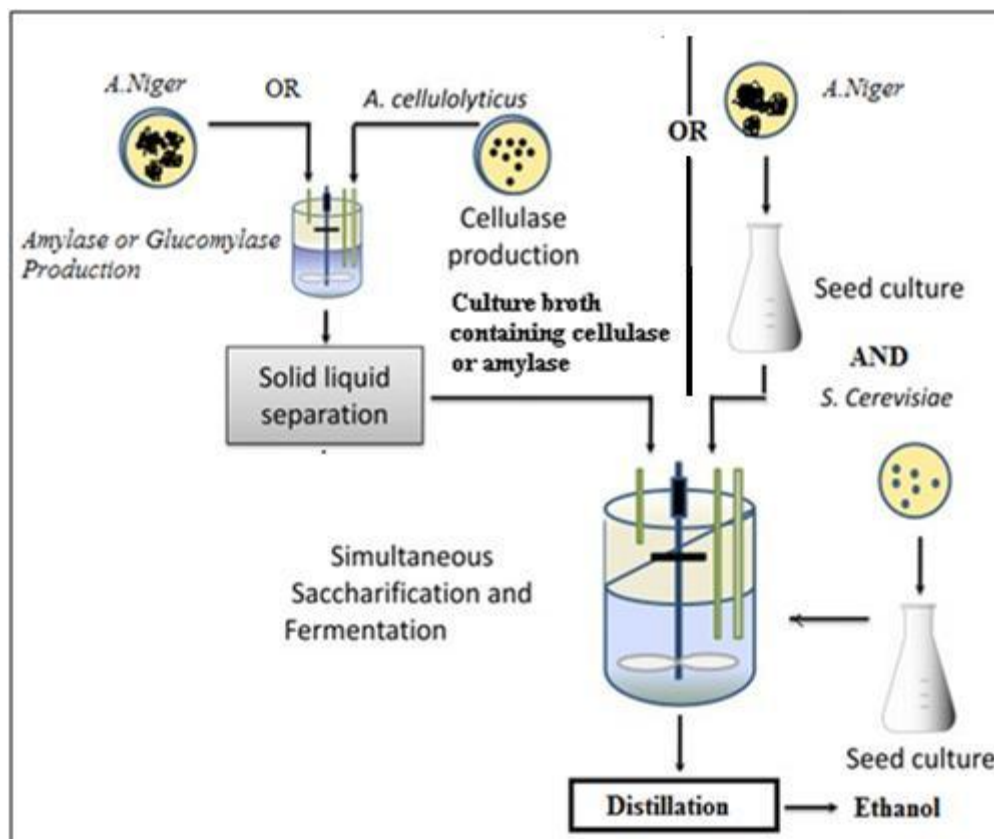


Fig.4 Diagrammatic Representation of SSF Process



Microorganism Participates In SSF Process of Bio-Ethanol Production

Starch molecule is basically hydrolyzed by the action of amylolytic enzymes: α -amylase (for liquefaction) and glucoamylase (for saccharification). There are numerous bacteria and fungi which can produce α -amylases and glucoamylase. In bacterial culture, α -amylase is mostly produced by *Bacillus* spp like *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus megaterium*. Amylases are also produced by various fungus species like *Aspergillus oryzae* and *Aspergillus niger*. In the similar way, glucoamylase can be produced by bacterial *Rhizopus species*, *Endomyces species* and few *Bacillus species*. In the second stage of the process *Saccharomyces cerevisiae* can be effectively used as it is the most promising

microorganism for ethanol production by fermentation. For cellulose, enzymatic hydrolysis is carried out by enzyme cellulase. Such enzyme can be effectively produced by bacteria like *Clostridium*, *Cellulomonas*, *Bacillus*, *Ruminococcus*, *Bacteriodes*, *Streptomyces* etc. The fungal species which can produce cellulases include *Sclerotium rolfisii*, *Trichoderma* Spp, few *Aspergillus* Spp. Rest fermentation step to produce bio-ethanol will be same just like starch based material.

Separate Hydrolysis and Fermentation (SHF)

The idea behind the most advanced research efforts towards SSF based bio-ethanol production was generated through analyzing disadvantages associated with separate hydrolysis and fermentation (SHF) process.

The process SHF consists of two distinct stages like starch or cellulose hydrolysis and glucose fermentation (as described in Figure. 3). Separate hydrolysis and fermentation (SHF) process has been successfully implemented for starch based ethanol production process. In this process, starch is initially catalyzed by the action of amylolytic enzymes for liquefaction purpose and glucoamylase for saccharification purpose.

The primary advantage of such configuration is that starch hydrolysis and sugar fermentation can be carried out separately which will minimize the interactions between these steps. However, α -amylase activities are normally inhibited by the accumulation of generated sugars during this process. Even instead of using commercial enzyme, using extracted enzyme from fermentation broth after microbial cultivation for initial scarification and second stage fermentation process for bio-ethanol production can be also carried out in SHF process. Even fully microbe based scarification and fermentation process instead of initial crude enzyme based scarification process also evolved in SHF process. SHF is basically an expensive and time consuming process therefore considerable efforts were directed to improve upon those problems including end-product inhibition problem associated with SHF process.

Efficient Simultaneous Saccharification and Fermentation (SSF) Process

To improve the existing production of bio-ethanol by SHF process, concept for simultaneous saccharification and fermentation was developed. Initially the concept of the process was configured with enzymatic hydrolysis of cellulose or starch and simultaneous fermentation, so-called simultaneous saccharification and

fermentation (SSF). The sequence of the process for SSF is virtually the same as the SHF process except that saccharification and fermentation steps are combined in a single stage in a single vessel. The presence of ethanol in this process makes the mixture less susceptible to contamination by unwanted microorganisms. In this process after liquefaction by α -amylase, immediately the enzyme glucoamylase is added to the slurry and consequently with yeasts. In microbe based advanced SSF process, both saccharification and fermentation are achieved simultaneously in a single vessel at optimized enzyme activity with least accumulation of sugars (Bothast and Schlicher, 2005). To make the process less time consuming, two organisms with only synergistic relationships are co-cultured together in the same vessel. The process has been diagrammatically represented in Figure 4. Simultaneous Saccharification and Cofermentation (SSCF) is another alternate process to SSF which facilitates pentose fermentation. Consolidated bioprocessing (CBP) is a similar approach which facilitates direct microbial conversion (DMC) and it integrates maximum biotransformation of biomass into ethanol in a single reactor by a single microorganism community (Carere *et al.*, 2008).

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