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Technologies and Factors Affecting Bioethanol Fermentation and its Commercialization

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Abstract

Fossil fuels are a major contributor to climate change and environmental pollution, and as the demand for energy production increases, alternative sources are becoming more attractive. Bioethanol reduce reliance on fossil fuels and can be compatible with the existing fleet of internal combustion engines. Bioethanol is typically produced via microbial fermentation of fermentable sugars. Traditional feedstocks (first-generation) include cereal grains, sugar cane, and sugar beets. However, due to concerns regarding food sustainability, lignocellulosic (second-generation) and algal biomass (third-generation) feedstocks have been investigated. Technologies such as Simultaneous Saccharification and Fermentation (SSF), Separate enzymatic Hydrolysis and Fermentation (SHF) and Fed- batch Fermentation for bioethanol hold tremendous potential for the production of bioethanol. The aim of this review focuses on the technologies and factors affecting bioethanol production and its commercialization.

1. Introduction

Bioethanol is among the most important products obtained for human needs through microbial sources. A large number of industrial and analytical processes in the area of industrial, environmental and biotechnology utilize bioethanol at some stage or the other. Current developments in biotechnology are yielding new applications for bioethanol. Technologies such as Simultaneous Saccharification and Fermentation (SSF), Separate enzymatic Hydrolysis and Fermentation (SHF) and Fed- batch Fermentation for bioethanol hold tremendous potential for the production of bioethanol. It can be of special interest to know some factors affecting bioethanol production to enhance its commercialization. This review focuses on the technologies and factors affecting bioethanol production and its commercialization. Following a brief discussion on the use of bioethanol as fuel, alcoholic fermentation, high cell density, glucose effects, catabolite repression, factors that affect bioethanl production and factors militating against commercialization of bioethanol.

The most commonly used and widely researched of the biofuel is bioethanol. Bioethanol is a type of alcohol that can be produced primarily through fermentation of any feedstock containing significant amount of sugar **(Kongkiattikajorn 2012)**. Bioethanol has widespread use as a solvent of substance intended for human contact or consumption, including flavorings, colorings and medicine. In chemistry, it is both an essential solvent and a feedstock for the synthesis of other product such as acetic acid. It has long history as a fuel for heat and light, and more recently as a fuel for internal combustion engine **(Demirbas 2009)**. Bioethanol can be blended with petrol or burned in nearly pure form in slightly modified spark-ignition. A liter of Bioethanol contains approximately two thirds of the energy provided by a liter of petrol. Bioethanol production process only uses energy from renewable sources and there is no net Co_2 emission to the atmosphere, thus making ethanol an environmentally friendly energy source. In addition, bioethanol is ethanol which can be derived or produced from several different biomass and conversion technologies **(Archibong 2016)**. Technologies such as Simultaneous Saccharification and Fermentation (SSF), Separate enzymatic Hydrolysis and fermentation (SHF) and Fed- batch Fermentation have tremendous potential for bioethanol production. In addition to the convectional applications in analytical chemistry and for sterilization or inactivation of microorganisms in container used food products. Bioethanol found its application in all aspect of human life, however there is need for its commercialization especially in Africa.

In an attempt to commercialize bioethanol production a lot of factors should be considered such as the strain, nutrient, environmental factors, cost of production, pretreatment technology and efficient recovery plants **(Myat and Ryu 2016; Ude and Kgatta 2013)**.

2. Historyofbioethanol

The use of ethanol as a motor fuel has a long history as the car itself. It began with the use of ethanol in the internal combustion engine invented by Nikolas Otto in 1897. The use of bioethanol for fuel was widespread in Europe and the United States until the early 1900s when it became more expensive to produce than petroleum-base fuel, and so was ignored until the oil crisis of the

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1970s **(Balat and Balat 2009)**. Since the 1980s, there has been an increased interest in the use of bioethanol as an alternative fuel. During World War II, when wartime conditions changed economy and priorities, several ethanol-from cellulose (EFC) plants were built in Germany, Russia, China, Korea, Switzerland and the US, among other countries, to provide an alternative fuel source **(Balat and Balat 2009)**. Since the end of the war, competition from synthetically produced ethanol has forced many of these plants to close **(Lin and Tanaka 2006)**. Since April 2004, the first demonstration plant using lignocellulosic feedstocks in Canada has been in operation **(Tampier *et al*., 2004)**. In 2006, for the Global ethanol market, Brazil installed more than 300 bioethanol-producing plants, producing 15 billion liters per year and supplying 3 million cars with pure ethanol. In the US, there were more than 80 plants producing 10 billion liters per year. However, USA has taken the lead with an impressive increase in the annual ethanol production. On the other hand, ethanol production in Europe represented 5% of the global production in 2008, with Germany and France being the main producers **(Wang *et al*., 2012)**. Despite the fact that there is an increasing interest in utilizing alternative sources for ethanol fermentation, the main sources of ethanol production in Europe are cereals and sugar beet. On the other hand, the US has produced about 50 billion liters in 2012 alone, the majority of the world's ethanol is produced by the US and Brazil together reaching values from 62 to 87% of the global ethanol production **(Balat *et al*., 2008)**. The vast majority of US ethanol is produced from hydrolyzed starch derived from corn, while bioethanol in Brazil is primarily derived from sugar originated from sugarcane. China, India, Eastern Europe, Western Europe and Canada are following **(Sivamani and Baskar 2015)**. Despite the recent advancement on bioethanol production, Nigeria and some African countries have not joined in the production of commercial bioethanol. Knowing some technologies, factors affecting production and militating against commercialization of bioethanol and development of some of these technologies will be of great interest especially in African countries.

3. Use of bioethanol as fuel

Bioethanol is an attractive alternative fuel because it is a renewable bio-based resource and it is oxygenated, thereby providing the potential to reduce particulate emission in compression- ignition engines **(Jimoh *et al*., 2013).** Bioethanol can be used as a fuel in cars, either in its pure form or blended with gasoline. In Brazil, alcohol from fermentation is blended with petrol to form gasohol for driving motor vehicles **(Okafor 2007)**. Bioethanol is most commonly blended with gasoline in concentration of 10% bioethanol to 90% gasoline called gasohol in USA **(Kim and Dale 2006)**. Blends having higher concentrations of bioethanol in gasoline are also used in flexible-fuel vehicles that can operate on blends of up to 85% bioethanol **(Malça and Fausto 2006)**. The presence of oxygen in bioethanol improves its combustion and therefore reduces hydrocarbon, carbon monoxide, and particulate emissions. Bioethanol has high octane number (108), broad flammability limits, high flame speeds and high heats of vaporization. These properties allow for a higher compression ratio, shorter time and leaner burn engine. Octane number is a measure of the gasoline quality and can be used to prevent early ignition which leads to cylinder knocks. Higher octane number is preferred in internal combustion engines thus an oxygenated fuel such as bioethanol provides a reasonable antiknock value **(Festel 2008)**. The disadvantage of bioethanol is that it has low cetane number (between 5 and 15). Low cetane number causes longer ignition delays, allowing more time for fuel to vaporize before combustion start **(Festel 2008)**. The use of bioethanol blended fuel for automobiles can significantly reduce petroleum use and greenhouse gas emission.

4. Alcoholic fermentation

The reducing power NADH produced during glycolysis has to be transferred to an electron acceptor to regenerate ethanol and carbon (IV) oxide which takes place within the cytoplasm where acetaldehyde serves as the terminal electron acceptor **(Zamora, 2009)**. With respect to glycolysis, alcoholic fermentation contains two additional enzymatic reactions. Pyruvate is initially decarboxylated into acetaldehyde by pyruvate decarboxylase. The cofactors are thiamine pyrophosphate (TPP) and magnesium. Then, acetaldehyde is reduced into ethanol NAD+ consumed by glycolysis. In the case of *S. cerevisiae* and other yeast species, this process is called recycling NADH to NAD+. This reaction is catalyzed by the alcohol dehydrogenase using zinc as cofactor. Both final products of alcoholic fermentation, ethanol and carbon dioxide, are transported outside the cell by simple diffusion **(Zamora 2009; Ribereau-Gayon 2006; Aggelis 2007)**.

5. Simultaneous Saccharification and Fermentation (SSF)

The glucose molecules are still imprisoned in long chain of cellulose and hemicelluloses and therefore not readily available for fermentation. This is why hydrolysis is necessary. Simultaneous Saccharification and Fermentation (SSF) is a method for producing ethanol that utilizes enzymatic bond breaking parallel to the enzymatic activity as the yeast are fermenting the sugar. In this process, the glucose produced by the hydrolyzing enzymes is consumed immediately by the fermenting microorganism present in the culture. This is a great advantage of SSF compared to SHF, since the inhibition effects of cellobiose and glucose on the enzymes and yeast are minimized by keeping low concentrations of these sugars in the media. SSF gives higher reported ethanol yields from cellulose than SHF and requires lower amounts of enzymes **(Saliu and Sani 2012)** This process is often effective when combined with dilute acid or high temperature hot- water pretreatment. In the process, both saccharifying enzymes or microorganism and fermenting microorganism are co- inoculated into the pretreated biomass. The sugar or glucose produced in hydrolysis is simultaneously fermented to ethanol which greatly reduces the product inhibition to the hydrolysis. Advantages of SSF includes: increase of hydrolysis rate by conversion of sugar that inhibit the yeast and cellulase activity; lower enzyme requirement; higher product yield; lower requirement for sterile conditions since glucose is removed immediately and ethanol is produced; short process time; and less reactor volume **(Sun and Cheng 2002)**. Some researchers have reported better yield using different substrates **(Sharma *et al.*, 2007; Apiwatanapiwat *et al.*, 2013; Apiwatanapiwat *et al*., 2011; Murata *et al*., 2015; Oberoi *et al*., 2012; Kaewkrajay *et al*., 2014)**. Another advantage of SSF when compared with SHF is the process integration obtained when hydrolysis and fermentation are performed in a single reactor, which reduces the number of reactors needed. An important strategy in SSF is to have the optimum conditions for the enzymatic hydrolysis and fermentation as close as possible, particularly with respect to pH and temperature. However, the difference between optimum temperatures of the hydrolyzing enzymes and fermenting microorganism is still a drawback of SSF. The optimum temperature for cellulases is usually between 40^{o} C and 50^{o} C, whereas *Saccharomycescerevisae* has an optimum temperature between 30^{o} C and 35^{o} C and is practically inactive at 40^{o} C and above. The development of thermotolerant yeast strain is expected to improve the performance of SSF **(Techaparin *et al.,* 2017)**.

6. Separate enzymatic Hydrolysis and Fermentation (SHF)

In this process, pretreated lignocelluloses are hydrolyzed to glucose and subsequently fermented to ethanol in separate units. The major advantage of this method is that it is possible to carry out the cellulose hydrolysis and fermentation at their optimum conditions. The optimum temperature for cellulase is usually between 40° C and 50° C, depending on the cellulase producing microorganism **(Prasad 2007)**. However the optimum temperature for most of the ethanol producing microorganism is between 30 and 35° C.
Inhibition of cellulase and other enzymes activity by released sugar, mainly cellobiose and glucose is the main drawback of SHF. At a cellobiose concentration as low as 6 g/l, the activity of cellulase is reduced by 60 %. On the other hand, glucose is a strong inhibitor for β-glucosidase. At level of 3 g/l of glucose, the activity of β-glucosidase is reduced by 75 %[30]. Another possible problem in SHF is that of contaminations. The hydrolysis process takes one to four days and a dilute solution of sugar always has a risk of microbial contaminations, even at rather high temperature such as 40° C and 50° C **(Taherzadeh and Karimi 2007)**.

7. Fed-batch fermentation

Fed-batch culture is, in the broadest sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the product(s) remain in the bioreactor until the end of the run. An alternative description of the method is that of a culture in which "a base medium supports initial cell culture and a feed medium is added to prevent nutrient depletion **(Ngibuini 2014; Kuhad *et al.*, 2010). It** is also a type of semi-batch culture. In some cases, all the nutrients are fed into the bioreactor. The advantage of the fed-batch culture is that one can control concentration of fed-substrate in the culture liquid at arbitrarily desired levels (in many cases, at low levels).
Generally speaking, fed-batch culture is superior to conventional batch culture when controlling concentrations of a nutrient (or nutrients) that affect the yield or productivity of the desired metabolite.In fed-batch fermentation, substrates and enzymes are added into reactors step wise as substrate is gradually degraded **(Kuhad *et al.*, 2010)**. Fed batch is expected to be a better procedure than batch in dealing with the situation of high solid substrate loading and enzyme concentration. Additionally, fed batch can generate high ethanol concentration for distillation resulting in a significant decrease in ethanol production cost.

8. High cell density (High cell concentration)

In a batch culture, to achieve very high cell concentrations, *e.g.* 50-100 g of dry cells/L, high initial concentrations of the nutrients in the medium are needed **(Shiloach and Fass 2005)**. At such high concentrations, the nutrients become inhibitory, even though they have no such effect at the normal concentrations used in batch cultures **(Yamanè and Shimizu 1984)**.

9. Glucose effect (Crabtree effect)

In the production of baker's yeast from malt wort or molasses, it has been recognized since early 1900s that ethanol is produced even in the presence of sufficient dissolved oxygen (DO) if an excess of sugar is present in the culture broth[35]. Ethanol is a main cause of low cell yield. Aerobic ethanol formation in the presence of glucose concentration is known as glucose effect or Crabtree effect. To reduce this effect, a fed-batch process is generally employed for baker's yeast production. In aerobic cultures of *Escherichia coli* and *Bacillus subtilis*, organic acids such as acetic acid, (and in lesser amounts, lactic acid and formic acid), are produced as byproducts when sugar concentration is high, and these acids inhibit cell growth as well as show deteriorating effect on the metabolic activities. The formation of these acids is called bacterial Crabtree effects **(Katie and Wei-Shou 2006)**.

10. Catabolite repression

When a microorganism is provided with a rapidly metabolizable carbon-energy source such as glucose, the resulting increase in the intracellular concentration of ATP leads to the repression of enzyme(s) biosynthesis, thus causing a slower metabolization of the energy source. This phenomenon is known as catabolite repression **(Katie and Wei-Shou 2006)**. Many enzymes, especially those involved in catabolic pathways, are subject to this repressive regulation. A powerful method of overcoming the catabolite repression in the enzyme biosynthesis is a fed-batch culture in which glucose concentration in the culture liquid is kept low, where growth is restricted, and the enzyme biosynthesis is depressed (**Katie and Wei-Shou 2006)**. Slow feeding of glucose in penicillin fermentation by *Penicillium chrysogenum* is a classical example in the category.

11. Factors that affects bioethanol fermentation

It is well known that the ability of yeast to produce ethanol depends on many factors such as strain, macro and micronutrient and environmental factors such as pH and temperature. Temperature has many effects on yeast such as growth rate, viability, rate of ethanol fermentation, length of lag phase, activity of enzyme and membrane function. Carbon and nitrogen are the main essential nutrients in fermentation media. Nitrogen is necessary for yeast growth and influence the rate of ethanol production and ethanol tolerance (**Deesuth *et al.*, 2012; Dasgupta *et al.*, 2013; Fakruddin *et al.*, 2012; Vohra *et al.*, 2014; Yuangsaard *et al.*, 2013)**. Apart from carbon and nitrogen sources, micronutrients or trace elements are also important factors for promoting cell growth and ethanol fermentation. Zinc ion and Magnesium ion were reported as trace element for yeast growth and ethanol fermentation **(Tograepi *et al.*, 2012; Yuan *etal.*, 2017)**. Zinc ion affects both cell growth and yeast metabolism. Magnesium ion is involved in physiological function, growth, metabolism and enzyme activity of yeast. It is a cofactor of some enzymes in yeast. Magnesium ion reduces the proton, especially anion permeability of the plasma membrane by interacting with membrane phospholipids, resulting in stabilization of the membrane bilayer **(Walker, 2004; Ude and Kgatta 2013)**. Manganese ion is important in the metabolism of yeast as a part of some enzymes relating to ethanol fermentation such as pyruvate carboxylase.
Other factors that affect ethanol production includes; organic acids such as acetic acid and accessible surface area of cellulose. Acetic acid is derived from the acetyl group in hemicelluloses. Several studies have shown a good correlation between the pore volume and population (accessible surface area for cellulose) and enzymatic digestibility of lignocellulosic materials **(Ezea 2019)**. The external surface area is related to the size and the shape of the particles. The internal surface area depends on the capillary structure of cellulosic fiber. Swelling of lignocelluloses with water and polar solvent creates large internal surface area **(Ezea 2019)**.

12. Factors militating against commercialization of bioethanol

There have been great progresses in development and commercialization of bioethanol in the last decade. However, ethanol selling cost is still higher than that of fossil fuel which can be attributed to the high cost of raw materials that are presently used for bioethanol production. For effective replacement of fossil fuel with ethanol, there is a need to further reduce the price of ethanol. Commercialization of lignocellulosic ethanol production is a promising way to reduce the price of ethanol. However, Conversion technologies for producing bioethanol from cellulosic biomass are still under development and have not yet been demonstrated commercially **(Chandel *et al.*, 2010)**. Some of the obstacles that impede the commercialization of ethanol include availability of raw materials for the production. Bio-ethanol production generally utilizes derivatives from food crops such as corn grain and sugar cane, but the limited supply of these crops can lead to competition between their use in bio-ethanol production and food provision **(Chandel *et al.*, 2010)**. Lignocellulosic biomass, on the other hand, is an abundant, cheap, renewable and potential feedstock for the sustainable production of bioethanol but their conversion rate to ethanol is much lower. It is critical to develop cost-effective technologies to ferment both hexose and pentose into ethanol. Apart from feedstock, pretreatment technology cost, cost of enzymes and robust ethanologenic microorganism are among the factors militating ethanol commercialization. Furthermore, an efficient recovery plants is of utmost important **(Chandel *et al.*, 2010)**.

Conclusion

Critical analysis of the literature shows that bio-ethanol production has a promising future if appropriate technologies are been applied during production. Bio-ethanol is a renewable energy that can be produced by utilization of renewable natural resources. Development, technologies and commercialization of bio-ethanol are very important and still the key especially in the developing countries where the wastes renewable natural resources are underutilized.

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