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REVIEW

A Brief Review of Some Current Improvements in ABE Production: New Development of Raw Materials, Strain Improvement, Fermentation Systems, and End Product Extraction

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Concerns over energy security, economic development and climate change are driving the search and development of bio-fuels as one of a number of possible alternatives to fossil fuels to meet increasing global energy demands. Biomass derived fuels or bio-fuels, such as ethanol and butanol, are thought to offer the only renewable liquid alternatives to petroleum based transportation fuels. Although ethanol has long been recognised as a typical bio-fuel, butanol is also being due to several advantages it has over ethanol as a liquid fuel. The production of butanol by acetone, butanol and ethanol (ABE) fermentation using mainly *Clostridium acetobutylicum* and *C. berjerinkii* has been used by industry for decades. However, ABE fermentation was replaced by cheaper petrochemical methods in the 1920's although by 1945 the ABE fermentation became second in importance only to ethanol production by yeast. Unfortunately, by the 1960's ABE fermentation was no longer in use industrially because of the high cost of substrate, low productivity and low solvent concentration due to butanol toxicity to the production strains, low yield, and high solvent recovery cost. Although recent advances in the use of cheaper and more abundant raw materials, strain developments through genetic engineering combined with advanced fermentation, and product recovery technologies are critical for overcoming some of these obstacles, the economics of butanol production is still affected by the type and cost of raw material, the type of fermentation system used, the butanol recovery techniques, by-product credit, solvent yield, concentration and productivity. This paper reviews some current progress/developments in ABE production in terms of fermentation technology, strain development, novel upstream and downstream processing in an attempt to make the ABE fermentation economically competitive and environmentally favorable.

Keywords: ABE Fermentation, Butanol, Ethanol, Biofuels, Batch, Biomass, Lignocelluloses.

CONTENTS

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1. INTRODUCTION

Fuel for transport makes up almost a third of the current world energy consumption. Bio-fuels-renewable liquid or gaseous transport fuels derived from plant or animal material—have emerged as one of a number of possible alternatives to fossil fuels that might help meet global

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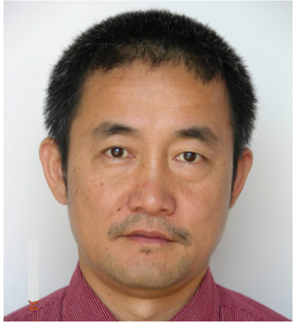

Lu Fan obtained the Ph.D. in applied phycology from Ben-Gurion University of Negev (Israel) in 1996. In the past twenty years, he has been focusing on microalgal biotechnology, dealing with mass cultivation of *Haematococcus pluvialis*, *Nostoc commune*, *Chlorella spp.*, *Pleurochrysis carterae* for the production of natural astaxanthin, health food, biofuels, and omega-3 fatty acids.

Wensheng Qin obtained his Bachelor and Master's degrees in China. He earned his Ph.D. in Molecular Biology and Biotechnology in 2005 from Queen's University Canada. He further received his postdoctoral training at Stanford University USA in Biochemistry and Biotechnology. Apart from the aforementioned studies, he worked in several other institutions including Zhejiang Academy of Agricultural Sciences in China, University of Waterloo and University of Toronto in Canada, National Polytechnic Institute in Mexico, Kansas State University and Yale University in USA. He holds expertise in biomass conversion, biofuels and bioproducts, microbial engineering, molecular biology and biochemistry. Qin has published 102 peer-reviewed scientific papers. He is now a professor at Lakehead University in Canada.

energy needs in an environmentally sustainable manner.[1] At the moment, bio-fuels make up a small proportion of the world's energy source, and production and use of biofuels is expected to increase due in part to targets and policies by various governments that are encouraging their use for transport.[1–3] In an attempt to curb carbon dioxide emissions, the EU has proposed in 2010 that biofuels should make up 9% of total fuel sales by 2020, which represents a huge increase in the market for biofuels.[1,4] The US government has mandated that 20 billion gallons of biofuels must be produced annually from non-corn biomass by the year 2020, and the US Department of Energy has also set goals to replace 30% of liquid petroleum transportation fuels with biofuels and to replace 25% of industrial organic chemicals with biomass derived ones by 2025.[5] In the UK, the Renewable Transport Fuel Obligation required fuel suppliers since 2008 to ensure that an increasing percentage of their total fuel sales to be made up of biofuels by 2020. The UK Government also intends for butanol to count as a renewable transport fuel.[6]

Biofuels derived from biomass such as ethanol and butanol are thought to offer the only renewable liquid alternatives to petroleum based transportation fuels.[7–9] Among the many biofuels, butanol is also being considered as a potential liquid biofuel in recent years due to the several advantages it has over ethanol, despite ethanol attracting the most attention world-wide.[10] Butanol is an attractive renewable liquid transportation biofuel or fuel additive that has the potential to substitute for both ethanol and biodiesel in the biofuel market, and is estimated to be worth $247 billion by the year 2020.[11]

2. A BRIEF HISTORY OF ABE FERMENTATION

The history of ABE fermentation has been covered thoroughly in many excellent reviews.[12–14] Briefly, ABE fermentation is one of the oldest known industrial fermentation methods with a history dating back more than 100 years. It dates back to Louis Pasteur (1861) who discovered that bacteria can produce butanol. This bacterium was isolated in 1912, named BY, and later re-named *C. acetobutyricum*.[15,16] Commercial production of butanol quickly spread around the world during the First and Second world Wars, first to produce acetone for ammunitions and then later to produce butanol for the paint industry. ABE fermentation became one of the largest industrial fermentation processes early in the 20th century.[17,18] In the former USSR, large scale ABE fermentation began in 1929 and continued until the late 1980's at least 8 industrial scale ABE fermentation plants were in operation. Countries such as China, Japan, Australia and South Africa also produced acetone and butanol by large scale ABE fermentation processes.[14,19]

However, ABE fermentation had lost its competiveness by the 1960's due to high substrate cost, low product yield, low productivity, low final product concentration due to butanol toxicity and the advent of more efficient petrochemical processes for butanol production.[20] Most of the ABE fermentation industry in Western Countries ceased to exist by 1960, although some production via fermentation continued in China, Russia and South Africa until the early 1980s.[14,19]

The rising cost of crude oil and increasing concerns over global warming combined with the increasing demand in recent times for the use of renewable resources as feedstocks for the production of chemicals and the advances in biotechnology have led to renewed interest in metabolic engineering and innovative process have renewed interests in the production of biofuels such as butanol by fermentation which can be used both as a chemical and as an alternative liquid fuel.[21]

3. PROPERTIES AND CURRENT USES OF BUTANOL

Butanol (butyl alcohol or 1-butanol or *n*-butanol, C_4H_9OH, MW, 74.12) is a four-carbon alcohol, a clear and neutral liquid with a strong characteristic odour. It is miscible with most solvents including alcohols, ethers, aldehydes, ketones, and aliphatic and aromatic hydrocarbons. Furthermore, it is a highly refractive compound and rather sparingly soluble in water (6.3%).[16] At present, butanol is mainly used as a solvent in the cosmetic and pharmaceutical industries as a valuable C4 feed stock for the chemical synthesis of butyl acrylate and methylacrylate esters for latex surface coatings and the production of enamels,

Table I. Properties of butanol compared with those of other fuels.

Properties	Methanol	Ethanol	Butanol	Gasoline
Energy density, MJ/L	16	21.2	29.2	32.5
Air-fuel ratio	6.5	9	11.2	14.6
Research octane number	136	129	96	91-99
Heat of vaporization, MJ/Kg	1.2	0.92	0.43	0.36
Flash point, °C	79	13	35	<-40

Note: Adapted from Buyondo and Liu.[24]

lacquers, butyl glycol ether, butyl acetate and plasticizers. Additionally, it is used as a solvent for the production of hormones, vitamins and antibiotics.[12]

4. BUTANOL AS A POTENTIAL BIOFUEL

The potential of butanol as a biofuel and its advantages over ethanol has been recently reviewed.[22,23] Briefly, butanol is an attractive renewable liquid transportation biofuel or fuel additive that has the potential to substitute for both ethanol and bio-diesel. Butanol is much less hydrophilic than ethanol and produces more energy per unit (can yield an extra 25% energy than ethanol). The energy density of butanol is 26.9–27.0 $MJ \cdot L^{-1}$, which is higher than that of ethanol (21.1–21.7 $MJ \cdot L^{-1}$) and so it can burn longer. It is also less volatile and less corrosive than ethanol, enabling easier transportation by currently used fuel pipelines. Also, butanol does not damage automobile valves and gaskets. Furthermore, butanol has lower vapor pressure, which makes it safer to use. It contains approximately 22% oxygen, which when used as a fuel extender will result in a complete fuel combustion producing only CO_2 and H_2O which have no negative impacts on the environment. About 85% butanol/gasoline blends can be used in unmodified petrol engines.[16]

A comparison of different alcohols and gasoline is shown in Table I adapted from Buyondo and Liu.[24] It can be seen from the table that butanol has several advantages over the other fuels. Butanol contains only 10% less energy than gasoline. The major disadvantage of butanol as a biofuel is its high flashpoint (butanol 35 °C, ethanol 13 °C, and gasoline <-40 °C). Butanol also has a lower octane number (butanol 96, ethanol 129, and gasoline 91–99) and a higher viscosity (varies with temperature) than the competitive fuels. Butanol also has a slower biodegradation rate than ethanol, particularly in water.[24] Currently, viable methods for the production of an array of oxygenated and fully saturated jet oil diesel fuels from butanol are also being investigated.[25]

5. CHEMICAL AND FERMENTATION PRODUCTION OF BUTANOL

Butanol can be produced either chemically from petroleum/petroleum products or through fermentation by a variety of *Clostridia* species. Currently, most butanol is chemically produced by either by the oxo process with propylene derived from petroleum as the staring material (with H and CO over rodium catalyst), or the aldol process in which two molecules of acetaldehyde undergo aldol condensation to yield the intermediate crotonaldehyde, which is then dehydrated and hydrogenated to give butanol. However, the chemical methods of butanol production require large-scale investment and high-technology equipment which makes them very expensive.[26]

Butanol can also be produced through anaerobic fermentation of carbohydrates in a process referred to as ABE fermentation, after its major chemical products: acetone, butanol and ethanol. ABE fermentation is a proven industrial process that uses solventogenic *Clostridia* species.[12,27] These gram-positive, spore forming anaerobic *Clostridia* constitute a diverse group of species with industrial, agricultural and medical uses. *Clostridium acetobutylicum* and *C.berjerinckii* are among the prominent solventogenic species capable of acetone and butanol production through fermentation.[27]

The fermentation occurs in two stages: the first is a growth stage in which acetate and butyric acids are produced and the second stage is characterized by acid re-assimilation to produce acetone, butanol and ethanol (ABE) in the ratio 3:6:1 respectively. With butanol being the major product, it is called butanol or ABE fermentation. During this stage, growth slows, and the cells accumulate granulose and form endospores. The fermentation also produces carbon dioxide and hydrogen. The metabolic pathways and enzymes involved in ABE fermentation by *C. acetobutylicum* are shown in Figure 1.[12,28,29]

These solvent forming bacteria, including *C. beijerinckii*, grow best between 30 °C and 40 °C. The pH varies during the fermentation and can drop from an initial value of 6.8–7.0 to about 5.0–4.5 (acidogenesis) and can rise up to 7.0 later in the fermentation (solventogenesis). It has been suggested that the switch to solvent production is an adaptive response of the cell to the low medium pH resulting from acid production.[30] The production of butanol is also limited by severe product inhibition. Butanol at a concentration of 10 g/L can significantly inhibit cell growth and fermentation. Consequently, butanol titers in conventional ABE fermentations are usually lower than 13 g/L. The low butanol yield and butanol concentration made butanol production from glucose by ABE fermentation uneconomical.[31,32] In addition, since the *Clostridium* species are strictly anaerobes, anaerobic conditions need to be established before beginning fermentation and need to be maintained during the process.[33,34]

6. PRODUCTION OF BUTANOL ON A COMMERCIAL SCALE

Commercial butanol fermentation processes have been developed by a few companies[35] and it is expected that the number of companies devoted to butanol production will increase worldwide as well as the development of new

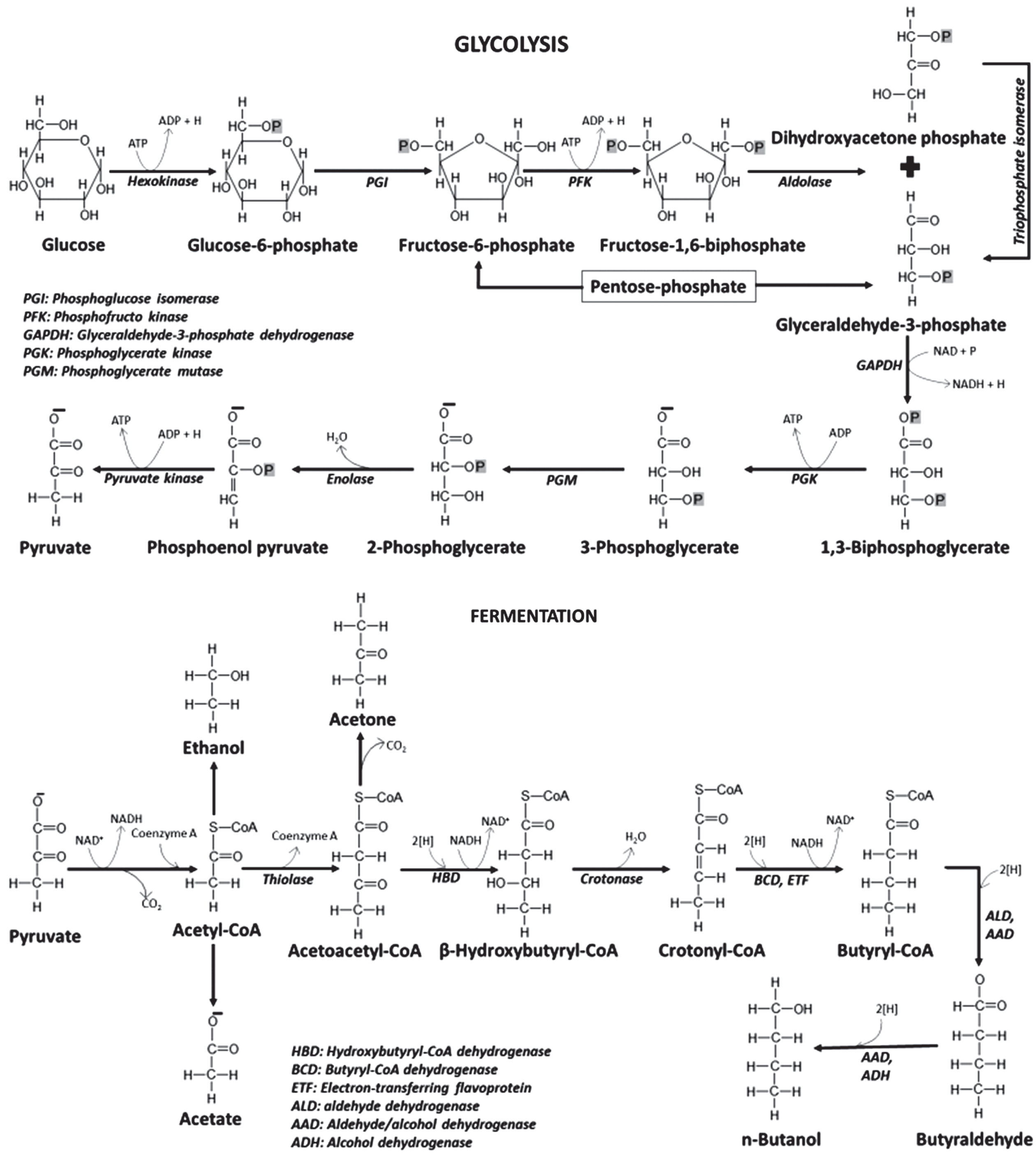

Fig. 1. Biochemical pathways in *C. acetobutylicum* for ABE production. Adapted from Jones and Woods.[12]

technologies to increase the butanol yield.[36] Since butanol is the preferred solvent, it attracts the highest price in the chemical market. In 2008, the global demand for butanol was 2.8 million tones, estimated to be worth approximately \$5 billion. The average growth is expected to be 3.2% pa with demand concentrated in North America (28%), Western Europe (23%) and north East Asia (35%).[11]

In 2006, DuPont (USA) and British Petroleum (BP) formed a partnership to develop a butanol production technology with biobased feedstock. They also established a £25 million advanced biofuels research centre in Hull (UK) to demonstrate butanol technology. BP has also provided a route for butanol into the transport fuel market and aims to blend butanol with petrol at its 1200 filling stations.[6] China is one of the countries that have started to commercialise ABE fermentation on a large scale with over \$200 million recently invested in the installation to 0.21 million tones/annum of butanol capacity with plans to expand to 1million t/a. There are six major plants that produce about 30000 t/annum of butanol from corn starch.[19] A relatively new plant built in Brazil operated by HC Sucroquimica produces 8000 tons of solvent p/annum from sugar cane.[37]

Cobalt Technologies ("Cobalt"), a leading developer of next generation bio-based chemicals, based in the US has announced the successful completion of a production plant of *n*-butanol at a scale greater than 100,000 liters.[38] Green Biologics, a leading global player in renewable *n*-butanol since 2012, had reported that it has commenced commercial shipments of bio-based *n*-butanol and acetone from its 21-million-gallon manufacturing facility in Little Falls, Minnesota, USA at the end of 2016.[39]

7. CHALLENGES TO ABE FERMENTATION AND SOME CURRENT IMPROVEMENTS

The technical and commercial challenges and possible solutions for conventional ABE fermentation have been extensively reviewed and are summarised by Green[11] in Table II. Generally, the economics of butanol production have been found to be strongly affected by the type and cost of raw materials (feedstock), the fermentation techniques (type of bioreactors), butanol recovery techniques, by-product credit, solvent concentration, yield, and productivity.[28,40] For industrial ABE production to be economical and sustainable there is the urgent need for alternative cheaper alternative feedstocks, better fermentation strains, improved fermentation techniques and cheap, effective and more sustainable process operations for solvent recovery and water recycling. Many scientists want to improve the progress of fermentation by ananlysing in system-level.[41–43] They simulated the progress by computational modeling using stoichiometric and kinetic approaches. Recently, another system-level perspective of ABE fermentation was reported by Chen Liao et al. They presented an integrated computational framework of clostridial ABE fermentation that combines metabolic reactions, gene regulation, and environmental cues.[44] This work provides a powerful tool for generating new hypotheses and for guiding strain design and protocol optimization, facilitating the development of next-generation biofuels. This may divide in to many independent subjects in the future.

Table II. The Challenges and solutions to ABE fermentation.

Challenge	Solutions
High feedstock cost significantly increase operating costs.	Transition towards cheaper (and more sustainable) feedstocks such as wastes and agricultural residues.
Low butanol titres increase recovery costs. Low titres also reduce sugar loadings and increase water usage.	Develop improved microbes with improved solvent titres and/or develop methods for *in situ* product removal to allevlate end product tolerance.
Low butanol yield increase feedstock costs.	Develop improved microbes with higher butanol yield and/or develop microbes with higher butanol: solvent ratios.
Low volumetric solvent productivities increase capital and operating costs.	Develop continuous fermentation processes that reduce down time and increase volumetric productivity.
Solvent recovery using conventional distillation is energy intensive and relatively expensive.	Develop low energy methods for solvent recovery and purification. Recovery can also be improved by improving the solvent titre.
High water usage is not sustainable and increase the cost of effluent treatment.	Recycle process water back through the fermentation.

Note: Adapted from Green.[11]

8. NOVEL FEEDSTOCKS FOR BUTANOL FERMENTATION

In past decades, the conventional substrates for ABE fermentation have been corn, molasses, wheat, millet, rye, glucose, starch, and whey permeate.[12,45] However, over time, these feedstocks became unaffordable because, in some cases, their use has led to deforestation and rising food prices.[4] The economics of ABE fermentation has been shown to be greatly affected by the type and cost of raw material used.[28] The cost of feedstock represents over 70% of the total production costs of biobutanol.[46,47]

The choice of substrate is therefore very important in ABE production and significant research has been performed over the years on using alternative substrates for fermentation such as maltodextrin, cracked corn, packing peanuts, starch, agricultural wastes, food waste and soy molasses and various other substrates such as low grade glycerol.[28,48–50] The idea of converting biomass-derived sugars to transportation biofuels was first proposed in the 1970's.[51] Lignocellulose from biomass is a substrate that offers great promise for the improvement of the economy of ABE fermentation. Lignocellulose is present in wood, and agriculture and forest wastes represent an abundant natural renewable carbon resource because of their renewable character and availability in large quantities at low cost.[52] Lignocelluloses are also less expensive than conventional agricultural feedstock and can be produced with lower inputs of fertilizers, pesticides and energy and their use could avoid the conflict between food and fuel production.[53]

Lignocellulose is a complex polymer composed of up to 75% carbohydrate mainly cellulose and hemicelluloses. The rest is lignin with a whole host of other components in small amounts such as protein, pectin, soluble sugars, vitamins and minerals. The composition of lignocelluloses varies significantly between different plants, different parts of the plant, age and growth conditions of the plant.[54]

Lignocelluloses from biomass offer great potential for improvement of the economy of ABE fermentation. Since *C. acetobutylicum* is not able to hydrolyse fibre-rich agricultural residues, effective pretreatment/hydrolysis of the cellulose and hemicellulosic fractions of lignocelluloses is required before it can be used as a feedstock. Pretreatment can be accomplished using acid, base, enzymes, or processes such as steam explosion and ammonia fibre explosion.[12,55] Pretreatment of any lignocellulosic biomass yields mainly the easily fermented hexose sugars (D-glucose) from the cellulose and also significant amounts of pentose sugars, mainly D-xylose and varying amounts of D-glucose, D-mannose, D-galactose, L-arabinose and L-rhamnose from the hemicelluloses depending on the starting material. Potential inhibitory products such as furfural, hydroxyl methyl furfural, acetic, ferulic, glucoronic and *p*-coumaric acids, phenolic compounds and salts are also produced. The ratio and concentration of these inhibitors depend on the selected pretreatment, biomass concentration and pretreatment conditions.[54,55]

Economically, it is important that all the sugars present in the hydrolysate are fermented to butanol and the fermenting microorganism is able to tolerate the inhibitors that are present in the hydrolysate. Solventogenic strains such as *Clostridium beijerinckii* exhibit cellulolytic and xylanolytic activities as well as desirable properties such as sugar co-fermentation.[12] Hydrolysates of various lignocellulosic biomass are increasingly being investigated as substrates for ABE fermentation. *Clostridium beijerinckii* BA101 can utilize cellobiose, glucose, mannose, arabinose and xylose. However, growth and ABE production of this strain decreases significantly in the presence of *p*-coumaric and ferulic acids, but, furfural and hydroxyl methyl furfural individually are not inhibitory but rather have stimulatory effects on the growth and ABE production. But a mixture of the two had a profound negative effect on growth and ABE fermentation and must be removed prior to fermentation.[56,57] Although significant progress has been made, it is clear that the production of butanol from biomass is still in its infancy.

9. CURRENT IMPROVEMENTS IN THE PRODUCTION STRAIN

The use of the good production strain will improve the economics of ABE fermentation by being able to tolerate high concentrations of the substrate, overcome inhibition by fermentation products (butanol and the other organic acids and alcohols). Furthermore, for the development of a cost-effective and efficient large-scale process to convert lignocellulosic biomass to butanol, production strains that can rapidly and efficiently ferment all the available sugars present in a biomass hydrolysate (D-glucose, D-xylose and the less predominant sugars such as L-arabinose) at high yield and high productivities are also required.[58] This could significantly improve the overall process cost by 20–25%.[59]

Up to now, *Clostridium acetobutylicum* and *Clostridium beijerinckii* are the two well-known microorganisms for butanol production.[60,61] Butanol production is carried out exclusively by members of the genus *Clostridia* or their mixtures.[62,63] Although the first strain that was used for commercial ABE fermentation was *C. acetobutylycum* P262,[12] other strains have been studied/used include *C. acetobutylicum*, *C. beijerinckii, C. thermosulfurogenes* EMI, *C. saccharolyticum* and *C. therosaccharolyticum*.[64,65] However, *Clostridia* are not ideal for ABE fermentation because of the relative lack of genetic tools to manipulate their metabolism, slow growth, their intolerance to butanol above 1–2% and oxygen, and the production of butyrate, acetone and ethanol as by products.[66]

The decades of the 1980s and 1990s saw tremendous progress in the development of genetic systems for the solventogenic *Clostridia*, which would enable the development of strains with improved fermentation characteristics. The completion of genome sequencing of *C. beijernkii* NCIMB 8052 by the Joint Gene Institute of the Department of Energy, USA, has opened up exciting possibilities of investigating the molecular mechanisms of solventogenesis on a genomic scale.[67,68] Furthermore, the comparison of gene expression patterns in the *C. beijerrinkii* 8052 parental strain and the *C. berijerinckii* BA101 hyperbutanol producing mutant strain provide insights towards engineering genetically modified *C. beijerrinkii* strain with improved butanol yields, titres and productivity.[69]

In recent years, *Clostridia* species and *Escherichia coli* have been routinely engineered to produce butanol via a CoA-dependent pathway[70] or by reversal of the fatty acid beta-oxidation pathway.[71] So far, the highest yielding CoA pathways utilize an oxygen sensitive enzyme, aldehyde/alcohol dehydrogenase (AdhE2) in *Clostridium*. The pathway has been studied and engineered in various host organisms such as *C. acetobutylicum*,[72] *Clostridium tyrobutyricum*,[73] *E. coli*,[74] *Saccharomyces cerevisiae*[66] and *Synechococcus elongatus* PCC 7942.[75] By optimizing engineered butanol pathways and native host metabolism, high butanol titers (20–30 g/L) have been achieved.[76] Some chemically mutated and genetically engineered solventogenic *clostridia* with improved solvent titre were compiled by Green.[11]

The best heterologous butanol-producing strains are presently derived from *E. coli.* By introducing an integrated pathway using *Ter* from *Treponema denticola*, as well as by blocking cellular NADH and acetyl CoA consuming pathways in *E. coli*, 14–15 g/L butanol with a yield of 31–33% (88% of the theoretical yield) was obtained.[77]

Finally, since butanol tolerance is a critical factor affecting the ability of microorganisms to generate economically viable quantities of butanol, the current *Clostridium* strains are sensitive to butanol concentration greater than 2%. Attempts made so far to increase butanol tolerance in *C. acetobutylicum* ATCC 824 using mutagenesis or serial enrichment resulted in only a marginal increase in butanol tolerance.[32] Product inhibition is also being addressed by the use of novel solvent removal techniques. Integrating reactors with separation techniques for simultaneous fermentation and separation decrease the inhibition thus increasing the ABE productivity by sometimes more than 2×.[78]

10. IMPROVEMENT IN BUTANOL FERMENTATION PROCESS

In addition to substrate, process technology (fermentation process) also impacts the economics of ABE fermentation.[28] Traditional ABE fermentation is carried out in two ways, batch fermentation and continuous fermentation. Batch fermentation is a simple and most commonly studied process for butanol production.[12,79] The usual batch fermentation requires large bioreactors and last from 48 to 72 h after which butanol is recovered usually by distillation.[80] Butanol productivity in batch bioreactors is often low (ranges from 0.1–0.3 g $L^{-1}h^{-1}$) which is due to the long periods of operation, product inhibition and downtime for cleaning, sterilizing, and filling.[81,82] The low productivity and low yields of batch culture can be addressed by fed batch or continuous processes.

Fed-batch is a technique that is applied to processes in which a high substrate concentration is toxic to the strain. Since butanol is toxic to *C. beijerinckii* and *C. acetobutylicum* cells, the fed batch fermentation technique cannot be used in this case unless one of the novel simultaneous fermentation and product recovery techniques is applied. In a number of studies, this technique successfully applied to ABE fermentation.[20,83]

Continuous bioreactors have been designed and processes have been developed to achieve higher ABE solvent productivities via continuous fermentation techniques. Continuous processes offer various advantages such as reduction in sterilization and inoculation time, high productivity, and reduction in butanol inhibition, but this reactor presents high product recovery costs due to low concentration of biofuel.[84,85] Large improvements with respect to the classical batch process have also been reported in optimized one-stage or two-stage systems.[86] The most commonly investigated bioreactor designs for continuous fermentation of butanol include: suspended free cell, immobilized cell, and membrane cell recycle.[78] Batch, fed-batch and continuous ABE fermentations using *C. acetobutylicum* were conducted and compared at pH 4.5 which is range for solvent production. While the batch mode provided the highest solvent yield, the continuous culture was preferred in terms of butanol yield and productivity.[87]

However, the major limitations of cell recycle bioreactors include membrane fouling with fermentation broth and high membrane cost.[88] Different membranes are researched for overcoming these problems. There are many conventional homogeneous polymeric membranes such as polysiloxane[89] and poly(1-trimethylsilyl-1-propene) (PTMSP)[90] etc.

Today, many scholars research on the PDMS membranes which are more promising than others with excellent hydrophobicity as well as good chemical and mechanical stability.[91–94] Various hydrophobic zeolites had been used as filler in enhancing membrane selectivity for gaseous separation and organic solvent separation.[95–97] Immobilized cell continuous culture systems were also found to improve bioreactor productivity.[78,98] Use of immobilized cell cultures and cell recycle reactor can further improve the reactor productivity even to the order of 40–50 times compared to batch rectors.[79]

11. PRODUCT ISOLATION AND PURIFICATION TECHNIQUES

One of the most significant challenges faced by commercial ABE fermentation by *C. beijerinckii* or *C. acetobutylicum* is the prohibitive cost of the recovery of butanol from the broth due to its low concentration and a higher boiling point than water.[99] At such a low concentration in the broth, the energy required for butanol separation by distillation is higher than the energy content of the product.[100] In ABE fermentation, the solvent is recovered by distillation, and since the boiling point of butanol is higher than water, distillation separates most of the water (98%) from the fermentation broth. However, the problem in using this process is the formation of an azeotrope that increases the energy cost thus making the distillation process very expensive, comprising about 20% of the total cost in the traditional ABE fermentation.[16,27]

In order to remove butanol during ABE fermentation in a relatively inexpensive and energy efficient way, a number of product removal methods investigated. These techniques include adsorption, gas stripping, liquid–liquid extraction, pervaporation, perstraction and reverse osmosis. These methods, coupled directly with the fermentation process, can separate the butanol *in situ*, thereby reducing the inhibition from butanol.[99,101,102] By applying most of these techniques, improved bioreactor productivities and higher sugar utilisation were obtained, thus making the ABE fermentation process more energy efficient.[82,101,103] Furthermore, among these removal techniques, gas stripping, liquid–liquid extraction and pervaporation have been identified as techniques that can be applied at commercial level.[102]

Studies have identified adsorption as a very simple technique that requires less energy for butanol separation compared to the other separation techniques. It is also a superior technique due to rapid adsorption, ease of desorption and regeneration of the adsorbents.[104] Among various adsorbents (silicate, bone charcoal and polyvinylpyridine) for butanol separation and concentration, silicate was found to be a more attractive and energy efficient recovery system.[105]

12. CONCLUSION

Concerns over energy security, economic development and climate change are driving the development of biofuels as one of many possible alternatives fossil fuels for helping to meet increasing global energy demands. Ethanol was the first liquid biofuel to attract attention for many years and is produced in large quantities and used as substitute for petroleum derived fuels in countries like Brazil and the USA. However, butanol produced by ABE fermentation with *Clostridia* species is also an attractive renewable liquid biofuel that has the potential to substitute for both gasoline and ethanol. As an alternative liquid biofuel, butanol offers distinct advantages over ethanol because of its high energy content, miscibility with gasoline, octane rating and low volatility which make it a substantially better biofuel than ethanol.

The ABE fermentation is one of the oldest known industrial fermentations with a history dating back 100 years. However, traditional ABE fermentation is not cost effective due to the high cost of raw materials, low product yield, low productivity, and low final product concentration which is mainly due to low butanol toxicity by the *Clostridia* strains used for the fermentation. The need for alternative cheaper feedstocks, better fermentation strains through genetic engineering, improved fermentation techniques and cheaper, effective and more sustainable process operations for solvent recovery and water recycle cannot be over-emphasised.

Research has been gradually progressing over the last 2 decades to make ABE fermentation environmentally favourable and economically competitive by trying to develop superior butanol production strains through advances in metabolic engineering along with the development and optimisation of novel state-of-the-art fermentation technologies and energy-efficient solvent recovery systems that are critical for overcoming the remaining obstacles in a cost-efficient butanol production. The use of sugars from biomass derived lignocelluloses such as wheat and barley straws, corn stover, switchgrass and dry distillers grains, and solubles that are cheap and abundant as feedstock have the potential to be carbon negative and to avoid the conflict between food and fuel production and at the same time, improve the competiveness of ABE fermentation.

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