Butanol and Ethanol production as Bio-fuel

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Abstract

Presently, shortage of petroleum reservoirs and pollution of the environment has forced countries to search for other fuel resources. Butanol and Ethanol (BE) are of great importance as fuels that are mainly produced by petrochemical processes. After the Second World War, bacterial production of these chemicals has attracted a great deal of attention by means of *clostridia* species. This article provides some necessary information on raw materials, medium characteristics and processes used for enhanced BE production.

Keywords: *Clostirdium Sp.*, BE production, Bio-fuel

1-Introduction

Recent interest in microbial production of Acetone and Butanol is based almost exclusively on investigations with strains of *sacharolytic clostridia* like *Clostridiumacetobutylicium*, *Clostridiumbeijerinckii* and *Clostridiumbutylicum* [3].
Bacterial solvent production using *clostridum Acetobutylicum* has been studied for many decades because of the economic importance of its fermentation end-products: Butanol, Acetone and hydrogen.
Strains of *Clostridiumbeijerinckii* are capable of producing a mixture of neutral solvents consisting either of isopropanol, 1-Butanol and Ethanol or of Acetone, 1-Butanol and Ethanol by those strains lacking isopropanol dehydrogenase.
The resurgence of interest in this organism has been due to projected future increase in oil prices and growing unreliability of supply. Any process using *clostridia* would be of benefit to the economy (and also to the environment) in those countries where excess biomass is available as a renewable energy source ;however, the bacterial solvent production process form biomass is not economically feasible with the currently available strains , needing considerable improvements in strains and a cheap carbon source [8].
The main constraint on the economic viability of solvent (ABE) fermentation is the cost of raw materials. About 60-70% of the total production cost in ABE fermentation is the cost of raw materials [1].

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Presently, this process (ABE production) is becoming very attractive for the production of chemicals and liquid fuels by using renewable resources such as palm oil waste and domestic wastes or abundant agricultural crops [4].
The process was first operated on a commercial scale, during the First World War, with maize as the substrate, subsequently, molasses became the preferred substrate also potato has been used for ABE production [7].

2-ABE production mechanism

The *clostridium* genus encompasses a collection of Gram-Positive, obligatory anaerobic, non-sulphate reducing, spore forming, and rod-shaped organisms. Over 100 species are currently recognized, displaying a wide range of phenotypes, including psychrophiles, thermophile and acidophile [8].
ABE fermentation by means of *Clostridiumsp.* occurs in two phases: acids are produced during the first phase, the pH decreases to around 4.8. Then the solventogenic phase is switched on and acids are reduced to Acetone, Ethanol and Butanol; the pH is around 5.5 during solventogenesis[8].
The pH value in broth decreases with butyric acid production during acidogenesis and then butyric acid reutilization and Butanol production result in a pH increase during solventogenesis [5].
Fig.1. shows the biochemical pathways involved in *clostridium beijerinckii* acidogenesis and solventogenesis [8].

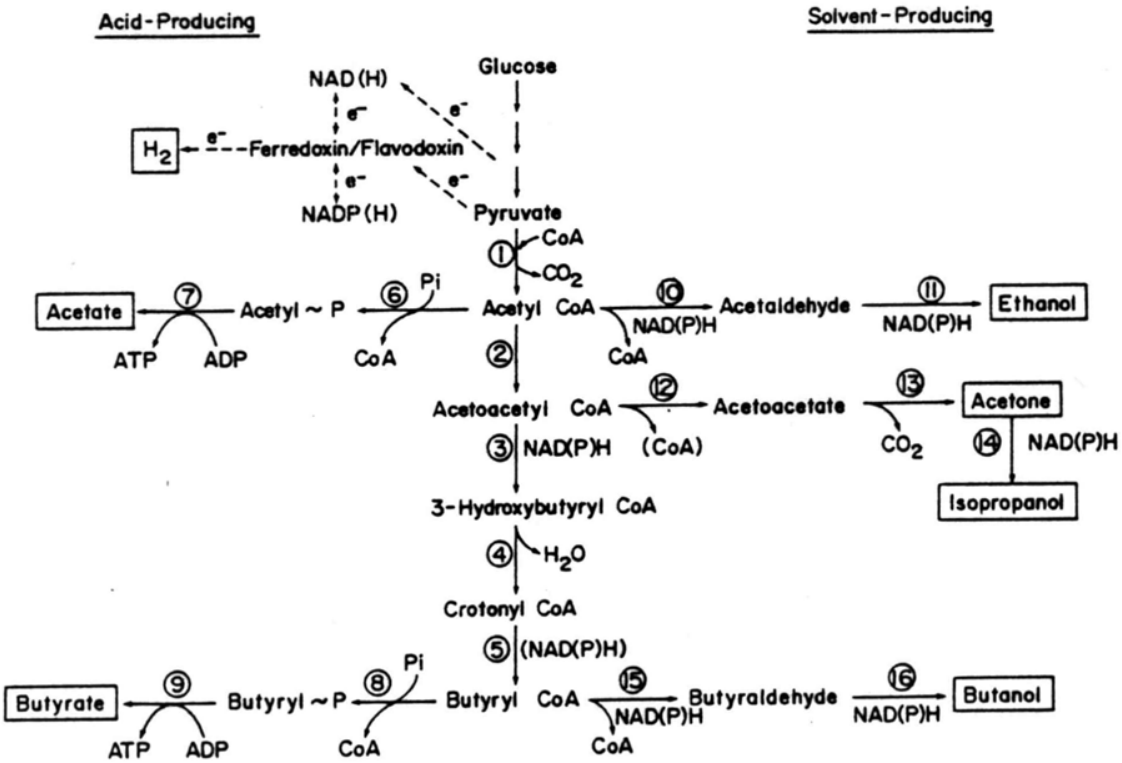

Fig 1. Metabolic pathway of acid and solvent production in *Clostridium Beijerinckii*.

During batch fermentation, there is a shift from acid to solvent formation towards the end of the exponential growth phase; shortly after the start of solventogenesis a decrease in biomass concentration is observed. This happens when the butyrate concentration reached its maximum and the glucose utilization rate declines for a short period of time, i.e., at the beginning of the acid detoxification process. After acid detoxification the biomass concentration rises again [3].

There are three main research areas to improve industrial scale ABE production: 1-Raw materials 2- Medium characteristics 3- the process used.

3-Raw material

Starch is an inexpensive carbon source. *Clostridum sp* can produce amylolytic enzymes such as amylases, pullulanase and glyco amylase that permit direct fermentation of starch to ABE. Utilization of various types of starchy materials for ABE production by *clostridum Acetobutylicum* has been examined by several researches [1].
In a typical process, a carbohydrate substrate is converted to a mixture of Acetone, Butanol, and Ethanol in the approximate ratio: 3-6-1 at a total broth concentration of around 20 g/l [7].
Starch is the major reserve carbohydrate in higher plants and is water soluble and granular that consists of a mixture of two polymers: 1- amylase: a linear chain of glucose 2- amylopectin: a branched polymer of glucose.
Amylopectin composition determines the viscosity [1].
Starches from different origins show different types of physicochemical properties depending on the relative amylase and amylopectin contents.
Corn and Sago contain 27-28% amylase and 72-73% amylopectin and the starch content of potato and tapioca is 17-21% amylase and 79-83% amylopectin. Reduced solvent production in fermentation of potato and tapioca starches may be attributed to their higher degree of branched polymerization as compared to corn and sago starch [1].
Madihah et al. used sago starch to produce solvents. They found that *C.Acetobutylicum* P262 was able to grow on sago starch and had a maximum total solvent production of 24.47 g/l. they also found that production of solvents by the bacterium was limited at high sago starch concentrations(>70 g/l) [1].
Gutierrez et al. compared solvent-productivity of several strains of solvent –producing clostridia from unsupplemented potato and form potato that had been pre-hydrolyzed with amylolytic enzymes. They concluded that prior treatment of potato with enzymes gave no improvement in solventogenic properties of the strains except *Acetobutylicum* ATCC 824, but it reduces considerably the viscosity, allowing higher solvent concentrations to be achieved, giving a reduction in the cost of the product recovery [7].

4-Processes used

Various systems of ABE production have been developed, including batch culture, Fed-batch culture integrated with a process of Butanol removal and continuous culture [4].
End-product inhibition, low product concentration and large volumes of fermentation broth, in addition to the high cost involved in generating the steam required to distill fermentation products from the broth largely contributed to the decline in fermentative ABE production [6].
Most production systems exhibited a very low volumetric ABE productivity due to low cell concentrations [4].
It has generally been observed that the cell concentration in anaerobic cultures is lower than in aerobic cultures, to overcome this critical defect, studies have been carried out on bioreactors with cell-Immobilization and these bioreactors exhibit improved ABE productivity in continuous culture with high cell density by cell immobilization on various types of carriers such as beads, brick pieces or fibrous matrices. On the other hand, a few studies have reported on bioreactors with cell-recycling by using membrane modules [4].
A continuous culture without cell-recycling could not be operated at a high dilution rate due cell wash-out which results in low ABE productivity.
Bioreactors with cell-recycling are advantageous due to the homogeneity of the broth that facilitates diffusion in the bioreactor as well as the total recycling of microorganisms [4].
Tashiro et al. produced ABE using a continuous production system with high cell density obtained by cell-recycling of *Clostridiumsaccharoperbutyliacetonicum N1-4*. They concentrated the solventogenic cells of the broth 10 times bye membrane filtration. Continuous culture with cell recycling was then started and the cell concentration increased to greater than 100 g/l. This cell concentration resulted in heavy bubbling and broth outflow, which made it impossible to carry out continuous culture. Therefore to maintain a stable cell concentration, cell bleeding was performed together with cell recycling [4].
In situ solvent extraction during fermentation has been shown to be one technique that enhances solvent production in ABE by reducing End-product inhibition. Different extractants like oleyl alcohol, decanol, propylene glycol have been used extensively in laboratory scale for *In situ* Butanol extraction. However, the present market value of these chemicals and the cost of subsequent solvent recovery have discouraged the scaling – up of these processes. Thus, one possible solution would be to identify cheap sources of extractant and end-uses of the solvent extraction process that would not require solvent recovery. Methylated fatty acids (MFAs) from vegetable oils are good alternatives and cheap sources of esters that could be used as extractants. In addition, they can be used as fuels (biodiesel) in agricultural machinery thereby eliminating the recovery step from solvent extraction [6].
Ishizaki et al. reported the use of methylated palm crude oil (CPO) as an extractant solvent to reduce end-product inhibition and to enhance solvent productivity in ABE fermentation. CPO did not inhibit the growth of the organism; otherwise, it could destroy the stability of the extractant and complicate the extraction process.
They observed that applying CPO as an extractant 47% of the total Butanol produced was extracted , glucose consumption was increased to 83% and high solvent productivities was obtained in comparison with conventional fermentation [6].
The pH-stat fed –batch cultures have also been used. These systems facilitate maintaining the substrate concentration at a low level in the broth or repressing undesirable byproducts caused by substrate at a high level [5].

5-Medium characteristics

It is reported that organic acid production is enhanced at higher pH while solvents are mainly produced at lower pH.
The addition of organic acids to the growth medium has been shown to stimulate solvent production and protect against the degeneration - Loss of capacity of the strains to produce solvents is called degeneration- of ABE producing *clostridia*. It is suggested that organic acids in broth trigger a metabolic shift from acidogenesis to solventogenesis, although the exact mechanism is still unknown [5].
Yukihiro et al. evaluated the addition of acetic acid and butyric acid to their system (a Fed-batch system) and concluded that butyric acid elevated the specific Butanol production rate, Acetone – Butanol production and yield of solvent. However, acetic acid only could enhance Acetone production. They also observed that when only butyric acid was fed, Butanol production was similar to that without feeding butyric acid after glucose depletion. However, when a solution of butyric acid containing glucose was fed, butyric acid utilization and Butanol production were observed. Also, Butanol flux was enhanced and Ethanol flux was very low in the presence of butyric acid [5].
One of the important factors that influenced cell growth, solvent and acid production is nitrogen level and carbon to nitrogen (C/N) ratio.
Solvent production does not occur if the undissociated butyric acid concentration does not increase and maintain at the critical level due to high pH or low Residual glucose concentration. Butyric acid in undissociated form is also inhibitory to cell growth and solvent production at concentrations higher than 0.5 g/l.
Madihah et al. suggested that a mixture of organic and inorganic nitrogen sources was required for good growth and to enhance solvent production [1].

6-The ABE fermentation in pilot plant and pre-industrial scale

Scale-up trials were performed during development and improvement of the traditional Acetone-Butanol fermentation at the beginning of the 20th century. For example, Weizmann's process, patented in 1915, was tested on a larger scale at Nicholson's distillery before the British government decided to erect a production facility. several years after the explosion in the established Acetone – Butanol plant at kings Lynn in England and its subsequent closure, the distillers company made a reassessment of the process in pilot-plant scale .the Acetone-Butanol production plant later established in Terre Haute, Indiana had a well equipped development division including a complete fermentation unit consisting of two fermenters and accessory equipment one-quarter of full plant size [2].
One aspect of the process develpement; however, is only sporadically described or mentioned – the scale – up step. This may be because results and experiences from pilot plant trials are sometimes less producible. It can also be assumed that commercial interests would tend to keep the results achieved confidential, at least as long as a competitive advantage is envisaged. In

addition, patients protecting a process offer generally relatively poor protection in most of the cases as modifications are easily made, thereby avoiding patent claims.
The most famous Acetone-Butanol fermentation pilot plants are: 1- Pilot plant at Terre Haute, Ind. 2- General purpose pilot plant at Curtis Bay, Maryland 3- Dokshukino plant, Former USSR 4- The biotechnology facilities at Soustons, France 5- A-B fermentation pilot plant in lower Austria [2].

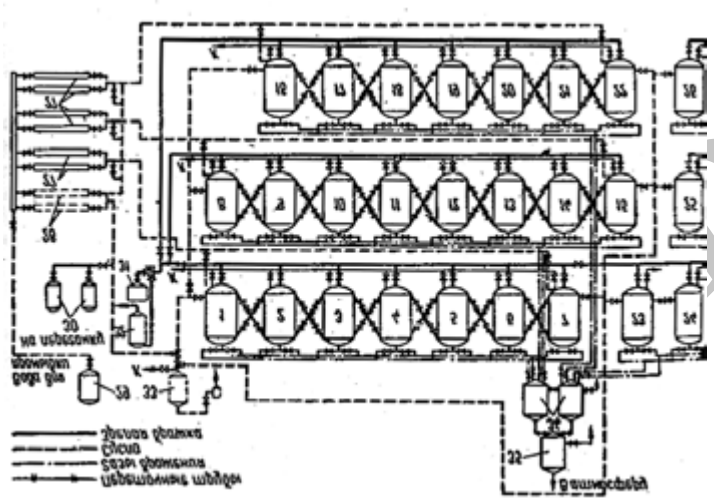

Fig 2. Schematical drawing of the Dokshukinski fermentation plant.

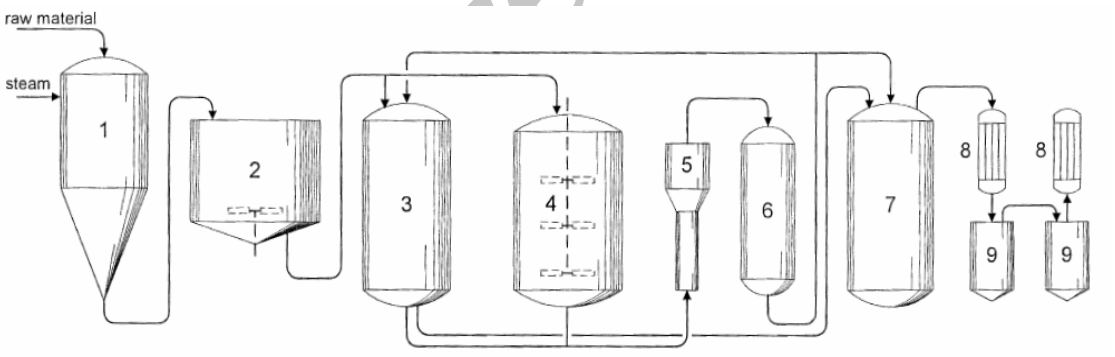

Fig 3. Principal flow sheet of ABE fermentation pilot plant in lower Austria.1-Henze-steamer 2- Mash tank 3- Substrate Vessel 4- Substrate vessel 5- First stage fermenter 6- Second stage fermenter 7- Finishing tank 8- Condenser 9- Condensate Collection Tank.

7-Conclusion

There have been a great deal of improvements in the process including the more exact classification of the solvent – producing strains , investigations toward a better understanding of the mechanisms of the clostridia cell, genetic improvements in order to enhance the solvent

tolerance or to improve the strain stability and at last engineering aspects of the fermentation have been improved dramatically.
Since there are almost no reports about the scale-up trials in recent years, it may be assumed that ABE fermentation is not economic. However, the complex nature of this fermentation is now well-known and the laboratory and pilot plant achievements are completely adaptable to industrial scale production.

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