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The Acetone-Butanol Fermentation in Pilot Plant and Pre-Industrial Scale

Dragan Nimcevic* and J. Richard Gapes

Vienna University of Technology
Institute of Chemical Engineering, Fuel Technology and Environmental Technology, Getreidemarkt 9/159
1060 Vienna, Austria

Abstract

A summary of literature data concerning pilot or pre-industrial scale trials of the acetone-butanol fermentation throughout its history is given. The recent pilot plant trials in Austria are also described for the first time. Some aspects of the current development of the acetone-butanol fermentation in general , especially from a technical point of view are also discussed.

Introduction

There are plenty of reasons why the introduction of a new idea (or process) directly in production plant scale is risky and probably expensive. For example, scaling-up of a microbial culture from laboratory work conducted in shake flasks requires translation to the kind of equipment used in a production plant, such as stirred vessel. Since the formal similarity between these two containers is lacking, there is a great deal of uncertainty in the scaling-up process, and the relatively small vessels in a pilot plant allow relatively risky experiments to be performed at relatively low cost. A second consideration is that staffing of a pilot plant is generally more skilled than a commercial unit can afford, which means that investigation and optimisation can be more quickly carried out. It is also possible to test the sophisticated problems of process control more cheaply (Hockenhull, 1975).

Scale-up trials were also performed during development and improvement of the traditional acetone-butanol fermentation at the beginning of 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 (Hastings, 1971). 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 (Gabriel, 1928).

Received October18, 1999; revised October 28, 1999; accepted October 28, 1999. *For correspondence. Email dnimc@mail.zserv.tuwien.ac.at; Tel. +43-1-58801-15935; Fax. +43-1-58801-15999.

The rapidly growing automobile industry between the two world wars along with it's demand for suitable lacquers based on butanol as raw material contributed to a huge development of the acetone-butanol fermentation industry which became second of importance only to the ethanol fermentation industry. The acetone-butanol fermentation operated commercially in a number of countries each of which more or less developed their own technologies, using local strains and exploiting local substrate supply opportunities and other prerequisites, indicating that pre-industrial scale tests were performed worldwide. The reports of runs are, however, scarce. Two notable exceptions are the report from the former USSR describing the experiments on the acetone-butanol fermentation on semi-industrial scale using corn flour substrate (Shaposhnikov *et al.,* 1931) and the report originating from Japan and describing the production of acetone-butanol on semi-industrial scale on sweet potato using two new isolates (Doi and Yamada, 1940).

The acetone-butanol fermentation declined very rapidly in most of the countries of the Western World after the 2nd World War, due to the both increased substrate prices and competition to the petrochemical industry based on the cheap raw material. Only the plant in South Africa continued to run until 1982 (Jones and Woods, 1986). The plant at the Organic Chemical Factories of the Egyptian Sugar and Distillation Company was apparently also running during the 1970s (Abou-Zeid *et al.,* 1978). The situation in the East was less clear, but it has been reported that in 1990 China still relied on the acetone-butanol fermentation for almost one-half of its supply of acetone (Santangelo and Dürre, 1996). Due to economic pressures the fermentation plants were all forced to recover all products possible and such integrated plants achieved a high degree of efficiency in operation. The investigations also led to the use of alternative, cheaper sources of fermentable carbohydrates, all of which proved successful at least in pilot plant tests (McNeil and Kristiansen, 1986).

The abrupt rise in petroleum prices during the 1970s led to a renewed interest in the feasibility of obtaining fuels or chemical feedstocks by microbial conversion, whereby special attention was paid to the acetone-butanol fermentation. Research was considerably intensified during 1980s and was directed toward the improvement of the complete process, including development of the improved strains, alternative fermentation substrates, improved cultivation techniques and improved product removal techniques. During the 1990s basic research on the genetics of solvent-producing clostridia and the efforts in order to improve the strains by genetic manipulation are especially intensive.

All the rises and falls of the acetone-butanol fermentation are very well documented in the literature in research papers, patents and reviews. One aspect of the process development, 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 reproducible. 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, patents protecting a process offer generally relatively poor protection in most of the cases as modifications are easily made, thereby avoiding patent claims.

In this review an attempt is made to summarise pilot trials on the acetone-butanol fermentation throughout its fascinating history. As already mentioned, the literature on this topic is relatively scarce and/or difficult to find, therefore this paper does not claim to present a complete review. With one exception, only pre-industrial test facilities for which more than one reference could be found are described. The acetone-butanol pilot plant in Austria, run by the authors and their co-workers and mentioned by Dürre (1998) is described in more detail for the first time.

Acetone-Butanol Fermentation Pilot Plants

Pilot Plant at Terre Haute, Ind.

During 1920s the Commercial Solvents Corporation of Maryland produced acetone and butanol in two plants, one of them at Peoria, Illinois and the other at Terre Haute, Indiana. While the Peoria Plant was devoted to the large-scale manufacture of butanol, acetone, ethanol and methanol from fermentation gases, the operations at Terre Haute were considerably more varied. In addition to the production of fermentation products, value-added products such as butyl acetate, butyraldehyde, dibutyl phthalate, butyl stearate and diacetone alcohol were also produced (Gabriel, 1928). Their Development Division was also concentrated at Terre Haute, among it the Bacteriological Research Department had a complete semi-commercial fermentation unit consisting of two fermenters and all accessory equipment in one-quarter of full size (Killeffer, 1927; Gabriel, 1928).

It can be readily assumed that the very first attempts to develop the continuous acetone-butanol fermentation in a large scale were undertaken by the Bacteriological Research Department at Terre Haute. Patents on a continuous acetone-butanol process appeared as early as 1930 in Great Britain and 1932 in the United States and both were submitted by the staff of Terre Haute plant (Wheeler and Goodale, 1930 and 1932). Both patents are very similar and they describe the possibility of running the existing production plant in continuous mode. The authors recognised the differences between the possibilities to run the ethanol fermentation and the acetone-butanol fermentation in continuous mode and they stated that the fact that the clostridia pass through various morphological and physiological forms has to be taken into account during process design. They proposed therefore the use of the series of 3 to 5 fermenters, or even more if desired. They also stated that the flow rate has to be maintained in such a manner that the bacteria remain in the first fermenter for a period less than the logarithmic growth period of the organisms in a normal batch fermentation. Using continuous fermentation as proposed it was apparently possible to reduce the residence time for complete fermentation from 52 hours in batch culture to 30 hours. It was stated that the continuous process may be continued for 30 days or longer and that a significant increase in the production capacity of the given plant and that considerable savings in labour, steam, electric power and other expenses are achievable.

Although it can be concluded from the patent text that the test trials were in fact conducted, no exact description of the work was published. The process described was apparently never been applied in large scale (McNeil and Kristiansen, 1986).

General Purpose Pilot Plant at Curtis Bay, Maryland

This pilot plant, described by Jacobs *et al.* (1948), was located in the fermentation department of the Curtis Bay plant of U.S. Industrial Chemicals, Inc. and it was operated for about three years on various types of fermentation, including ethanol, acetone-butanol, organic acids and certain mold cultivations. The acetone-butanol fermentations conducted were mainly subjected to rigorous examination to determine whether conditions existed which would lead to contamination. The high yield of solvents and the pour plates made from the three fermentation stages showed no contamination and testify to the clean, satisfactory fermentations obtained in this plant. It is not clear from the report whether these acetone-butanol fermentation trials were conducted in batch mode or if the continuous operation was also tested.

Dokshukino Plant, Former USSR

This plant is mentioned in a number of reviews on acetone-butanol fermentation (*e.g.* Ennis et al., 1986; Jones and Woods, 1986; Maddox, 1989). The pilot unit was built in 1958 and it included a battery of 11 fermenters of 3.5 m^3 each (Yarovenko, 1964). The first fermenter was equipped with a stirrer and it served as inoculator and activator. The equipment was operated for 8 days without trouble at a residence time of 30 hours. After 8 days the acidity in the final fermenters started to increase.

Based on the results of the pilot plant the continuous acetone-butanol fermentation was introduced in 1960 into the Dokshukino production plant. Since the results in the pilot plant showed that a smaller number of fermenters could be employed all at an identical flow rate, lowering the total residence time, then three batteries of 7 to 8 fermenters with a volume of 220 and 270 m^3 were installed. Compared to batch fermentation, the plant yielded a 20% productivity increase and saved 64.4 kg of starch per ton of solvents produced (Hospodka, 1966).

The work on acetone-butanol fermentation in USSR from this period resulted generally in several very interesting articles concerning problems of infection in continuous culture (*e.g.* Yarovenko, 1964), stability of the continuous culture (Ierusalimsky, 1964) and the use of alternative fermentation substrates (Nakhmanovich and Shcheblykina, 1959, 1960).

The Biotechnology Facilities at Soustons, France

According to the French plans up until 1990 to replace 10% of petrol by gasoline substitutes, the teams from Institut Français du Pétrole and its engineering subsidiary Technip started a pilot project in order to evaluate the industrial potential of the production of solvents for gasoline substitute fuels. The project included the erection of the plant for conversion of biomass to acetone and butanol (Lloyd, 1984).

The pre-industrial scale plant at Soustons was initially designed to use mainly cereal straw and corn stover as

the raw materials and the aim was to produce 1 ton of solvents from 6 to 7 tons of raw material. The biomass was pre-treated by steam explosion and the pre-treated substrate was then hydrolysed using a cellulase complex produced in the plant by *Trichoderma reesei* CL 847 grown on lactose. The hydrolysates were then fermented to acetone and butanol by strains of *Clostridium acetobutylicum*. The solvents were separated by heteroazeotropic distillation and the stillage was concentrated, anaerobically digested or recycled (Marchal *et al.,* 1985b). Meanwhile the substrate spectrum was extended and the use of sugar beet and Jerusalem artichoke as fermentation substrates was mentioned (Marchal *et al.,* 1985a). The experimental facilities and the diverse activities in the plant are summarised and well described by Nativel *et al.* (1992).

Experiments in batch mode operation on the acetone-butanol fermentation in pilot scale (2 m^3) and in the demonstration scale (50 m^3) were described. Yields of 1 ton solvents from 7.7 tons corn cob were reported. The economic evaluation of the process stated that the costs of acetone-butanol fermentation from lignocellulosics are strongly dependent on market value of the by-product lignin (Marchal *et al.,* 1992).

Another study showed that, although lignocellulosics currently led to the highest production costs both for acetone-butanol and ethanol fermentations, they offered the greatest potential process improvement. It was also stated in this report that conversion to ethanol from either C_6 sugars or C_5+C_6 sugars is more economically favourable than the acetone-butanol production (Desmarquest and Requillart, 1992).

Acetone-Butanol Fermentation Pilot Plant in Lower Austria

During 1997 and 1998 the authors and their co-workers erected and run a pilot plant at a commercial agricultural distillery in Lower Austria. The plant was run from 1998 to 1999 in this distillery, parallel to the production of crude ethanol from different starchy raw materials, including potato, wheat, rye and maize.

The decision to erect the plant in the existing distillery was governed by financial considerations. In particular major savings could be achieved because it was not necessary to design, buy and install the most of the plant infrastructure (including boiler, process water and air supply), the substrate storage and much of the substrate preparation equipment. The major disadvantage was total subordination of infrastructure access to production operations. The result was unreliable supply of water, steam and substrate.

The flow sheet of the pilot plant is presented in Figure 1. The complete plant, was made of quality stainless steel, excluding the condensate collection tanks (vessels 9 in Figure 1) which were made of glass. The two substrate vessels (3 m^3 and 3.5 m^3, vessels 3 and 4 in Figure 1) were fed directly from the mash preparation equipment of the distillery (vessels 1 and 2 in Figure 1). Unhydrolysed starchy mashes, as well as liquefied and saccharified mashes were obtainable. Substrate was diluted and sterilised in the substrate vessels as required.

The first stage fermenter was designed as a gas-lift bioreactor with working volume of 50 litres (vessel 5 in Figure 1). The second stage fermenter had a working volume of up to 300 litres and was equipped with an on-line gas-stripping unit (vessel 6 in Figure 1). The 3 m^3 finishing tank was designed for solvent separation by distillation or vacuum distillation and for re-circulation of the fermentation mixture to the second stage fermenter if necessary (vessel 7 in Figure 1). The 3 m^3 substrate vessel (vessel 3 in Figure 1) was also connected to the second stage fermenter and to the finishing tank and it was possible to use it as the third stage fermenter.

Both substrate preparation vessels and the first stage fermenter including all piping, fittings and connections were of sterile design and were sterilised using saturated steam at at least 2 bar_{gauge} before starting each fermentation run. Diaphragm valves were exclusively installed. Flanged connections were avoided where possible and the quality inert gas welding was performed instead. The second stage fermenter, the finishing tank and piping were sterilised by saturated steam at at least 1 bar_{gauge} and in these sections

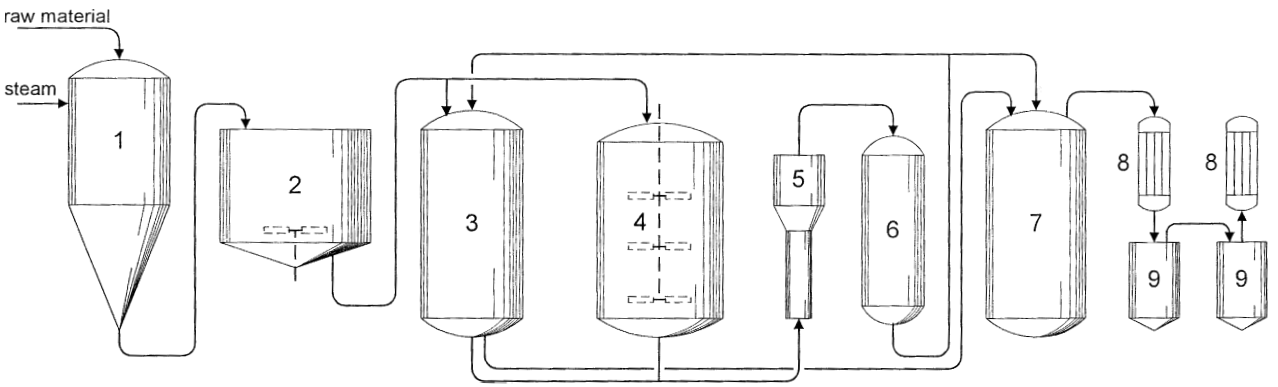

Figure 1. Principal flow-sheet of the acetone-butanol fermentation pilot plant at the commercial distillery in Lower Austria. The real sizes of the equipment do not correspond to the size relationships on the drawing. 1) Henze-steamer, 5 m3 (ownership of the Distillery); 2) mash tank, 7 m3 (ownership of the Distillery); 3) 3 m3 substrate vessel; 4) 3.5 m3 substrate vessel; 5) first stage fermenter, 50 litres; 6) second stage fermenter, 300 litres; 7) finishing tank, 3 m3; 8) condenser; 9) condensate collection tank, 50 litres.

mostly the ball valves were installed. These less strict sterilisation conditions were sufficient, since it was assumed that the fermentation in the later phase would be less sensitive to contamination and contamination problems generally appear to originate in the first fermentation stage (Yarovenko, 1964).

The plant was designed for different modes of operation, including batch, fed-batch and continuous, however the substrate was always prepared batchwise.

Although it was possible to use unhydrolysed, liquefied or saccharified mashes, only liquefied mashes were tested. Unhydrolysed starchy mashes are also readily fermentable also without supplementation (Nimcevic *et al.,* 1998) but when used in the pilot plant they caused blockages of the equipment due to the starch disposition. It was found that a rapid liquefaction using relatively small amounts of amylase prevents blockages and interruptions during continuous operation and may therefore be more economic. Full saccharification includes the use of an additional enzyme (amyloglucosidase) and requires a longer reaction time. Complete saccharification of the mash (as for the ethanol fermentation by yeasts which cannot ferment glucose polymers) does not improve the fermentation (unpublished results). Moreover, sugar monomers are more sensitive than short chain polymers to thermal degradation during sterilisation and the degradation products can adversely affect the fermentation. The strain *Clostridium beijerinckii* NRRL B592 was used and the longest continuous fermentation conducted with this organism lasted about 4 weeks. It was not difficult to maintain the continuous operation and interruptions were caused by external circumstances.

Special attention was paid to product removal and recovery. Different on-line and off-line techniques were tested, including distillation, vacuum distillation, rectification, gas stripping and reverse osmosis. Although recent investigations report significant improvements in membrane systems (*e.g.* Qureshi and Blaschek, 1999) this technique was not tested in the pilot plant since it was assumed that because of relatively low solvent fluxes through the membrane the required membrane area is too high for commercial operation, contributing to the high investment and operating costs. Membrane evaporation has been tested by the authors in laboratory scale (Gapes *et al.,* 1996) and found to be technically possible. Additionally both pervaporation and membrane evaporation can suffer from the membrane fouling problems, which would cause additional operating costs, although the authors have not encountered such problems. Among the product purification techniques tested rectification with reflux yielded as expected the best results with regard to solvent selectivities and throughput.

Concluding Remarks and Future Prospects

The acetone-butanol fermentation was the first large-scale fermentation process developed which is sensitive to contamination. Hence this fermentation contributed much to the knowledge of how to run sterile processes in an industrial scale. The engineers involved learned to cope with the new demands of industrial microbial cultivation, including problems with sterilisation and aseptic procedures. These experiences were of essential importance for the further development of the industrial microbial cultivation and in particular for the start of the antibiotic era during 1940s.

The period of the increased interest in the potential of the acetone-butanol fermentation during the last 20 years has resulted in many improvements in the process, including the more exact classification of the solvent-producing strains (Ref. *e.g.* Jones and Keis, 1995), investigations toward a better understanding of the mechanisms of the clostridial cell (*e.g.* Dürre *et al.,* 1995; Mitchell *et al.,* 1995), genetic improvements in order to enhance the solvent tolerance (*e.g.* Formanek *et al.,* 1997) or to improve the strain stability (*e.g.* Kashket and Cao, 1995). Not only classification, understanding of the genetics and microbiological aspects were significantly improved, but also engineering aspects of this fermentation were widely investigated and many improvements have been achieved: long-term stable continuous cultures were developed, cell immobilisation and cell recycle techniques for enhanced solvent productivity were investigated and a variety of alternative product removal and recovery techniques were tested (Ennis *et al.,* 1986; Maddox *et al.,* 1993).

All these achievements have until now almost exclusively been only of academic significance, since recent reports about the scale-up trials are almost non-existant. One can generally assume that the acetone-butanol fermentation because of its relative complex nature and apparently low yields cannot be economic and therefore the scale-up trials would actually have little sense. However, the complex nature of this fermentation is now well-known and laboratory scale experiments and our work in pilot plant scale have shown that the problems of degeneration, contamination or phage attack can be overcome when the appropriate techniques are used. Moreover, these techniques are not necessarily extremely expensive or too complicated for use in production facilities.

Another problem is what is commonly quoted as an apparently low yield. This is in fact not truly the case. The yield of products is limited by the First Law of Thermodynamics which says that energy cannot be created nor destroyed. Since approximately 95% of the energy in the substrate are present in the products, the yield of the fermentation is in fact very high. The bacteria have merely concentrated this energy into compounds of higher energy content than the substrates while using about 5% for their own growth and metabolism. It is therefore neither theoretically possible nor reasonable to expect significantly higher yields, although some improvements were achieved *e.g.* when the culture was sparged with CO, whereby it was possible to reduce the yield of gaseous products and to increase the butanol yield (Datta and Zeikus, 1985). Approximately 10% of the energy is present as hydrogen and its use is important for the energy house-hold of a production facility and its economics. Alternatively it can be used for value-added products or cleaned and sold.

Nevertheless, according to the numbers of reports both solvent productivity and final solvent concentration can be increased, at least under laboratory conditions. Some further improvements are possible when using continuous mode of operation with on-line product removal. When the products are separated on-line, the solvent concentration in the fermentation mixture can be maintained at lower levels, thereby contributing to reduced product inhibition, increased culture stability, improved substrate utilisation

and enhanced volumetric productivity- of course, a compromise has to be made because the product separation would operate more efficiently when the solvent concentrations are higher. Such improved systems have been tested often, whereby probably the most promising results were achieved when on-line membrane separation techniques were used (Gapes *et al.,* 1996; Qureshi and Blaschek, 1999). However, before the membrane techniques can really find their use for large-scale operation, a number of drawbacks have to be considered. First of all, these techniques (and especially membrane evaporation) remove large amount of water from the fermentation mixture in addition to the solvents, thereby contributing to the medium concentration and eventually the salt accumulation both of which can affect the fermentation. Further, membranes still suffer from fouling and membrane fluxes and selectivities are still a matter for further development.

The use of continuous fermentation is frequently said to improve the economic viability. Significant improvements in continuous acetone-butanol fermentation in the laboratory scale, including increased productivity, long-term stability and lack of degeneration changes have been demonstrated by a number of authors. Pilot experiments and the large scale continuous operation in the Dokshukino plant for example have shown that this mode of operation can yield considerable savings and it is generally assumed that a four-week continuous operation should already be cost-effective. However, experience from industrial microbial cultivation practice show that commercial technologists actually do not prefer this mode of operation and consider it to be more difficult, especially because of the contamination problems. On the other side, modern equipment for aseptic operation can allow for a stable pure clostridial culture propagation and activation under continuous conditions, as shown during tests in the Austrian pilot plant. This fact can contribute significantly to the achievement of one of the crucial feasibility prerequisites-reliability. The reliability means that once when the acetone-butanol fermentation does function in the industrial scale the cultivation conditions have to be maintained in such a way that the complete conversion of the substrate is guaranteed, *i.e.* the danger of contamination (either by phages or by other microorganisms) must be reduced to the level as low as possible and these conditions must be maintained through the whole operation period. Only a reliable acetone-butanol fermentation can be expected to be commercially successful (D. T. Jones, International Conference on the Applied Acetone Butanol Fermentation, 16-18 Sept. 1999, Krems, Austria).

Other aspects which determine the economic success of the acetone butanol fermentation, including analysis of the substrate markets, process costs and product markets are discussed in more detail in this issue by Gapes *et al.* (2000).

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