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The History of the Acetone-Butanol Project in Austria

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Abstract

The acetone-butanol fermentation and the closely related 2-propanol-butanol fermentation are of interest to Europe in particular for environmental and socio-economic reasons, but its economic and technical feasibility must be proven before reestablishment as a commercial proposition. In particular the reestablishment of a new, fledgling fermentation industry selling into markets presently serviced by the mature, firmly established and highly capitalised petrochemical industry will require a significant driving force or else commercial users, who frequently have long-term supply contracts, will remain with known and proven suppliers. Hence the present state-of-the-art of the acetone-butanol fermentation is best described as technically and economically difficult but possible in niche markets. The most likely future is for decentral fermentation facilities processing locally made substrates and selling into niche markets.

Introduction

The meeting “International Conference on the Applied Acetone-Butanol Fermentation” in Krems near Vienna, Austria on 16th to 18th September 1999 was the largest in a series of three international meetings and was intended to draw together the international scientific community studying this process and to present the results of two research projects financed by the Commission of the European Union with support from the Austrian Ministry of Science and Transport. While the smaller of the two projects covered only mobility and meeting activities, the larger project was concerted with the building and testing of a pilot-plant for the fermentation of agricultural starch products to acetone and butanol. The pilot plant was built at the site of an ethanol plant run by a farmers’ co-operative fermenting these substrates.

The meeting attracted many scientists well known from the scientific literature and representing almost every group in the world researching this field. Delegates came from as far afield as New Zealand, Japan, South Africa and the USA along with those from Europe. Members of the farming community, the ethanol industry and from government made up the balance of the delegates. Christian Schuster has given a short summary of their scientific contributions in his introduction.

Europe’s interest in the acetone-butanol fermentation is reflected world-wide by continued research activity. This interest is based on the use of renewable biomass from the local agricultural community to provide renewable bulk chemicals. The goals are not only to reduce carbon dioxide emissions in line with the international targets set at Rio and Kyoto, but also nationally to help reverse stagnation of rural agricultural communities and resulting unemployment and mass migration from rural to urban areas. The concurrent reduction in imports and related balance-of-payment considerations is seldom emphasised in this context. The acetone-butanol fermentation addresses all these problem areas, however, solutions to difficult economic and technical problems are required prior to reindustrialisation of the process. The advances in technology presented at Krems may very well have supplied enough solutions to allow for economic operation of an acetone-butanol fermentation plant in niche markets. A discussion of biological technologies addressing these problems can be found in Gapes *et al.* (1997).

History

The development history leading up to the Austrian program goes back long before the two EU-projects and can be traced back to 1980 when Ian Maddox and Vidar Larsen began investigations into this fermentation at Massey University in New Zealand for the use of whey-permeate for the dairy industry. Richard Gapes soon joined them after completing a degree in chemical and materials engineering at Auckland University. Amongst other publications an engineering costing analysis (Gapes, 1982) and work on process reliability (Gapes *et al.*, 1983) resulted. Experimental work with batch fermentations also suggested that the protonated fatty acid groups, headspace pressure, both hydrogen and carbon dioxide and the minimum pH reached were all important for the butanol concentration, while ethanol and acetone concentrations were affected by other factors (Gapes, 1982). Richard Gapes then worked in the family consulting engineering practice, the dairy industry, the ethanol fermentation industry, and in the gold and silver mining industry. This experience was later invaluable while building the fermentation pilot plant in Austria. When Richard Gapes began work at the Vienna University of Technology in 1987 with Alfred Schmidt the institute already had interest in the acetone-butanol fermentation – Helmut Effenberger had investigated the process for the fermentation of pentose sugars. Richard Gapes continued this work using a two-stage continuous fermentation. Not only xylose but also sucrose and glucose were used and particular attention was paid to process stability and reliability and to recovery after disruptions typical in industrial operation. The two-stage process was advantageous not only to dampen out the oscillations frequently observed but, also to permit greater experimental freedom with the second stage than possible in a single stage system.

The Austrian project application for an acetone-butanol pilot-plant was submitted to Brussels in January 1994 two weeks after Austria entered the European Monetary System as a precursor to entry to the European Union and became

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the first European Union shared-cost project co-ordinated by an Austrian Group. The second European Union project was a concerted action project intended to draw together European groups interested in the process. These two projects included partners in Germany (Peter Dürre), United Kingdom (Mike Young, Nigel Minton), France (Jacque Meyer), Spain (Juan Carrassco), Holland (Pieternel Claassen) and Hungary (Kati Reczey) and involved extensive co-operation with New Zealand groups (Ian Maddox, David Jones).

Research Philosophy

Economic modelling and sensitivity analyses of the acetone-butanol fermentation process had shown those major cost factors typical of many fermentation processes - purpose-grown substrates make up three quarters of production costs followed by capital related costs, energy and personnel. Further consideration of the theoretical boundary conditions for economic feasibility based on the laws of thermodynamics, the metabolic reaction stoichiometry and the market size and pricing structure then permits identification of niche-markets which can support an acetone-butanol fermentation process. Not surprisingly these niche-markets have in part been filled by the ethanol fermentation industry for many decades, but due to the current glut of ethanol in Europe this industry is undergoing restructuring and diversification. This provides an opportunity for the modernised acetone-butanol fermentation process, where the ethanol and the acetone-butanol fermentations can operated as production alternatives sharing equipment rather in competition. The South African plant at Germiston near Johannesburg which shut down during the 1980's had exploited this co-operation. These economic considerations are summarised briefly by Gapes (2000) and an overview of substrate and product market possibilities in Schuster *et al*. (1997) and Gapes (1995) respectively.

The sensitivity analyses also underlined the overriding importance of reliability in production, which guided laboratory work on this topic. Regardless of the cause of loss of production, for example due to loss of yields or productivity, reliability is of the utmost importance for the economic feasibility. The importance of reliability is recognised by industry, which shies away from continuous fermentations in favour of batch forms of production. The advantages of continuous fermentation along with its associated problems in a demonstration scale acetone-butanol fermentation facility were shown by Yarovenko (1964) and David Jones illustrated these problems from a historical perspective by analysis of production logs from commercial acetone-butanol fermentation plants operating early last century (Jones *et al.* 2000). The historically documented phage problematic and degeneration in its diverse forms are both technical difficulties over and above restrictive economic limitations. For these reasons laboratory work in Vienna has concentrated on process reliability during the fermentation of low-quality substrates available in rural areas. The 1999 meeting in Krems included a number of contributions relating to the question of reliability and degeneration.

Laboratory Research

Laboratory work up until the EU project was performed by Richard Gapes followed by Dragan Nimcevic, and then during the project initially by Hedwig Swoboda followed by Reinhard Thayer. Long-term, continuous fermentation requires particular dedication although on-line data-logging and data-processing using a personal computer reduces the necessity for work during nights, week-ends and public holidays. The computer was programmed to alert the operator by modem and telephone in case of non-standard conditions and it was possible to view the fermentation curves from outside the laboratory by modem and telephone.

In total the equivalent of over four years of continuous fermentation have been performed and have concentrated on conditions and procedures effecting fermentation reliability and reproducibility and have demonstrated the ability to reproducibly perform stable fermentation for extended periods. The substrates used have included both semi-synthetic media containing xylose, glucose and sucrose (Gapes *et al*, 1996; Mutschlechner *et al*., 2000) along with agricultural products such as whey, potato, corn, silage and wheat (Claassen *et al.,* 1998; Gutierrez *et al.,* 1998; Nimcevic *et al.,* 1998). Investigations directly into the causes of these problems also yielded interesting results (Maddox *et al*., 2000; Steiner *et al*., 1999; Swoboda *et al*., 2000).

While the effects of phage attack and degeneration are frequently rapid and can halt production within a day or even just a few hours both phenomena can also occur gradually and result in little more then a reduction in productivity and culture vigour. It was necessary therefore to develop more sensitive methods of detection, in particular methods for the prediction of an impending loss of production. The ratio of butanol to acetone and the fermentation gases can provide, for example, warnings of trouble-to-come, however, more informative methods have also been developed. For example, analysis of protein concentrations using electrophoresis reveals characteristic patterns associated with acid production and solventogenisis (Schuster *et al*., 1998). While electrophoresis can describe the state of the fermenting culture as a whole, resolution of near-infrared and raman spectra into the contributing spectra can provide quantitative information at a cellular level (Schuster, 1999; Schuster *et al.,* 1999a; Schuster *et al.,* 1999b). Christian Schuster has been carrying out these analyses in co-operation with the spectroscopy instrument suppliers Bruker (Karlsruhe, Germany; contact: F. Mertens) and Dilor (Bensheim, Germany, contact: E. Urlaub), and several university groups (B. Lendl, Institute of Analytical Chemistry, Vienna University of Technology; M. Grube, Institute of Microbiology at the University of Latvia, Riga; M. Young and R. Goodacre, Institute of Biological Sciences, Aberystwyth, Wales). The extent and degree of starch hydrolysis to short chain and monomeric units can also be analysed using these techniques, which is commercially important due to the economic ramifications of over or under dosing enzyme, particularly in ethanol facilities (Schuster *et al*., 1999c). These techniques are also suitable in part for on-line use in commercial facilities, where early warning of an impending loss of productivity can not only allow better diagnosis of possible causes but also allow for recovery procedures to be started before significant loss of production occurs (turn-around time for fermenters can be minimised, for example).

The Pilot-Plant Project

Nimcevic and Gapes (2000) have given a summary of scale-up work described in the literature. The Vienna group in the EU projects lead by Richard Gapes was responsible for designing, building and running the pilot plant and for supporting work in the laboratory. Initially the organic fraction of municipal solid waste was the focus of attention, however, by the end of the project low-cost starch produce as used for decades by the European ethanol industry become the focus of attention. This change of emphasis came about as interest was discovered in the fermentation by an ethanol fermentation facility run by a farmers' co-operative shortly before construction of the pilot plant began. The pilot plant itself was therefore redesigned and constructed in a commercial ethanol plant. This brought two significant advantages; firstly substrate delivery, storage and preparation was all performed by the production facility, and secondly research performed in an operating production environment incurs invaluable practical experience under conditions which cannot be reproduced in a laboratory. There were, however, three significant disadvantages; firstly the group was split across two sites which greatly reduces symbioses amongst the personnel, secondly access to analytical and other research infrastructure was inhibited and thirdly substrate delivery and availability of services such as water and electricity were governed by site production requirements. This latter become increasingly problematic as the ethanol-production facility was fully rebuilt and modernised concurrent with the pilot-plant project and it became impossible to operate the pilot-plant for more than several weeks without day-long breaks to essential services.

Pilot plant experiments were intended to test the performance and reliability of fermenting commercially available agricultural substrates in an industrial environment and to provide scale-up experience. In spite of difficulties associated with continuous acetone-butanol fermentation the pilot-plant was designed to be able to run in fully continuous operation, but also capable of batch-wise processing. The sterile sections of the pilot plant itself were constructed of weldable stainless steel whereby particular attention was paid to sterility and to cleanliness. The scale-up factor was approximately 50 to 100. In general the pilot plant results matched or were superior to laboratory results. This appears in part to be due to the large working volumes - laboratory experiments have shown that larger fermentation volumes tend to yield better results (unpublished data). The first stage had a working volume of 50 litres and the second stage approximately 150 litres. Dragan Nimcevic was the engineer who carried out the detailed design, supervised construction and lived on-site during continuous operation of the pilot-plant. Hans Baumgartner provided materials and mechanical engineering advice.

The Future

In the immediate future testing in a demonstration scale is necessary to prove that the engineering design philosophies and fermentation procedures used in the pilot-plant can provide reliable production and prevent contamination by phages and other micro-organisms in larger scale equipment. The process as presently envisaged does appear to be economic in the targeted niche markets and work in Vienna is currently concentrating on organisation of such a demonstration plant.

Research further into the future must continue to provide better reliability and to develop technologies operating nearer to theoretical limits. The theoretical limits governing substrate yields and usage are already being approached and significant economic improvements can only by achieved by use of newer, cheaper substrates. As shown in Gapes (2000) purpose-grown crops are at present unlikely to allow for an economic process in competition with oil-derived products at the present price of crude. Additionally the quantities of low-quality substrates targeted at present are limited. Growth of a future acetone-butanol fermentation industry is therefore only possible if a new low-cost, high-volume substrate can be found. One possible hope in this respect comes from genetic engineering. While genetic engineering of butyric acid bacteria has generally not yet been able to contribute major advances for the acetone-butanol fermentation, basic research using genetic engineering techniques has been contributing to our understanding of control mechanisms governing cell metabolisms including solventogenisis. Genetic engineering has, however, recently supplied the hope of a major breakthrough in substrate availability. The complete analysis of the *Clostridium acetobutylicum* genome performed by Genome Theropeutics Inc. and supported by the U.S. government revealed genetic sequences apparently coding for cellulose enzyme complexes. If this endogenous genetic material can be reactivated and demonstrates sufficient activity then a whole new class of substrates would be available for acetone-butanol production.

An area where theoretical limits are not closely approached is the energy required for product separation. It the relevant theoretical limit is defined by the energy of solution of the components including entropy considerations, then current technologies such as distillation and pervaporation cannot approach this limit (Friedl and Gapes, 1990). Even technologies not requiring a phase change such as extraction and nanofiltration are relatively energy intensive. A fundamental break-through in this area is required.

The energy demands of the process are of importance not only from the point of view of economic feasibility but also if acetone/2-propanol and butanol are to be used as a liquid energy carrier. (The fermentation products can be used as mixtures in diesel and in petrol/gasoline for the transport industry and can also be used to increase the temperature and water tolerance of ethanol/fuel mixtures, to improve the octane value and to help improve most emission values, in particular the net production of carbon dioxide. The recognition of MTBE as carcenogenic may also increase interest in this area.) Although the fermentation products can be used in transport fuels this is of limited interest if their production consumes more energy than the mechanical energy gained in use. For this reason the energy requirements for product separation must be greatly reduced.

The group in Vienna has also looked at novel uses for the fermentation products as winter diesel supplements (Puntigam *et al*., 1998; Nimcevic *et al*., 2000) and for the fermentation by-products as biodegradable plastics (Parrer *et al*., 2000).

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