

Applied Acetone-Butanol Fermentation

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The International Conference on the Applied Acetone Butanol Fermentation, held in September, 1999, in Krems, Austria, brought together a group of scientists from four continents with interest in the bacterial group of solvent-producing Clostridia. A wide range of topics from basic molecular microbiology to applied bioprocess technology was covered.

The acetone-butanol (ABE) process, formerly a large industry, declined in the competition with the oil-based chemical industry. However, in the search for sustainable process alternatives based on renewable resources, an economic revival appears desirable and promising, in order to produce the basic chemicals 1-butanol and acetone from biological materials. The aim of the conference was to highlight all aspects of the current progress towards this goal, combining the findings of molecular microbiology and microbial physiology with modern bioprocess technology for an enhanced performance of the overall process.

In the historical forward, Richard Gapes, chairman of the conference, tells the story of the acetone-butanol fermentation project in Vienna from the beginnings up to the recently realised pilot plant and the conference. The list of scientific contributions from the conference which were written down for this symposium volume is started with the topics in basic science, follows on to more and more applied work and ends with the technology work.

The interest of basic microbiological science in solvent-producing Clostridia lies first in the complex metabolic pathways and their regulation. The minireview by Jacques Meyer on clostridial metalloproteins introduces into the wide field of redox reactions in these anaerobic prokaryotes, but also connects to very different groups of microbial and higher organisms, due to the very conserved nature of this class of proteins. The work of Behrens and Dürre describes the biochemical and functional characterisation of a response regulator protein involved in potassium uptake in *Clostridium acetobutylicum*, and a comparison to the different functionality of the corresponding protein in *E. coli*. The groups of Mike Young and Eva Kashket tackle in transatlantic cooperation in the work of Liyanange *et al.* the issue of tolerance of *C. beijerinckii* to its toxic products, finding a relationship to the down-regulation of glycerol dehydrogenase in more tolerant mutants; although the mechanism of this effect is yet to be clarified, the work shows new insights into mutagenesis and gene regulation in this organism. The issue of strain degeneration, the loss

of the ability to produce solvents on a genetic level, is addressed for *C. beijerinckii* by the second work of Liyanange *et al.*, looking for a possible role of transposable genetic elements (insertion sequences) in the rearrangement of the genome leading to degeneration.

The second topic of very practical interest in basic and applied research covered in the symposium is the development of transformation vectors for Clostridia, here especially reporter gene vectors, which are analytical tools to study the regulation of physiological activities in an organism. Quixley and Reid describe a reporter gene based on the activity of a clostridial β -1,4-glucanase. The constructed plasmid can be used for monitoring the expression from cloned promoters in non-pathogenic clostridia, by an enzyme activity easily detected in plate tests as well as a quantitative photometric assay. Davis *et al.* developed a reporter gene assay for the indirect detection of toxin production in the pathogen *C. botulinum*. The detection of toxin production - related transcription activity is based on luminiscence or β -galactosidase activity. The assay was developed for studying the physiology of food poisoning activity, but can be generally applied to related organisms and to other metabolic activities.

A third group of "basic" topics are the works in functional genomics, directly inspired by the findings from the "genomic revolution", in this case the complete genome sequence of *C. acetobutylicum* which is available since about a year and a half. Tangney and Mitchell compare the organisation of a sucrose transport operon in *C. acetobutylicum*, in its sequence lately known from the genome project, with the better characterised corresponding operon in *C. beijerinckii*. Huang *et al.* show the characterisation of a second butyrate kinase in *C. acetobutylicum*, of which a gene was found in the genome, and the function could be shown in recombinant *E. coli*, even though no protein production could be detected in *C. acetobutylicum*.

Moving on to the more "applied" studies, physiological investigations on *Clostridium* have received more attention again since the process development has come closer to a new industrial application. Shaheen *et al.* show comparative fermentation studies with different strains on varied substrates; they used the new, genetic-based taxonomy of solventogenic clostridia to identify relevant industrial strains from all four different species and compared data from literature and industrial reports with results obtained under consistent and comparable conditions in their own laboratory, coming up with practical suggestions for the optimal utilisation of strains on specific substrates. Maddox *et al.* clarify the confusion in the terminology in literature between different types of "misbehaving" *Clostridium* cultures, the phenomena of degeneration and "acid crash", and provide a mechanistic explanation for the occurrence of the latter in batch culture. Mutschlechner *et al.* describe an approach to transfer a model for the ABE batch fermentation directly to continuous fermentation by running two coupled continuous

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bioreactors at working points corresponding to early and late time points in batch fermentation.

Practical problems which were encountered in the classical ABE fermentation industry and are expected to reoccur in a revived industrial process are also addressed in the symposium. Jones *et al.* review the problem of bacteriophage infections, especially from the vast amount of experience gathered in the records of the fermentation industry, which are outside the usually available scientific literature. They go back to industry reports of the 1920s, through the first systematic research on clostridial phages, and to latest experiments with artificial phage infections of cultures and genomic studies of known phages. The possible implications of phage infections for the continuous operation of an industrial-scale plant are discussed. Parrer *et al.* report an approach to minimise the organic content of waste water from the ABE process by conversion of the organic acid by-products to bacterial polyesters.

Some strategies to enable an economically viable ABE fermentation are the topics of the last articles in the symposium volume. Experiments on the use of a cheap waste substrate, here domestic organic waste after pretreatment and hydrolysis, are reported by Claassen *et al.* The development of the ABE fermentation in pilot plant scale is reviewed by Nimcevic and Gapes, ending at the current pilot plant for the modern continuous process with integrated product removal; this plant situated in Austria was visited by the conference participants. Finally, the economic prospects of the modern ABE process are reviewed by Richard Gapes on both a theoretical basis and by market considerations; also included is a sensitivity analysis, giving hints on which improvement in the performance of the organism and the process make the highest contribution to the economic outcome.

From the results of the pilot plant trials and the economic analysis, the technical realisation of the modern ABE process at least for niche market applications appears feasible. Combined with the application of the increasing insights in physiology, and especially of the understanding on the molecular organisation of metabolic regulation, it should be possible to bring the process back to a viable economic enterprise.