BIOBUTANOL

D. T. Jones
Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

Keywords: biofuels, biobutanol, bioethanol, biodiesel, solvents, acetone, butanol, ethanol, gasoline, diesel, ABE fermentation, solvent-producing, *Clostridium*, *acetobutylicum*, *beijerincki*, sugar-based substrates, molasses starch-based substrates, corn, butanol toxicity, genetic manipulation, genetic engineering, metabolic engineering, biomass, lignocellulose, distillation, continuous fermentation.

Contents

Summary

The increasing cost of crude oil, concerns about energy security and the realization that fossil fuels are a finite resource have resulted in renewed focus on biofuels as a sustainable fuel resource. The use of biofuels is also seen to have advantages in countering further increases in carbon dioxide emissions. Bioethanol, and biodiesel are already being blended into gasoline and diesel in a number of countries and many countries have set targets for increasing the biofuel content of transportation fuels.

Butanol is an important industrial chemical and chemical feedstock but it also has potential for use as a biofuel. Currently virtually all butanol is produced chemically using either the oxo process from propylene or the aldo process from acetaldehyde. Like ethanol, butanol can be produced both by petrochemical and fermentative processes.

The production of biobutanol by fermentation for use as a biofuel is generating considerable interest as it offers certain advantages in comparison with bioethanol. These include higher energy content, lower water adsorption and corrosive properties, better blending abilities and the ability to be used in conventional internal combustion engines without the need for modification. Biobutanol can be produced from starch or sugar based substrates by fermentation utilizing various species of solvent-producing anaerobic bacteria belonging the genus *Clostridium* (see also– *Basic Strategies of Cell Metabolism*). The industrial production of butanol by *Clostridium spp*. in the Acetone-Butanol-Ethanol (ABE) fermentation process flourished during the first half of the last century and continued into the second half until the availability of cheap crude oil made petrochemical synthesis more economically competitive.

Renewed interest in the production of biobutanol from biomass has lead to the re-examination of the ABE fermentation. Currently a number of companies and scientific institutions are investigating the possible revival of the conventional ABE process or

the development of new bio-processes. Two major companies recently announced that they have committed themselves to the production of biobutanol as a biofuel additive.

The ABE process, however, has a number of limitations which render it uneconomic, at present, as compared with the ethanol fermentation. Key problems associated with the bio-production of butanol are the cost of the substrate, along with toxic inhibition of the fermentation by butanol limiting the yields and concentration of solvent that can be produced. Research involving genetic engineering, metabolic engineering, process engineering and alternative methods of solvent extraction and recovery is being undertaken to improve the production of biobutanol by fermentation. Strategies include reducing butanol toxicity and manipulation of the fermentation and the cultures to achieve better product specificity and yields along with improved substrate utilization.

The most intensively studied solvent-producing species is *Clostridium acetobutylicum*. Its genome has been sequenced and the regulation and genetic manipulation of solvent formation is being extensively investigated. Molecular biology has provided a detailed understanding of genes and enzymes involved in solvent production and the engineering of recombinant strains with superior biobutanol–producing ability is now fast becoming a reality. Advances in continuous culture technology, integrated fermentation processes, *in situ* product removal and improved downstream processing are providing new approaches to improving substrate utilization, reducing butanol toxicity, reducing process stream volumes and obtaining overall improved bioreactor performance.

The medium to long term future for biofuels is likely to be dependent on the ability to ferment lignocellulose substrates. Current technologies for the degradation of plant biomass tend to be slow, inefficient and only marginally economic. Solvent-producing *Clostridium* strains offer a number of advantages in that they can produce a variety of hemicellulase and cellulase enzymes naturally, although they are not able to ferment crystalline cellulose. Another plus is that it they able to ferment both hexose and pentose sugars whereas industrial yeast is only able to ferment hexose sugars. Certain Clostridial strains have a powerful complex of cellulase enzymes known as a cellulosome. Genetic engineering research is aimed at transferring this complex to butanol producing strains to improve the efficiency of cellulose degradation.

Both the current price of crude oil, and the environmental aspects relating to carbon dioxide emissions, could speed up a swing back to fermentation processes using renewable plant biomass. The high price of crude oil had made the biotechnological route for butanol production economically competitive once again. Thus the future of the industrial processes for the production of butanol by fermentation can be predicted to be on the increase.

1. Introduction

Recently biofuels have become the focus of worldwide interest and discussion. The increasing cost of crude oil, coupled with concerns about energy security, the contribution of carbon emissions to global warming and the realization that fossil fuels are a finite resource, have resulted in renewed interest in the production of liquid biofuels as a sustainable energy source (see also – *Biorefineries*). Bioethanol, produced mainly from corn or sugar cane and biodiesel produced from a variety of oil based crops and wastes (such as soya bean, rapeseed, sun flower, palm oil, jatropha, lard, used cooking oil etc.) are already being added to gasoline and diesel (see also – *Biodiesel*), in a number of countries. Many countries have now set targets for increasing the biofuel content of fossil-base liquid transportation fuels. Currently biofuels account for around 2% of all liquid transportation fuel consumption. Some sources are predicting that biofuels could account for 20-30% of all liquid fuel consumption by 2020. Optimistic estimates indicate that the global market for biofuels could reach 87 billion gallons by 2020, up from just under 11 billion gallons currently.

Biobutanol produced by fermentation has the potential to provide a new generation biofuel that is seen to offer a number of attractive features as a liquid transportation fuel. The reason that butanol has attracted renewed interest is that it offers certain advantages compared with ethanol. Butanol is a four carbon alcohol and it is widely used as an industrial solvent and as chemical feedstock precursor for the production of a variety of organic chemicals.

These are used in the manufacture of a wide range of products that include paints, lacquer finishes, thinners, plastics, butyl rubber, resins, adhesives, elastomers, emulsifiers, flocculants, absorbents, brake and hydraulic fluids, dicing fluids and cleaning fluids. They are also used in the production a variety of cosmetics, perfumes and other personal hygiene products as well as in textiles, leather, printing, paper, pesticide and safety glass manufacture.

Most butanol is produced by petro-chemical synthesis from propylene using the oxo process or from acetaldehyde using the aldol process. Currently butanol sells at around 4.00 $US per gallon and the United States industrial butanol market is approximately 350-400 million gallons per year (worth around 1.4 billion $US).

The global market is approximately 1050-1200 million gallons per year. This in itself represents a sizable market independent of butanol’s potential as a biofuel. As an industrial solvent and chemical feedstock butanol can command a higher price than it would if utilized as a biofuel.

Although butanol is currently produced from petrochemicals, during much of the 20th century butanol, along with acetone, and ethanol was produced worldwide on a commercial scale using and industrial fermentation process. The fermentation utilised either corn (along with other starch-based raw materials) or molasses (along with other sugar-based raw materials) as the substrate employing various anaerobic bacterial species belonging to the genus *Clostridium*.

The classical industrial Acetone Butanol Ethanol (ABE) fermentation process does however suffer from a number of limitations, which, make it less economically competitive, in comparison with bioethanol. Currently investigations are being undertaken by a number of companies and scientific institutions aimed at re-assessing and possibly re-introducing the butanol fermentation process in one form or another.

A new generation of research employing genetic engineering, metabolic engineering and advances in process engineering along with the possible use of cheaper fermentation substrates, is being undertaken with the hope of improving the economic viability of biobutanol production by fermentation.

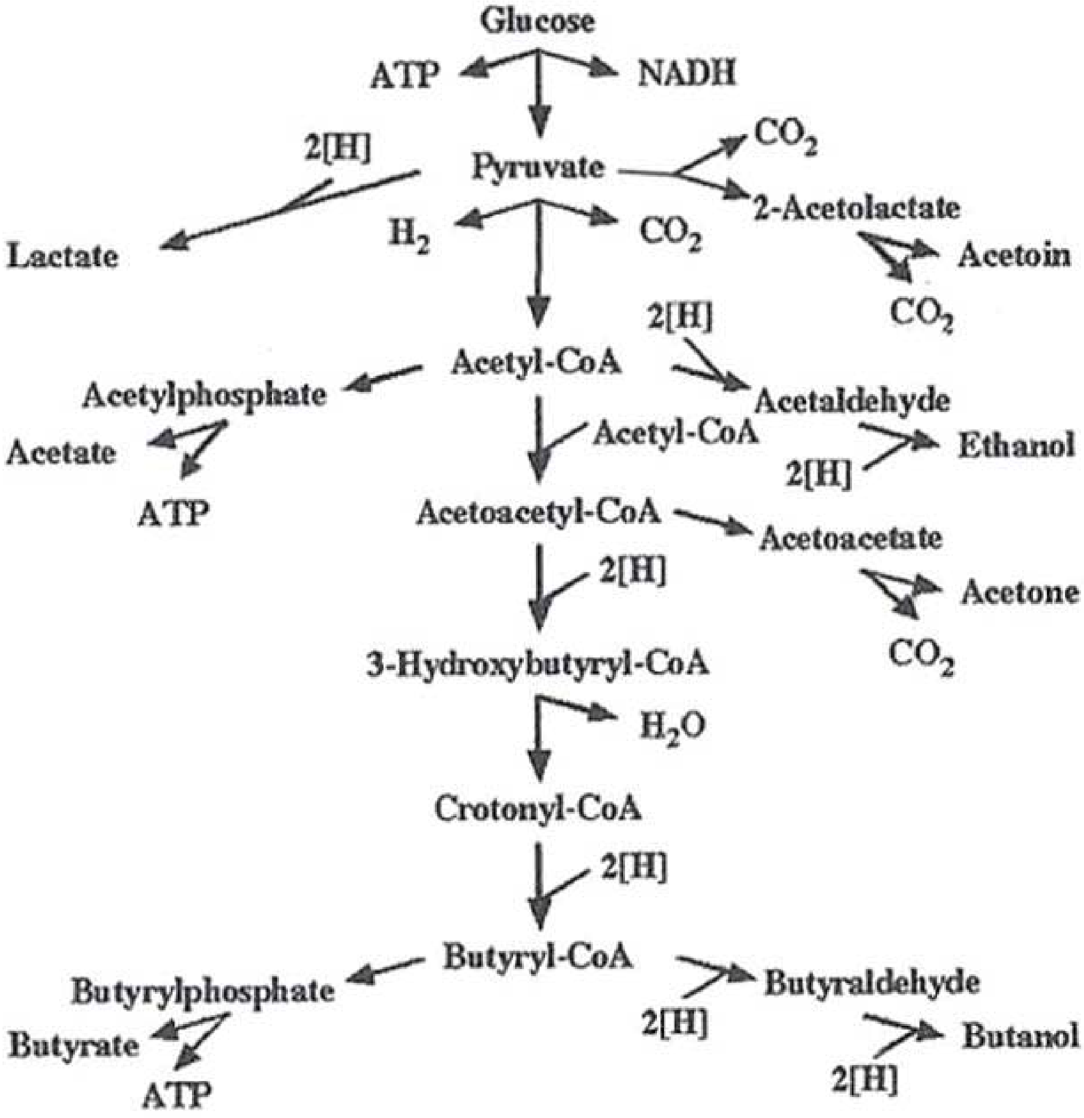

Figure 1. Fermentation pathways in solvent-producing clostridia.

2. Biobutanol as a Biofuel

2.1 Properties of Biobutanol as a Liquid Transportation Fuel

Like ethanol, n-butanol (biobutanol) is an alcohol, with a molecular weight of 74.12 composed of four carbon atoms and ten hydrogen atoms in comparison with ethanol which contains two carbon and six hydrogen atoms. Butanol has a number of intrinsic properties that make it attractive for use as a biofuel. The relevant properties of n-butanol compared with other liquid fuels are summarised in Table 1.

Property	Gasoline	Ethanol	Butanol	Biodiesel	Diesel

Reid Vapour Pressure kPa	62	15	2.3	-	<3
Lower Flamibility limit					
Concentration Vol%	1.4	3.3	1.4	-	0.6
Temperature ^{0}C	-45	13	36	-	64
Upper flamibility Limit					
Concentration Vol%	7.6	19	11.2	-	5.6
Temperature ^{0}C	-20	42	-	-	150
Flash Point	-43	13	36	>120	64
Autoignition Temprature	300	366	343	-	230
Cloud Point	NA	NA	-89	0	-26
Density kg/L	0.791	0.785	0.81	0.86	0.863
Vapour Specific Gravity	3.5	1.6	2.6	-	5.5
Kinematic Viscosity mm^2/sec	NA	NA	3.7	3.5-5	2-8
Lower Heating Value					
Mass MJ/kg	43.9	27.0	33.22	37.8	42.6
Volume MJ/L	32.7	21.2	26.9	32.5	36.7
BTU per Gallon	115,000	110,000	84,000	120,000	13,000
Research Octane Number	90-100	108	96	NA	NA
Cetane Number	NA	2-12	17	>51	40-47.5

Table 1 Properties of n-Butanol (Biobutanol)

2.2 Energy Content

Butanol has an energy content closer to that of gasoline and has 25% more energy density per litre than ethanol. The higher energy content of butanol means that in gasoline terms it contains 90% of the BTU per gallon of gasoline in comparison with ethanol at 60% BTU per gallon. Butanol costs more to produce than ethanol, but gives a higher performance in engines so cars would get better mileage on butanol. This becomes more important as the amount of biofuel in the fuel blend increases. Its higher energy content could provide possible premium biofuel applications by charging more at the pump for a superior fuel. A further energy advantage is that in the production of biobutanol, by fermentation, considerable quantities of hydrogen are produced resulting in 18% more energy produced from the same amount of fermentable substrate as ethanol.

2.3 Octane Rating and Vapour Pressure

Butanol has a similar octane rating to gasoline but not as high as that of ethanol (it burns more slowly but is harder to ignite). One advantage is it does not have the toxicity problems of MTBE. Compared with ethanol, butanol has a lower vapour pressure. The vapour pressure (pounds per square inch at 100 F, Reid VP) is 0.33 for butanol, 2.00 for ethanol and 4.50 for gasoline. The low vapour pressure point coupled with a high flash point makes butanol safer to use at high temperatures and it is also generally safer to handle than ethanol.

2.4 Water Tolerance

Butanol has a much lower affinity for water (7.8%) in comparison with ethanol (100%) giving it greater tolerance to moisture and water contamination. This makes it less corrosive that ethanol and since it is safer than ethanol or gasoline to handle, it can be blended directly with gasoline and transported via existing gasoline pipelines. Currently ethanol has to be transported separately and mixed at the fuel outlet.

2.5 Compatibility with Existing Internal Combustion Engines

Butanol is well suited to current vehicle and engine technologies. It does not require automakers to compromise on performance to meet environmental regulations. Butanol uses fuel to air ratios which are closer to that of gasoline than ethanol and butanol does not attack the piping of internal combustion engines. One distinct advantage of biobutanol over bioethanol is it can be used as a direct one for one replacement for gasoline without making any vehicle modifications.. Butanol's performance as a transportation fuel has long been recognized. It was, for example, used to fuel vehicles during World War II. Recently it was used as the fuel it in an unmodified car that was driven across the USA. Ethanol is limited to around a 10% mixture before internal combustion engine modifications are required. To use higher concentrations of ethanol, car engines have to be modified to flexi fuel vehicles. Due to its low vapour pressure, butanol can be blended into gasoline at higher concentrations than existing biofuels without the need to retrofit vehicles or require specially adapted vehicles.

2.6 Co-blending Features

Another feature that makes biobutanol attractive is its ability, to be used as a co-blending agent with ethanol and gasoline. Furthermore, butanol ester-based biofuels have the potential to be total replacements for gasoline, diesel, and possibly aviation and jet fuels. It is regarded as being environmentally friendly since its combustion, in an internal combustion engine, does not yield any toxic compounds such as SOX, NOX or carbon monoxide. Butanol can also be blended directly with diesel fuels. Currently biobutanol can be blended up to 10%v/v in European gasoline and 11.5%v/v in US gasoline. With butanol there is the potential to greatly increase the maximum allowable use in gasoline. Enhancing the performance of the fuel blends in this way could speed up growth of the overall biofuels market along with the agricultural markets that support it.

2.7 Handling and Distribution Advantages

Biobutanol can be blended effectively with both gasoline and ethanol. The structure of butanol gives it a tolerance to water contamination so it can be transported in pipelines. Ethanol which absorbs water tends to corrode pipelines and must be transported by trucks, trains or barges in relatively small batches to terminals for blending with gasoline. This gives biobutanol certain advantages over ethanol, including the ability to be mixed at the oil refinery, making distribution easier, avoiding the need for additional large-scale supply infrastructure. Butanol is less susceptible to separation in the presence of water than existing ethanol-gasoline blends and therefore allows it to use the industry's existing distribution infrastructure without requiring modifications in blending facilities, storage tanks or retail station pumps.

2.8 Synergies with Bioethanol and Biodiesel *:*

Biobutanol has synergies with both bioethanol and biodiesel. It is produced from the same agricultural feedstocks as ethanol (corn, wheat, sugar cane, sugar beet, sorghum, cassava etc.). Existing bioethanol plants could be cost-effectively retro-fitted for biobutanol production requiring relatively minor changes to fermentation and distillation facilities. There is also a vapour pressure co-blend synergy using biobutanol in gasoline containing bioethanol, which facilitates ethanol blending and delivers better fuel economy than gasoline ethanol blends alone. Butanol can also be blended with petrodiesel and vegetable oils to facilitate the conversion to biodiesel. In comparison with biodiesel biobutanol has a lower cloud point and can provide more consistent quality and potentially better production costs. In summary, biobutanol offers biomass producers and biofuel converters the option of upgrading to a higher value bio-molecule. It is also compatible with and facilitates the introduction of bioethanol into the fuel pool.

2.9 Feedstock Flexibility and Agricultural Benefits

Feedstock flexibility is seen to be another advantage. Biobutanol can be produced from the same agricultural feedstocks as used for ethanol. Existing production technologies can utilize a variety of conventional sugar-containing crops such as sugar cane and sugar beet and starch-containing crops such as corn, wheat, sorghum and cassava, supporting its global implementation. A further advantage of clostridial based butanol fermentations compared with yeast-based ethanol fermentations is that clostridia readily utilize pentose sugars and plant hydrolysates. This should ensure that production processes would be compatible with future lignocellulosic biofuel feedstocks such as fast growing energy crops (e.g. grasses and trees) or agricultural by-products (e.g. corn stalks, bagasse). The lignocellulosic technologies being developed within a biorefinery concept will have a natural fit with biobutanol. It has also been suggested that the expanding biofuels market could be beneficial for global farming as it provides additional marketing opportunity for key agricultural products, thus enhancing value to farmers.

-
-
-

Bibliography

Atsumi S., Hanai T., Liao J. C. [2008] Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature.* 451: 86-89. [This is recent article reporting the genetic engineering of *E. coli* to produce butanol].

Chiao, J.-S., Sun Z.-H. [2007] History of the acetone butanol ethanol fermentation industry in China: development of continuous production technology. *J. Mol. Microbiol. Biotechnol*. 13: 12–14. [This is recent report covering the ABE fermentation in China].

Cornillot E., Soucaille P. [1996] Solvent-forming genes in clostridia. *Nature*. 380: 489. [This was the initial announcements that the solvent genes in *C. acetobutylicum* were located on a large plasmid].

Cornillot, E. *et al.* [1997] The genes for butanol and acetone formation in *Clostridium acetobutylicum* ATCC 824 reside on a large plasmid whose loss leads to degeneration of the strain. *J. Bacteriol*. 179: 5442–5447. [This was the key paper giving the details of the finding that the solvent genes were located on a large plasmid in *C. acetobutylicum*].

Dürre P., *et al*. [1995] Solventogenic enzymes of Clostridium acetobutylicum: catalytic properties, genetic organization, and transcriptional regulation. *FEMS. Microbiol. Rev*. 17: 251-262. [This is an earlier review providing the details of solvent pathway genes and enzymes].

Dürre P., Bahl H. [1996] Microbial production of acetone/butanol/isopropanol. In *Biotechnology*, Ed. M. Roehr, Vol. 6, 2ndedn. VCH, Weinheim, 229–268. [This is a general review dealing with solvent producing systems in the clostridia].

Dürre P. [1998] New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. *Appl. Microbiol. Biotechnol.* 49: 639–648. [This is report covering new approaches and development in the ABE fermentation].

Dürre P. [2005] Formation of solvents in clostridia, In: *Handbook on Clostridia*. Ed D"urre, P., CRC Press, Boca Raton. 671–693. [This article provides a general coverage of the molecular mechanisms involved in solvent production].

Dürre P. [2007] Biobutanol: An attractive biofuel. *Biotechnol. J.* 2: 1-10. [This report provided a general overview of he utilization of the ABE fermentation for biofuel production].

Dürre P. [2008] Fermentative butanol production: bulk chemical and biofuel. *Ann. N. Y. Acad. Sc*. 1125: 353-62. [This is recent review focusing on the features of biobutanol as a biofuel].

Ezeji T. C., Qureshi N., Blaschek H. P. [2004] Butanol fermentation research: upstream and downstream manipulations. *Chem. Rec*. 4: 305–314 .[This report provides and overview of development in process technology].

Ezeji T. C., Qureshi N., Blaschek H. P. [2007] Bioproduction of butanol from biomass: from genes to bioreactors. *Curr. Opin. Biotechnol.* 18: 220–227. [This review provides a general coverage of the more applied aspects of the ABE fermentation including advances in process technology].

Fischer R. J., Helms J., Dürre P. [1993] Cloning, sequencing, and molecular analysis of the *sol* operon of *Clostridium acetobutylicum*, a chromosomal locus involved in solventogenesis. *J. Bacteriol.*, 175: 6959–6969. [This report provides a detailed analysis of the specific regions of the genome that encode for solvent production].

Huang W-C., Ramey D.E., Yang S-T. [2005] Continuous production of butanol by *Clostridium acetobutylicum* immobilized in a fibrous bed reactor. *Appl. Biochem. Biotechnol.* 113: 887-898. [This is a report of a new approach to solvent production that has been protected by patenting].

Jones D. T., Woods D. R. [1986] Acetone-butanol fermentation revisited. *Microbiol. Rev.* 50: 484–524. [This review provides a very broad-based coverage of the ABE fermentation with extensive earlier references].

Jones D. T. [2001]. Applied acetone-butanol fermentation. In *Clostridia. Biotechnological and Medical Applications*. Eds H. Bahl & P. D¨urre. Wiley-VCH. Weinheim.125–168. [This article covers the ABE fermentation in South Africa coupled with a more general review of the ABE process].

Keis S., Shaheen R., Jones D. T. [2001] Emended descriptions of *Clostridium acetobutylicum* and *Clostridium beijerinckii*, and descriptions of *Clostridium saccharoperbutylacetonicum* sp. nov. and *Clostridium saccharobutylicum* sp. nov. *Int. J. Syst. Evol. Microbiol.* 51: 2095–2103. [This report summarizes the modern molecular taxonomy of the solvent producing clostridia].

Lop´ez-contreras A.M., *et al.* [2003] Production by *Clostridium acetobutylicum* ATCC 824 of CelG, a cellulosomal glycoside hydrolase belonging to family 9. *Appl. Environ. Microbiol*. 69: 869–877. [This paper gives a more detailed analysis of component making up the cellulosome system].

Mitchell W.J. [1998] Physiology of carbohydrates to solvent conversion by clostridia. *Adv. Microb.Physiol*. 39: 31-130. [this is a general review that has a major focus on sugar uptake and transport systems in *Clostridium*].

Nölling J., *et al.* [2001] Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*. *J. Bacteriol.*, 183: 4823–4838. [This paper encompassed the joint effort involved in analyzing the genome sequence of *C. acetobutylicum*].

Paredes, C. J., Alsaker, K. V., Papoutsakis, E. T., [2005] A comparative genomic view of clostridial sporulation and physiology. *Nat. Rev. Microbiol.* 3: 969–978. [This is a general review comparing and contrasting the genes involved in sporulation in *Clostridium*].

Sabathé F., Bélaich A., Soucaille P. [2002] Characterization of the cellulolytic complex (cellulosome) of *Clostridium acetobutylicum*. *FEMS. Microbiol. Lett.* 217: 15–22. [This paper provide detailed information on the genes encoding for an apparent cellulosome in *C. acetobutylicum*].

Sauer U., Fischer R., Dürre P. [1993] Solvent formation and its regulation in strictly anaerobic bacteria. *Curr. Top. Mol. Genet.* 1: 337–351. [This paper provides a general overview of the regulation of solvent formation].

Schwarz W.H. [2001] The cellulosome and cellulose degradation by anaerobic bacteria. *Appl. Microbiol. Biotechnol*. 56: 634–649. [This report provides a general coverage of cellulosomes and the role they play in lignocellulose degradation].

Sullivan L., Bennett G. N. [2006] Proteome analysis and comparison of *Clostridium acetobutylicum* ATCC 824 and Spo0A strain variants. *J. Ind. Microbiol. Biotechnol.* 33: 298–308. [This paper deals with the linkage between sporulation and solvent production].

Tomas C. A., *et al.* [2005] Metabolic engineering of solventogenic clostridia, In: *Handbook on Clostridia*. Ed D”urre, P. CRC Press, Boca Raton. 813–830. [This articles covers strategies for the metabolic engineering of solvent production].

Zverlov V.V., *et al.* [2006] Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery. *Appl. Microbiol. Biotechnol.* 71: 587–597. [This is recent report covering the ABE fermentation in Russia].

Biographical Sketch

David Jones was born and educated in South Africa and obtained a PhD in microbiology from Rhodes University. He held academic positions at the University of Natal, Rhodes University and the University of Cape Town and a visiting fellowship at Oxford University before moving to New Zealand in 1989 to take up the post of Professor and Head of Department of Microbiology at Otago University. Subsequently he held the position of Dean of the Otago School of Medical Sciences for a period of 11 years.

For the last 30 years his major focus of research has been the solvent-producing clostridia. He initially became involved with the industrial Acetone, Butanol fermentation process though his association with National Chemical Products in South African. He gained hands-on experience on the industrial fermentation process operated by the company and undertook research on strain improvement through genetic and physiological manipulation and improvements in fermentation process technology. After moving to New Zealand his research focused on the molecular taxonomy and characterization of the industrial solvent-producing clostridia, comparative genomics, comparative studies on fermentation characteristics and studies on phage infections and lytic degeneration in the fermentation process.

He has been active in fostering biotechnology in New Zealand and has been a long standing member of the international solvent-producing Clostridium research community. He has been a regular attendee of international conferences. symposia and workshops and has maintained a number of international research collaborations and linkages with colleagues working in the field. He is now an emeritus professor and has continued his involvement in the industrial Acetone Butanol fermentation as a consultant and scientific/technical advisor.