Bottlenecks in Bacterial Production of Fuel Butanol

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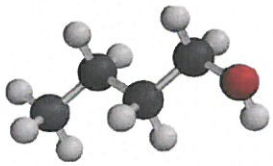
Butanol belongs to bulk chemicals which could be produced via fermentation. Nowadays it attracts a special attention because of its quite good properties as a fuel. From main advantages over ethanol the following are worth mentioning :

1. Modification of engine is much simpler or even not necessary – depending on the type of engine [**Alasfour, 1997**]
2. Butanol can be burned in both internal combustion engines and spark-ignition engines
3. Butanol has higher flash point: 35 ^{0}C (ethanol: 13 ^{0}C)
4. Butanol has lower vapor pressure and is less miscible with water
5. Butanol is less corrosive and safer to handle
6. Higher energy content than ethanol.
7. Not as corrosive as ethanol.
8. Uses an air/fuel ratio which is close to that of gasoline. Ethanol does not.
9. Can be shipped through existing fuel pipelines where ethanol must be transported via rail, barge or truck.
10. Can replace gasoline any percentage up to 100%. Ethanol can only be used up to 85%.
11. Gives better mileage than ethanol.
12. Safer to handle than ethanol.
13. Will also assist in the conversion of vegetable oils into biodiesel.



The main disadvantages of butanol comparing with other fuels are

a) Lower octane number rating
b) Higher viscosity

Following table shows the most important technological parameters

Table I Comparison of some fuels and fuel additives [Ladisch, 1991]

Fuel	Energy density MJ/L	Air/fuel ratio	Specific energy MJ/kg air	Heat of vaporization MJ/kg	RON	MON
Gasoline	32	14.6	2.9	0.36	91 - 99	81 - 89
Butanol	29.1	11.2	3.2	0.43	96	78
Ethanol	19.6	9.0	3.0	0.92	129	102
Methanol	16	6.5	3.1	1.2	136	104

RON … research octane number, MON … motor octane number

1 History of acetone–butanol–ethanol (ABE) fermentation, its industrial development and the present state of development

First reliable information about ABE fermentation dates back to Louis Pasteur who in 1861 discovered anaerobiosis in investigation with *Vibrion butyrique:* name of organisms living in the absence of air "anaerobies" **[Pasteur, 1861]**. This consortium of microbes represented a mixed culture of anaerobes were most probably prevailed *Clostridium butyricum.* Martinus Beijerinck called these microbes *Granulobacter saccharobutyricum, G. butylicum, G. lactobutylicum, G. polymyxa.* Industrial production of butanol went through several up-and-downs. Time period before the WW I is noted for a high need of butanol for technology of butadiene and rubber production. However, during WW I lack of acetone as a solvent for production of explosives (cordite) drew attention of manufacturers from butanol to acetone and butanol became an unwanted product. From period 1912 – 1939 a series of patents appeared. The fundamental patent of Chime Weizmann (later the first president of the state Israel) from 1915 put the foundation for a fast development of this area of biotechnology **[Weizmann, 1915]**. Canada, U.S. and Great Britain took the lead in production of these products in the 30th. Also increased demands for butyl acetate as an important solvent for production of car lacquers characterized this époque. ABE fermentation is not an easy microbial process which has several narrow spots which will be discussed later. One of them - later diagnosed as bacteriophage infection – was for the first time encountered in 1923 **[McCoy et al., 1944]**. After intensive research finally in 1935 a bacteriophage-immunized strain was introduced. As raw materials were mostly exploited grain, corn, molasses (sugar beet or sugar cane with addition of soybean meal and other proteins) and in some countries also potatoes. Literature from this time gives date about using initial 6 % sugar (starch) in medium and after fermentation the broth contained around 2 % total solvents. The WW 2 increased interest in butanol and acetone production and many new plants were built in Japan, India, Australia, South Africa, Taiwan, Brazil, Egypt and a few another countries producing smaller volumes of solvents. During the 50th and 60th then started ABE boom in USSR, Czechoslovakia, Poland, China and several other countries. However, this successful and fruitful period did not last long. Decline of ABE production had started and by 60th production ceased at first in U.K. and U.S. Reasons why it was stopped must be found above all in production of synthetic ethanol which was used for butadiene synthesis. Plants in South Africa, Soviet Union and China were operating long after cease in Europe and U.S.

ABE plant built in former ***Czechoslovakia*** (1952 – 65) was considered mainly 1) for verification of research work carried out at the Institute of Chemical Technology Prague (Department of Fermentation Chemistry and Technology), incl. testing strain isolates, 2) for development of technology and engineering. Series of distillation columns was designed for removal, concentration and purification of all solvents. Butanol and acetone were produced in very high purities; however ethanol was transferred to ethanol distillery plant for further processing. It has a very low purity. Annual production was not clearly fixed because of its R & D status but on average it was around 1000 t till 1500 t of solvents produced batchwise from potatoes, grain (mainly rye) or eventually sugar beet molasses. If the fermentation ran well then the process was prolong by withdrawing one half of the liquid followed with addition of one half of fresh and cool medium. This process called "tank cutting" contributed to shortening fermentation by about 6 – 8 hrs. An average fermentation time was 36 – 38 hrs.

Producing strains of *Cl. acetobutylicum* were derived from soil and other natural sources. Hundreds of isolates were collected by Professor J. Dyr during 15 years research prior to plant installation. Selection and testing were carried out with respect to properties of raw materials. During time of ABE investigation and technology development a new idea of a continuous process occurred. The continuous fermentation should have been organized as a so called battery-operated fermentation. Number of interlinked tanks could be 9 - 11 or more.

The last but very intensive boom started very recently connected with energy crisis and with search for alternative fuels and energy sources **[Dürre, 2008, Blaschek et al., 2002]**. Nevertheless, by now the process is not fully introduced in an industrial scale to be economically acceptable and profitable. Many companies announced high industrial production and daring perspectives very soon, e.g. BP/DuPont partnership and British Sugar – production of 30000 t /y butanol, pilot plant trials in Austria, in 2008 plant or pilot plant should have been built in Brazil (sugar cane) **[Afschar et al. (1990)]**. In China operate now 11 plants with annual production of 185 000 t. Small companies like Green Biologics, Cobalt Biofuels, Butalco and some others also proclaimed ABE production. Long delays in planned terms give evidence about difficulties and complexity of industrial solution and/or economic uncertainty.

Butanol as one of examples of biofuels meets many arguments against its production from agricultural raw materials and therefore manufacturers search for processing of new generation of raw materials such as lignocellulosic wastes, wood from fast growing trees etc. Economics of lignocellulosic treatment is not yet sufficient for cheap ABE process. Nevertheless, there appeared very promising data about butanol price from lignocellulosics. **Festel [2008]** already proclaims 0.35 EURO/ L butanol produced from wheat straw (plant capacity 200 kt/y, process start-up already planned in 2008).

2 Process bottlenecks, experimental approach and overall discussion

Objective of our presentation is to look at the process drawbacks in view of our present laboratory investigation, current state of the art and some results received during industrial process several decades ago. Among the main bottlenecks belong:

- Bottleneck I: Raw material and overall composition of fermentation medium,
- Bottleneck II: Strain of genus *Clostridium* (metabolic pathways and their regulation, high tolerance to butanol). Stability of the culture (e.g. sporulation, resistance against phage contamination),
- Bottleneck III: Selection of cultivation technique - (batch, fed-batch, various modes of continuous fermentation)
- Bottleneck IV: Reduction of inhibitory effect of biosolvents, especially of butanol.

2.1 Raw material and overall composition of fermentation medium

Various carbon substrates can be fermented by bacteria of genus *Clostridium.* These bacteria retain enzymes with wide range of saccharolytic activities. Therefore the raw materials should contain starch and/or mono- and di-saccharides like glucose, fructose, sucrose, maltose, lactose. Some strains can exploit even arabinose, xylose and glycerol. Cellulose cannot be fermented despite a finding that some clostridia possess cellulosome – macromolecular

aggregate (rich in β-glucosidase activities) responsible for cellulose hydrolysis but so far no indication about activation of this gene in the cells of solventogenic clostridia was published **[Leschine, 2005]**. For the selection of raw materials it must be borne in mind that for each raw material must be found and adapted a proper bacterial strain. Interesting raw material could be glycerol which forms a substantial part of wastes produced in processing of methyl ester of rape seed oil **[Venkataramanan and Johnson, 2009].** No strain we have tested so far provided such property. Another important factor which must not be forgotten in context with raw materials is the necessity to enrich medium containing raw material with nutrients (inorganic and complex organic, e.g. yeast extract, protein hydrolysis's). Bacteria used in most of our experiments were selected primarily with the purpose of attaining the high production parameters. There is no universal strain manifesting the same or similar results for all types of media. The key criteria always used for culture and/or fermentation assessment are both the final concentration of solvents, esp. butanol, butanol productivity and yield of butanol (grams of butanol formed from 100 g of utilized saccharide).

Table II presents results of screening obtained with all 9 collection strains origin of which is given in Tab. III and growing on different media.

Tab. II Experimental results of screening experiments with 9 tested strains of the genus *Clostridium*

	Butanol				Acetone				Ethanol			
Medium →	A	B	C	D	A	B	C	D	A	B	C	D
Strain of *Clostridium* Exp.No.	g/L				g/L				g/L			
C1	2.32	4.35	**10.05**	**2.06**	0.71	1.17	**4.42**	0.51	0.25	0.39	**0.79**	0.21
C2	2.02	**2.35**	**9.42**	2.50	0.61	**0.54**	4.33	0.36	**0.10**	0.35	0.48	0.17
C3	**1.83**	-	**6.07**	4.79	**0.53**	-	2.45	0.53	0.11	-	0.21	0.15
C4	3.61	3.86	6.24	4.38	0.92	0.60	3.38	**0.64**	0.22	0.21	0.35	0.22
C5	4.02	5.61	5.82	4.38	1.18	1.75	2.91	0.59	**0.41**	**0.19**	**0.13**	0.15
C6	5.28	**8.81**	5.02	4.25	**1.43**	**2.24**	1.75	0.52	0.23	**2.65**	0.22	**0.68**
C7	3.96	5.12	5.28	3.65	0.84	1.68	1.62	0.41	0.18	0.34	0.16	0.14
C8	**5.57**	5.61	4.40	3.34	1.41	1.75	2.91	**0.26**	0.27	0.19	0.26	0.09
C9	2.07	-	**2.65**	**4.91**	0.60	-	**1.32**	0.64	0.16	-	0.14	**0.12**

A – TYA (recommended medium for growth of clostridia), B – molasses medium, C – corn medium, D – glucose/xylose medium

Very important parameter often used for selection of bacterial strains is not only concentration of butanol in the fermentation broth but also ratio B : A : E and minimal content of acetone and ethanol. Mainly acetone content should be minimized as much as possible with respect to fuel quality. It seems that some strains need starch for solvent production and do not produce them in required quantities even on media containing glucose as a carbon source.

Conclusion to bottleneck I: Raw materials (incl. type of a carbon source) must be tested with respect to suitability for a strain. Glucose/sucrose containing media need not be always the best ones for all strains. Raw materials based on lignocellulosic hydrolyzate must be carefully studied regarding complexity of inhibitors.