Review

Biobutanol: An attractive biofuel

Peter Dürre

Institut für Mikrobiologie und Biotechnologie, Universität Ulm, Ulm, Germany

Biofuels are an attractive means to prevent a further increase of carbon dioxide emissions. Currently, gasoline is blended with ethanol at various percentages. However, butanol has several advantages over ethanol, such as higher energy content, lower water absorption, better blending ability, and use in conventional combustion engines without modification. Like ethanol, it can be produced fermentatively or petrochemically. Current crude oil prices render the biotechnological process economic again. The best-studied bacterium to perform a butanol fermentation is *Clostridium acetobutylicum*. Its genome has been sequenced, and the regulation of solvent formation is under intensive investigation. This opens the possibility to engineer recombinant strains with superior biobutanol-producing ability.

Received 1 August 2007
Revised 23 August 2007
Accepted 24 August 2007

Keywords: Biobutanol · Biofuel · Butanol · *Clostridium acetobutylicum* · Solvents

1 Introduction

The world's energy system is still largely based on fossil fuels. A very controversial public discussion focuses on the question of how long proven, probable, and possible petroleum reserves will last. The date of the peak oil (highest production rate) is continuously pushed into the future (2010 and later), since higher prices will render unconventional sources such as tar sands and oil shales economically producible. However, it is generally accepted that crude oil prices have reached sustained highs and might well continue to increase. In addition to availability and price of petroleum-based diesel and gasoline, there is growing concern on the effect of greenhouse gas emissions on the world's climate. Burning of fossil fuels leads to a (meanwhile dramatic) increase of CO_2 in the atmosphere. This dangerous CO_2 output, however, could be counterbalanced by using renewable biomass for biofuel production. All CO_2 released from such substances stems from organic material and can eventually be recaptured by bacteria and plants. As mobility is still increasing, especially in countries such as China and India, and replacement of combustion engines by fuel cells will still take many years, the demand for liquid fuel is growing enormously. However, it is clear that due to limitation of arable land, biofuels from biomass will only represent a fraction of the total fuel required. Nevertheless, this share (for Germany, 25% is estimated in 2020 [1]) will substantially help to extend availability of crude oil and to reduce greenhouse gas emissions. As a consequence, political actions have been taken. The European Union, for example, has passed the directive 2003/30/EC. It states that the share of biofuels must reach 2% in 2005 and 5.75% in 2010 (a 10% minimum target for the biofuel market share by 2020 has been proposed). This will mostly be achieved by blending fossil fuels with bioethanol or biodiesel, or by using biodiesel directly. However, these substances have several disadvantages, which can be overcome by alternative biofuels such as biobutanol.

Correspondence: Professor Peter Dürre, Institut für Mikrobiologie und Biotechnologie, Universität Ulm, 89069 Ulm, Germany
E-mail: peter.duerre@uni-ulm.de
Fax: +49-731-5022719

Abbreviation: **BtL**, biomass-to-liquid

2 History

The use of biofuels is no novel invention. Rudolf Diesel tested peanut oil as a fuel in his newly invented engine already in 1912 [1]. Nikolaus August Otto, inventor of the accordingly named combustion engine, and Henry Ford showed pure ethanol to be able to run the respective motors. The use of butanol as a biofuel has only recently been reported, when in 2005 a car driven by David Ramey toured across the United States, using this alcohol instead

of gasoline. Although consumption was ~9% higher, emissions of CO, hydrocarbons, and NO_x decreased enormously (http://www.butanol.com/, as of 22 August 2007).

However, the history of biological butanol formation dates back much longer. Already in 1862, the well-known French scientist Louis Pasteur reported this alcohol to be a fermentation product of "Vibrion butyrique" [2], which did not represent a pure culture but presumably contained clostridia of the *Clostridium butyricum* or *C. acetobutylicum* type [3]. The first pure culture of such an organism was probably isolated by Albert Fitz in Strasbourg, who published a series of papers on *Bacillus butylicus*. This anaerobic, spore-forming bacterium fermented glycerol, mannitol, and sucrose to butanol, butyrate, CO_2, and hydrogen (plus minor amounts of acetate, ethanol, lactate, and propanediol), produced typical clostridial forms, and was killed by higher concentrations of butanol [4–7]. These pioneering studies paved the way for isolation of further solvent-forming bacteria around the turn to the twentieth century. Numerous microbiologists were involved, among them the famous Martinus Beijerinck and Sergei Winogradsky (for a detailed list see [8]). At that time, the genus name *Clostridium* was a mere morphological description, meaning small spindle [9]. Thus, it is not surprising that a variety of different designations were used, including *Granulobacter saccharobutyricum*, *Amylobacter butylicus*, and *Bacillus orthobutylicus* [8]. Meanwhile it is known that mostly clostridia (strictly or moderately anaerobic, spore-forming, Gram-positive bacteria, incapable of dissimilatory sulfate reduction) perform a butanol fermentation. Well characterized and extensively used and investigated are *C. acetobutylicum*, *C. beijerinckii, C. saccharoperbutylacetonicum*, and *C. saccharobutylicum* [10]. A few other microorganisms form butanol as a major fermentation product. *Butyribacterium methylotrophicum* belongs to the taxonomic kingdom bacteria [11], whereas *Hyperthermus butylicus* is an archaeon [12].

Microbial solvent formation also made its way to industrial use. The so-called ABE fermentation (for acetone-butanol-ethanol) became the second largest biotechnological process ever performed. It is beaten in volume only by ethanol fermentation, usually performed in much smaller facilities. The story dates back to the beginning of the twentieth century, when a number of scientists tried to produce rubber artificially [8, 13, 14]. The idea was to use isoprene for polymerization, which could be produced from isoamyl alcohol. An alternative was butadiene, derived from butanol. In England, the company of Strange and Graham had employed Fernbach and Schoen from the Institut Pasteur in Paris and Perkins and Weizmann from Manchester University to work on that project. In 1911, Fernbach and Strange applied for two patents describing fermentation of various organic materials to mainly amyl, butyl, and ethyl alcohols by a mixed culture as well as recovery of the products [15, 16]. Weizmann left the research group in 1912, but continued to work in this field. In his memoirs, he described his success of isolating a new culture, producing large amounts of a liquid that smelled like amyl alcohol. In fact, it turned out to be a mixture of acetone and butanol [17]. Ironically, the price of natural rubber started to decrease during these years, due to increasing production from plantations in Asia. However, the disregarded by-product acetone helped the ABE fermentation process to an industrial breakthrough. World War I had started in 1914, and in November of the same year two British cruisers (Good Hope, flagship of admiral Sir Christopher Cradock, and Monmouth) were sunk off Coronel in Chile. They were part of a fleet getting into combat with the German East Asia squadron, commanded by vice-admiral Maximilian von Spee. It is reported that the British ammunition suffered from bad preparation, resulting in shells that plopped harmlessly into the water far short of enemy ships (http://butanol.com/docs/Weizman-Terre_Haute.doc, as of August 22, 2007). For preparation of cordite, the propellant of cartridges and shells, acetone was required in large quantities, and there was a tremendous shortage of chemically produced acetone in England at the outbreak of the war. Weizmann applied for a patent in 1915, describing production of acetone and alcohols from starchy material by a mixed culture, mainly consisting of what later became known as *C. acetobutylicum* [18, 19]. Advantages over the Fernbach strain were higher productivity with respect to acetone and the ability to use a variety of starch-containing substances. This process guaranteed a constant supply of acetone in Great Britain, Canada, and the United States during the war. Weizmann denied personal honors from the British government, but, being a member of the Zionist movement, made clear that he favored to repatriate Jewish people in Palestine. Without doubt, this influenced the British Foreign Secretary and led eventually to the famous Balfour declaration of 1917. Several years later, Chaim Weizmann became the first president of the newly founded State of Israel.

There was almost no use of butanol during the war, but the substance had been stored in immense vats (one later to be used as a swimming pool [20]). Upon introduction of prohibition in the United States in 1920, there was an immediate shortage of amyl alcohol, a by-product of alcoholic fermentation. It had been used to produce amyl acetate, a solvent for quick-drying lacquers, needed by the growing automobile industry in large amounts. Butanol proved to be a perfect replacement, forming the starting point for production of butyl acetate. At a consequence, all ABE production plants closed after the armistice were reopened and new ones were built. Numerous countries used the fermentation to fulfill the respective industrial needs, and up to 1950 approximately two thirds of world's butanol supply stemmed from the biological process [21]. The largest plant was located in Peoria, IL, USA, and had a capacity of 96 fermenters with a

volume of 50 000 gallons or 189 000 L each. Thus, the total volume was 18 168 m^3 [13]. Facilities in South Africa, the former Soviet Union (using lignocellulose hydrolysates as a substrate), and China (using continuous culture technology) were smaller, but operated until the 1980s or even until 2004 (in China) [22–24]. The decline of the fermentation was caused by increasing substrate costs (molasses) and low crude oil prices, rendering chemical production cheaper. Until around 2005, butanol was only considered to be a bulk chemical precursor for production of acrylate and methacrylate esters, glycol ethers, butyl acetate, butylamines, and amino resins. Their use is manifold: production of adhesives/scalants, alkaloids, antibiotics, camphor, deicing fluid, dental products, detergents, elastomers, electronics, emulsifiers, eye makeup, fibers, flocculants, flotation aids (*e.g.*, butyl xanthate), hard-surface cleaners, hormones and vitamins, hydraulic and brake fluids, industrial coatings, lipsticks, nail care products, paints, paint thinners, perfumes, pesticides, plastics, printing ink, resins, safety glass, shaving and personal hygiene products, surface coatings, super absorbents, synthetic fruit flavoring, textiles, as mobile phases in paper and thin-layer chromatography, as oil additive, as well as for leather and paper finishing. Meanwhile, however, BP und DuPont have declared that they are restarting the industrial ABE fermentation in 2007 in Great Britain, to produce butanol for use as a biofuel in the United Kingdom (http://www2.dupont.com/Biofuels/en_US/, as of 22 August 2007). Also, China is reopening its fermentation plants, and a new one has been built in Brazil. Thus, prospects for the biological butanol production are really bright.

3 Biofuels: Butanol *versus* ethanol

The most widespread biogenic fuels are biodiesel (used in pure form or as a blend) and bioethanol (used as a blend) [1]. Biodiesel is generated from vegetable oil, animal fats, or recycled restaurant greases. The fatty acid components are converted into methyl esters (FAME, fatty acid methyl esters). Glycerol is a by-product. Bioethanol stems from alcoholic fermentation of sugar-containing materials by yeast. Both, biodiesel and bioethanol produced in the described way are considered first-generation biofuels. Another approach uses lignocellulosic biomass as a feedstock, these are second-generation biofuels (BtL, biomass-to-liquid). In principle, the process is based on generating synthesis gas (consisting mostly of CO and hydrogen) and reacting it into liquid alkanes, alkenes, and alcohols by the Fischer-Tropsch method. The desired product formation can be adjusted by process conditions and use of appropriate catalysts. The same principle is used for CtL (coal-to-liquid) and GtL (gas-to-liquid) fuels, which, however, are not biofuels. BtL fuels currently focus mostly on diesel and are known under the brand names SunFuel® and SunDiesel® (cooperation of Choren Industries, Shell, Volkswagen, and Daimler). A disadvantage is that the process currently works best with wood, which excludes other biomass such grass, stalks, leaves, *etc.*, from usage. In Germany, a pilot BtL plant (15 000 t/a) is scheduled for operation at the end of 2007/beginning of 2008 and a large one (200 000 t/a) is planned for 2009. Since 2004, ethanol can also be produced from cereal straw and corn stover at an industrial and competitive scale, using a combination of pretreatment, enzyme addition, and fermentation (cooperation of Iogen, Petro-Canada, and Shell; http://www.iogen.ca/news_events/press_releases/2004_04_21.html, as of 22 August 2007). Pretreatment involves chopping of the biomass and treatment with steam, and applying cellulases stemming from *Trichoderma reesi*; fermentation is performed using recombinant microorganisms. Due to its origin, the generated fuel is called cellulose ethanol. Abengoa Energy also produces bioethanol by enzymatic hydrolysis and fermentation as well as thermo chemical conversion of biomass (http://www.abengoabioenergy.com/, as of 22 August 2007). Biofuels of currently minor importance are vegetable oils (used directly instead of converting it to biodiesel) and methane.

Recently, another player entered the stage and that is biologically produced butanol (biobutanol, ButylFuel™). David Ramey is planning to produce this compound by a two-stage fermentation process. First, biomass feedstock is converted to butyrate and hydrogen by *C. tyrobutyricum*, and then *C. acetobutylicum* converts the acid into butanol (http://www.butanol.com/, as of 22 August 2007, patented by Environmental Engineering, Inc.). Probably based on the traditional process and using *C. beijerinckii* (details have not yet been released), BP and DuPont, in cooperation with British Sugar, announced biobutanol production to be started in 2007. An existing ethanol plant in Wissington, Norfolk, UK, will be remodeled for this purpose. Sugar beet will serve as a feedstock (http://www.britishbioethanol.co.uk/IsolatedStorage/94175874-67b5-4c33-9f38-380233f14049/ContentAssets/Documents/BP%20release%20200606.pdf, as of 22 August 2007).

Using butanol instead of ethanol as a biofuel provides a number of significant advantages. First, the substance can be used in pure form or blended in any concentration with gasoline. Ethanol can be blended only up to 85%. Second, neither combustion of butanol as a sole fuel nor as a fuel extender will require modification of existing car engines. Third, it has a lower vapor pressure and is thus safer to handle. Fourth, it is not hygroscopic. This allows blending of gasoline at the refinery, well ahead of storage and distribution. Blending with ethanol must occur shortly before use. This feature of butanol will also prevent contamination of groundwater in case of spills. Fifth, it is less corrosive. This means that the complete existing infrastructure (tanks, pipelines, pumps, filling stations, etc.)

Table 1. Comparison of gasoline to biofuels

Fuel	Caloric value (MJ/L)	Air-fuel ratio	Research octane number
Gasoline	32.5	14.6	91–99
Ethanol	21.2	3.0	129
Butanol	29.2	11.2	96

can be used. Sixth, the energy content is higher, increasing the mileage/gasoline blend ratio. Seventh, the dibutyl ether derivative has the potential for a diesel fuel. Table 1 shows a comparison of some characteristics of the important biofuels with gasoline. In principle, butanol could be produced *via* a BtL process as well. However, the fermentation seems to be economically the method of choice.

4 Fermentation pathways and enzymes involved

The original Weizmann strain of *C. acetobutylicum* was best suited for growth on starchy materials. Later isolation of similar species focused on molasses as a substrate. Originally, these strains were also considered to be *C. acetobutylicum* and were kept under this name in publications and strain collections. Only at the beginning of the 1990s was it discovered that besides the true *C. acetobutylicum* three other species were in use, namely *C. beijerinckii*, *C. saccharoperbutylacetonicum*, and *C. saccharobutylicum* [10]. It is important to crosscheck older literature with this publication to identify the proper organism. Most data are available for *C. acetobutylicum*. This bacterium typically performs a standard butyric acid fermentation, with acetate, butyrate, CO_2, and H_2 as major products (Fig. 1). About twice as much butyrate is produced compared to acetate. Some ethanol is formed constitutively, and also some acetoin. Lactate formation depends on specific growth conditions. As starch serves as a good substrate, it is not surprising that two amylases have been isolated from *C. acetobutylicum* and analyzed in detail [25, 26]. However, only one of the respective genes could be unambiguously identified from the genome sequence so far [27]. Inspection of the genome yielded surprising findings: Genes were found, whose products should allow degradation of two major plant wall materials, namely cellulose and xylan [28]. Whereas the latter compound had previously been shown to serve as a substrate for *C. acetobutylicum* and respective enzymes had been isolated and characterized [29–32], cellulose cannot be metabolized [33]. An inactive cellulosome complex was detected [34], but single components could be functionally overexpressed [35–37]. This phenomenon still remains to be clarified. Glucose transport into the cell is mediated by a phosphoenolpyruvate-dependent phosphotransferase system [38]. Hexoses are further metabolized *via* glycolysis, pentoses *via* the pentose phosphate pathway. A detailed description of what is known about the respective enzymes and their genes has recently been published [27].

At the end of the exponential growth, a major metabolic switch takes place in *C. acetobutylicum*. The organism slows down acid production, and takes up excreted acetate and butyrate and converts them into the solvents acetone and butanol (approximately two times more butanol than acetone) (Fig. 2). It is a response to endangering environmental conditions. Anaerobic bacteria are in general unable to keep their internal pH constant, it is ~1 unit higher than the external pH [39–41]. Due to the acids formed during fermentation, the external pH will drop. Starting at about a value of 4.5, considerable amounts of undissociated acetic and butyric acids will be present, which are able to diffuse into the cytoplasm. There, they

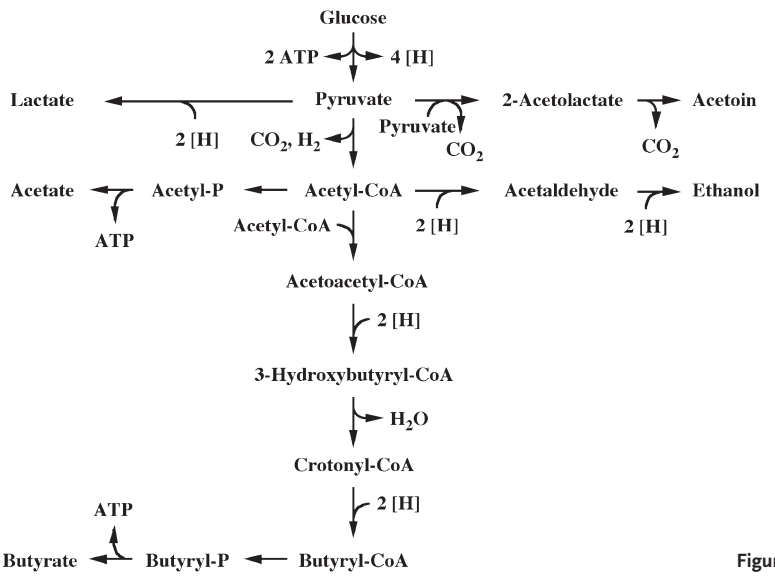

Figure 1. Acidogenic fermentation performed by *C. acetobutylicum*.

will dissociate again and thus cause the proton gradient between inside and outside to collapse. This H^+ gradient (external higher than internal) is essential for living cells. Its breakdown results in cell death. Being able to convert acids into solvents and thus increasing the pH again, provides *C. acetobutylicum* with a distinct growth advantage over species that only perform an acid fermentation. However, it is only a limited advantage, as especially butanol has a damaging effect on membranes and some membrane proteins [42, 43]. Thus, the bacteria in parallel start the synthesis of endospores to guarantee long-term survival. This explains why the metabolic networks of solventogenesis and sporulation are regulatorily linked (see next paragraph).

The first enzyme, required for solventogenesis, is an acetoacetyl-CoA:acetate/butyrate-coenzyme A transferase (CoA transferase, CtfA/B) that converts reinternalized butyrate and to a lesser extent also acetate into butyryl-CoA and acetyl-CoA. The latter is recycled to acetoacetyl-CoA, the starting point of the reaction. The respective enzyme, however, a thiolase (ThlA) is also needed for butyrate formation. This has implications for the regulation of its gene, as discussed below. Acetoacetate, one of the products of the CoA transferase action, is converted into acetone and CO_2 by acetoacetate decarboxylase (Adc), a reaction required to "pull" the thermodynamically unfavorable butyryl-CoA formation. This can also be achieved using different substrates, resulting in massively increased butanol production over acetone [44]. It is noteworthy that Adc occurs in two different forms in the cell, possibly indicating a regulation *via* protein modification [45]. Finally, butyryl-CoA is reduced to butyraldehyde and butanol by several enzymes. AdhE is a bifunctional butyraldehyde/butanol dehydrogenase and catalyzes initiation of solventogenesis, whereas BdhB, a butanol dehydrogenase, takes over later and is responsible for the massive production. BdhA is an alcohol dehydrogenase that uses acetaldehyde and butyraldehyde almost equally well [46], assuming its physiological role to be just a sink for surplus reducing equivalents and resulting in minor ethanol and butanol formation. AdhE2 is another bifunctional butyraldehyde/butanol dehydrogenase that is only formed when *C. acetobutylicum* is growing on reduced substrates (*e.g.*, a mixture of glucose and glycerol), which leads to a so-called alcohologenic fermentation (only butanol and ethanol are formed, but no acetone) [47, 48]. This reaction can also be induced by adding the redox dye methyl viologen [49, 50]. All of the aforementioned enzymes are regulated at the level of their genes, some of them by additional mechanisms as well.

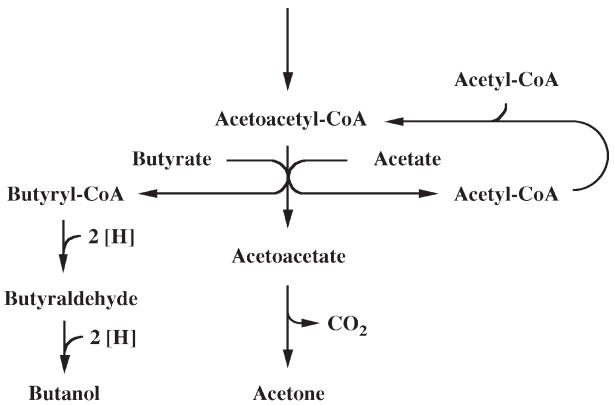

Figure 2. Solventogenic fermentation performed by *C. acetobutylicum*.

5 Operon structure and regulation of solventogenesis genes

The genome of *C. acetobutylicum* consists of a 3.94-Mbp chromosome and a 192-kbp plasmid (pSOL1) [28]. Genes encoding solventogenic enzymes are located on both elements. The megaplasmid harbors three operons, two of which are organized in a cluster. The *sol* operon consists of the genes *orfL* (encoding a peptide of still unknown function), *adhE* (encoding the bifunctional butyraldehyde/butanol dehydrogenase, the gene is sometimes also designated as *aad*), *ctfA*, and *ctfB* (encoding the two subunits of the CoA transferase) [51, 52]. Transcription of the operon stops at a typical rho-independent terminator, which serves the same function for the downstream located, but convergently transcribed monocistronic *adc* operon, encoding the acetoacetate decarboxylase [53–55]. This organization is unique within the solventogenic clostridia, as all other species contain a *sol* operon starting with a butyraldehyde dehydrogenase gene (*ald* or *bld*), followed by *ctfA*, *ctfB*, and *adc* [56, 57]. In *C. acetobutylicum*, the organization in two different operons explains the ability of the organism to change the acetone/butanol production ratio. The *sol* operon of *C. acetobutylicum* is transcribed from a single, inducible promoter, which shows typical α^A-dependent motifs [51]. The transcript is processed after its formation [58], representing another stage of regulation. A binding site for Spo0A~P, the master transcription factor of sporulation, is located upstream of the control region (in reverse orientation). This represents the regulatory link between solventogenesis and sporulation [59]. The suggestion that an upstream located gene, *orf5*, served as a repressor (*solR*) of the *sol* operon [60], turned out to be an error [58, 61]. However, the existence of additional regulators is very likely [58]. It is also important to note that the *adhE* part of the transcript is translated into both the complete bifunctional enzyme and a separate, C-terminal butanol dehydrogenase domain. It may be that this counteracts instability problems [58]. The promoter strength of the *sol* promoter is ~2 orders of magnitude lower than those of the *adc* and *bdhB* operons [62], possibly reflecting the physiological function of AdhE in initiation of solventogenesis (and implying higher stability of the other encoded enzymes) [63].

The *adhE2* gene is also located on the megaplasmid. It forms a monocistronic operon and two transcriptional start points have been determined [48]. As one of these also seems to be an mRNA processing site (very little homology of deduced "promoter" sequences to the consensus) and a Spo0A~P binding site (0A box) precedes the proper promoter, the regulation seems to be very similar to the *sol* operon [27].

The other solventogenesis genes reside on the chromosome. *bdhA* and *bdhB* are organized in contiguous, monocistronic operons [64]. Both promoters seem to be α^A-dependent, the *bdhA*-controlling one having only ~6% of the strength of the *bdhB* one [62]. As expected, upstream of the *bdhB* promoter a 0A box is found [65], as the gene product is important for butanol formation [63]. However, such a motif is present before the *bdhA* control region as well (two 0A boxes) [59], although under usual experimental conditions this promoter turned out to be constitutive [63]. That could indicate the involvement of additional regulators or point to dangerous conclusions drawn from *in silico* analysis.

As mentioned before, thiolase is involved in both acidogenesis and solventogenesis. Two respective genes are present in *C. acetobutylicum*, *thlA* and *thlB*, of which the former encodes the metabolically active enzyme. *thlB* is part of a polycistronic operon, which is expressed at very low level, and the physiological relevance of its gene products is not yet known [65]. However, the monocistronic *thlA* operon shows a so far unique expression pattern in solventogenic clostridia: massive transcription during acidogenesis, drastic decrease at initiation of the shift, and again massive transcription during solventogenesis [65]. The two different expression phases are explained by the need for this enzyme in both pathways (compare Figs. 1 and 2). The regulator(s) responsible for this control is(are) still unknown.

Two global approaches for investigation of regulatory links inside a cell are proteome analysis and transcriptional profiling (DNA microarrays). Both have been started for *C. acetobutylicum*. Comparing acidogenic and solventogenic cells, several proteins were found to increased under the latter conditions. Among them were only one true solventogenic enzyme, Adc (in two modifications, see before), several stress proteins [DnaK, GroEL, Hsp18 (also in two modifications), PdxY (possibly quenching singlet oxygen)], and three proteins catalyzing serine biosynthesis and incorporation into proteins [45]. The physiological relevance of the latter phenomenon is unclear. Another study compared the wild-type and *spo0A*-negative and overexpression mutants [66]. Stress proteins again were found to be affected, protein modifications again were noticed, and six proteins were missing in the *spo0A*-knockout mutant [66]. While proteome analysis still suffers in part from the fact that a number of proteins cannot be resolved on the gels in use, DNA microarrays cover the complete transcriptome. The group of Papoutsakis (Northwestern University, Evanston, IL) has performed pioneering studies with *C. acetobutylicum* in this field. Microarray analysis has been used to study a *spo0A*-knockout and a degenerated strain [67], overexpression of the *spo0A* gene [68], the effects on antisense RNA down-regulation of *ctfB* [69], overexpression of *groESL* [70], butanol stress and tolerance [68, 71, 72], and early sporulation and stationary phase events [73]. These experiments yielded a vast amount of data, confirming in part earlier results obtained by other methods as well as resulting in unexpected findings. Mining, however, has just begun, and we are still far from understanding the system biology of *C. acetobutylicum*. Nevertheless, first comparisons of physiology and sporulation between bacilli and clostridia became possible [74].

An open question still is also the nature of the signal(s) responsible for initiation of solventogenesis. In continuous culture, a very reliable method to induce the shift under controlled conditions, a surplus of glucose, a limiting concentration of phosphate, and a pH value ≤4.3 are required [9]. Approximately neutral pH values are possible if higher concentrations of acetate and/or butyrate are added [75, 76]. In addition to salt concentration, temperature also plays an important role. All these parameters affect DNA topology, so that the DNA might act as a sensor for environmental changes and even trigger transcription [77]. Support for this theory stems from experiments showing transcription of the *sol* operon to be directly dependent on DNA relaxation [78]. Intracellular metabolites, whose concentration is changing during the shift to solventogenesis, also might be involved in signaling. Several groups suggested butyryl-CoA, butyryl phosphate, NAD(P)H, and/or ATP/ADP [39, 79–81].

6 Developmental potential of the biological process

Although the fermentation meanwhile seems to be economically competitive or even superior to the petrochemical synthesis of butanol (as demonstrated by building new plants and reopening old ones), there is still potential for further improvement. Usually, most of the total costs of a biotechnological process are required for substrate delivery and processing. Currently, sugar (molasses, sugar cane, sugar beet) will be used as a substrate (new plants in Brazil or the UK, see above) or starch (stemming from maize) will serve this purpose (pilot plant in the US). These compounds are at present comparatively cheap. First, this might change in future. Second, although there is still plenty of arable land that could be used for such crops, there is also an ongoing public discussion about the ethics of using sugar or corn for fuel preparation instead of nutrition. For future industrial use of the butanol fermentation, the search for an abundant substrate that does not compete with food offers a tremendous econom-

ic benefit. Such a substance might well be cellulose. Already in the former Soviet Union, the early butanol fermentation had been based on lignocellulose as a substrate (which only later become economically non-competitive) [23]. The cellulosome systems of clostridia and other anaerobic bacteria are meanwhile well characterized [82, 83]; substrate conversion over time, however, is still unsatisfactory for industrial purposes. This renders metabolic engineering of solventogenesis genes into such bacteria less promising. However, a combination of cellulose pretreatment and butanol fermentation certainly has a lot of potential that awaits exploitation. Examples of economically suitable pretreatments are the Iogen process (cellulose ethanol, see above) as well as methods that have been developed by Green Biologics (Abingdon, Oxfordshire, UK, http://www.greenbiologics.com/biofuels.asp, as of August 22, 2007) and Green Sugar GmbH (Dresden, Germany, http://www.green-sugar.eu/products_03_de.html, as of August 22, 2007). They claim to be able to convert all cellulosic components of biomass (including grass, leaves, etc.) into sugars that can be used for fermentation.

The second focus of development is on metabolic engineering. In principle, there are two different options: (i) the genes required for butanol formation can be cloned into a desired production organism, and (ii) a butanol producer such as *C. acetobutylicum* can be mutated in a way that it only produces butanol (homobutanol producer) plus some CO_2 and H_2. The first possibility would be suitable for biotechnology workhorses such as *Escherichia coli*. Since this organism does not possess the ability to synthesize C_4 compounds, the respective genes would have to come from *C. acetobutylicum* as well. A problem might become the expression of oxygen-sensitive essential clostridial enzymes, such as AdhE. Such an approach also does not take into account tolerance of the envisaged production strain against butanol. As already mentioned before, this alcohol damages membranes and certain membrane proteins. This seems to be less of a problem in *C. acetobutylicum*. There have already been a number of attempts to improve solvent tolerance in this organism [70–72]. Another approach used overexpression of cyclopropane fatty acid synthase. Tolerance could indeed be improved, but butanol production was decreased [84]. However, even without such targeted constructs, a number of other mutants have produced butanol in concentrations considered a few years ago impossible [27]. The current best performer (238 mM butanol) is a *orf5*-negative strain, overexpressing *adhE* [85]. Thus, it may be wise to start with *C. acetobutylicum* to construct a tailor-made production strain. Acetate and butyrate production could be inactivated by mutations in the phosphotransacetylase and phosphotransbutyrylase genes or the corresponding kinase genes, respectively. Lactate formation can be inhibited by inactivating the lactate dehydrogenase gene, acetone synthesis by knockout of *adc*, and acetoin production by a mutation in the 2-acetolactate synthase gene. A gene knockout procedure has recently been developed for the clostridia (https://hcai.nottingham.ac.uk/minton.pdf, as of 22 August 2007). Finally, there is still another approach. A patent from the Fraunhofer-Institute in Stuttgart, Germany, describes butanol formation from butane by means of a specific monooxygenase (http://www.igb.fhg.de/WWW/GF/Biokatalyse/en/GFBK_233_Butanol.en.html, as of 22 August 2007). Overexpression of the respective gene might result in a different, yet suitable recombinant production strain.

The third area for improvement is downstream processing. Previously, distillation has been the method to separate butanol from the fermentation broth. However, this procedure has a high requirement for energy, as butanol has a boiling point higher than water. Therefore, other recovery methods have been intensively tested (reviewed in [86–89]). Alternative possibilities are adsorption, gas stripping, liquid-liquid extraction, perstraction, pervaporation, and reverse osmosis. Adsorption with molecular sieves such as silicalite is attractive, as the energy requirement is lower than for gas stripping and pervaporation (heat is needed for desorption) [90]. Disadvantages, however, are removal of nutrients from the broth, low selectivity, and high prices of the resin. Gas stripping seems to be a simple, yet successful method. It has a low selectivity and does not completely remove solvents from the fermentation broth, but continuous use is possible. Recovery of products is achieved by condensation. The gases do not interfere with the microorganisms. A recent study showed it to be better suited than pervaporation [88]. However, foam formation might cause problems [91]. All other methods have a much higher selectivity, but suffer from other problems. Liquid-liquid extraction requires a solvent that is nontoxic to the bacteria. In perstraction, the same principle is used, but a membrane separates culture and extracting solvent. In general, membranes are expensive and often suffer from clogging and fouling problems. This limits also the use of pervaporation, by which products such as butanol selectively diffuse across a membrane and are evaporated for recovery, and reverse osmosis, in which high pressure is used to separate the water *via* a semipermeable membrane and thus to yield a concentrated product broth. However, it is obvious that numerous recovery methods are available that allow a much more economic butanol purification than in the past.

7 Conclusions

Biobutanol has numerous advantages when used as a biofuel on a large scale. It can already be economically synthesized as demonstrated by the recent increase in industrial production. Its use will help to extend fossil fuel reserves and to reduce greenhouse gas emissions. In ad-

dition, there are a number of different approaches for further improvements of the biotechnological process, using both molecular biology and advanced process technology. Thus, there is tremendous potential for future development that awaits elucidation and exploitation.

Work in my laboratory was supported by grants from the BMBF GenoMik and GenoMikPlus projects (Competence Network Göttingen), and the SysMO project COSMIC (PtJ-BIO/SysMO/P-D-01-06-13), www.sysmo.net.

The authors have declared no conflict of interest.

8 References

[1] Paul, N., Kemnitz, D. (Ed.), *Biofuels*, Fachagentur Nachwachsende Rohstoffe e. V. (FNR), Gülzow 2006

[2] Pasteur, L., Quelques résultats nouveaux relatifs aux fermentations acétique et butyrique. *Bull. Soc. Chim. Paris* 1862, *May*, 52–53.

[3] Sauer, U., Fischer, R., Dürre, P., Solvent formation and its regulation in strictly anaerobic bacteria. *Curr. Top. Mol. Genet.* 1993, *1*, 337–351.

[4] Fitz, A., Über die Gährung des Glycerins. *Ber. Dtsch. Chem. Ges.* 1876, *9*, 1348–1352.

[5] Fitz, A., Über Schizomyceten-Gährungen II [Glycerin, Mannit, Stärke, Dextrin]. *Ber. Dtsch. Chem. Ges.* 1877, *10*, 276–283.

[6] Fitz, A., Über Schizomyceten-Gährungen III. *Ber. Dtsch. Chem. Ges.* 1878, *11*, 42–55.

[7] Fitz, A., Über Spaltpilzgährungen. VII. *Ber. Dtsch. Chem. Ges.* 1882, *15*, 867–880.

[8] Dürre, P., Bahl, H., Microbial production of acetone/butanol/isopropanol, in: Roehr, M. (Ed.), *Biotechnology*, vol. 6, 2nd edn., VCH, Weinheim 1996, pp. 229–268.

[9] Dürre, P., From Pandora's box to cornucopia: Clostridia – a historical perspective, in: Dürre, P. (Ed.), *Clostridia. Biotechnology and Medical Applications*, Wiley-VCH, Weinheim 2001, pp. 1–17.

[10] Keis, S., Shaheen, R., Jones, D. T., 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.* 2001, *51*, 2095–2103.

[11] Grethlein, A. J., Worden, R. M., Jain, M. K., Datta, R., Evidence for production of *n*-butanol from carbon monoxide by *Butyribacterium methylotrophicum*. *J. Ferment. Bioeng.* 1991, *72*, 58–60.

[12] Zillig, W., Holz, I., Wunderl, S., *Hyperthermus butylicus* gen. nov., sp. nov., a hyperthermophilic, anaerobic, peptide-fermenting, facultatively H_2S-generating archaebacterium. *Int. J. Syst. Bacteriol.* 1991, *41*, 169–170.

[13] Gabriel, C. L., Butanol fermentation process. *Ind. Eng. Chem.* 1928, *28*, 1063–1067.

[14] Jones, D. T., Woods, D. R., Acetone-butanol fermentation revisited. *Microbiol. Rev.* 1986, *50*, 484–524.

[15] Fernbach, A., Strange, E. H., Improvements in the manufacture of products of fermentation. *Br. Patent* 1911, No. 15,203.

[16] Fernbach, A., Strange, E. H., Improvement in the manufacture of higher alcohols. *Br. Patent* 1911, No. 15,204.

[17] Weizmann, C. (Ed.), *Memoiren*, J. P. Toth, Hamburg 1951, pp. 203–204.

Professor Peter Dürre obtained his PhD in 1981 at the University of Göttingen, Germany and performed postdoctoral research in 1981–1983 at the University of California, Berkeley. Since 1995 he is Professor of Microbiology and Director of the Institute of Microbiology and Biotechnology at the University of Ulm, Germany. Since 2003 he is also Vice President for Research of the University of Ulm. Peter Dürre is Editor of *Applied Microbiology and Biotechnology*, Senior Editor of *Journal of Molecular Microbiology and Biotechnology* and Member of the Editorial Boards of *Applied and Environmental Microbiology* and *Genome Letters*. His research focuses on obligate anaerobic bacteria. Major projects involve spore formation in clostridia, regulation of acetone and butanol formation in *Clostridium acetobutylicum*, metabolic engineering of solvent-producing strains for industrial use, construction and application of clostridial recombinant endospores for cancer treatment, and identification of acne-causing enzymes in *Propionibacterium acnes* for selective inhibition and disease therapy. Peter Dürre is author and co-author of more than 80 research articles and reviews and edited several books.

[18] Weizmann, C., Improvements in the bacterial fermentation of carbohydrates and in bacterial cultures for the same. *Br. Patent* 1915, No. 4845.

[19] McCoy, E., Fred, E. B., Peterson, W. H., Hastings, E. G., A cultural study of the acetone butyl alcohol organism. *J. Infect. Dis.* 1926, *39*, 457–483.

[20] Killeffer, D. H., Butanol and acetone from corn. A description of the fermentation process. *Ind. Eng. Chem.* 1927, *18*, 46–50.

[21] Rose, A. H. (Ed.), *Industrial Microbiology,* Butterworths, London 1961

[22] Jones, D. T., Applied acetone-butanol fermentation, in: Bahl, H., Dürre, P. (Ed.), *Clostridia. Biotechnological and Medical Applications*, Wiley-VCH, Weinheim, 2001, pp. 125–168.

[23] Zverlov, V. V., Berezina, O., Velikodvorskaya, G. A., Schwarz, W. H., Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: Use of hydrolyzed agricultural waste for biorefinery. *Appl. Microbiol. Biotechnol.* 2006, *71*, 587–597.

[24] Chiao, J.-S., Sun, Z.-H., History of the acetone-butanol-ethanol fermentation industry in China: Development of continuous production technology. *J. Mol. Microbiol. Biotechnol.* 2007, *13*, 12–14.

[25] Paquet, V., Croux, C., Goma, G., Soucaille, P., Purification and characterization of the extracellular α-amylase from *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* 1991, *57*, 212–218.

[26] Annous, B. A., Blaschek, H. P., Isolation and characterization of α-amylase derived from starch-grown *Clostridium acetobutylicum* ATCC 824. *J. Ind. Microbiol.* 1994, *13*, 10–16.

[27] Dürre, P., Formation of solvents in clostridia, in: Dürre, P. (Ed.), *Handbook on Clostridia*, CRC, Boca Raton 2005, pp. 671–693.

[28] Nölling, J., Breton, G., Omelchenko, M. V., Makarova, K. S. *et al.,* Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*. *J. Bacteriol.* 2001, *183*, 4823–4838.

[29] Lemmel, S. A., Datta, R., Frankiewicz, J. R., Fermentation of xylan by *Clostridium acetobutylicum*. *Enzyme Microb. Technol.* 1986, *8*, 217–221.

[30] Qureshi, N., Li, X.-L., Hughes, S., Saha, B. C. *et al.,* Butanol production from corn fiber xylan using *Clostridium acetobutylicum*. *Biotechnol. Prog.* 2006, *22*, 673–680.
[31] Lee, S. F., Forsberg, C. W., Gibbins, L. N., Xylanolytic activity of *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 1985, *50*, 1068–1076.
[32] Lee, S. F., Forsberg, C. W., Rattray, J. B., Purification and characterization of two endoxylanases from *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* 1987, *53*, 664–650.
[33] Lee, S. F., Forsberg, C. W., Gibbins, L. N., Cellulolytic activity of *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 1985, *50*, 220–228.
[34] Sabathé, F., Bélaich, A., Soucaille, P., Characterization of the cellulolytic complex (cellulosome) of *Clostridium acetobutylicum*. *FEMS Microbiol. Lett.* 2002, *217*, 15–22.
[35] Sabathé, F., Soucaille, P., Characterization of the CipA scaffolding protein and *in vivo* production of a minicellulosome in *Clostridium acetobutylicum*. *J. Bacteriol.* 2003, *185*, 1092–1096.
[36] Lopéz-Contreras, A. M., Martens, A. A., Szijarto, N., Mooibroek, H. *et al.,* Production by *Clostridium acetobutylicum* ATCC 824 of CelG, a cellulosomal glycoside hydrolase belonging to family 9. *Appl. Environ. Microbiol.* 2003, *69*, 869–877.
[37] Perret, S., Casalot, L., Fierobe, H. P., Tardif, C. *et al.,* Production of heterologous and chimeric scaffoldins by *Clostridium acetobutylicum* ATCC 824. *J. Bacteriol.* 2004, *186*, 253–257.
[38] Tangney, M., Mitchell W. J., Characterisation of a glucose phosphotransferase in *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* 2007, *74*, 398–405.
[39] Gottwald, M., Gottschalk, G., The internal pH of *Clostridium acetobutylicum* and its effect on the shift from acid to solvent formation. *Arch. Microbiol.* 1985, *143*, 42–46.
[40] Huang, L., Gibbins, L. N., Forsberg, C. W., Transmembrane pH gradient and membrane potential in *Clostridium acetobutylicum* during growth under acetogenic and solventogenic conditions. *Appl. Environ. Microbiol.* 1985, *50*, 1043–1047.
[41] Dürre, P., Bahl, H., Gottschalk, G., Membrane processes and product formation in anaerobes, in: Erickson, L. E., Fung, D. Y.-C. (Eds.), *Handbook on Anaerobic Fermentations*, Marcel Dekker, New York 1988, pp. 187–206.
[42] Moreira, A. R., Ulmer, D. C., Linden, J. C., Butanol toxicity in the butylic fermentation. *Biotechnol. Bioeng. Symp.* 1981, *11*, 567–579.
[43] Bowles, L. K., Ellefson, W. L., Effects of butanol on *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 1985, *50*, 1165–1170.
[44] Bahl, H., Gottwald, M., Kuhn, A., Rale, V. *et al.*, Nutritional factors affecting the ratio of solvents produced by *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 1986, *52*, 169–172.
[45] Schaffer, S., Isci, N., Zickner, B., Dürre, P., Changes in protein synthesis and identification of proteins specifically induced during solventogenesis in *Clostridium acetobutylicum*. *Electrophoresis* 2002, *23*, 110–121.
[46] Welch, R. W., Rudolph, F. B., Papoutsakis, E. T., Purification and characterization of the NADH-dependent butanol dehydrogenase from *Clostridium acetobutylicum* (ATCC 824). *Arch. Biochem. Biophys.* 1989, *273*, 309–318.
[47] Girbal, L., Soucaille, P., Regulation of solvent production in *Clostridium acetobutylicum*. *Trends Biotechnol.* 1998, *16*, 11–16.
[48] Fontaine, L., Meynial-Salles, I., Girbal, L., Yang, X. *et al.*, Molecular characterization and transcriptional analysis of *adhE2*, the gene encoding the NADH-dependent aldehyde/alcohol dehydrogenase responsible for butanol production in alcohologenic cultures of *Clostridium acetobutylicum* ATCC 824. *J. Bacteriol.* 2002, *184*, 821–830.
[49] Rao, G., Mutharasan, R., Alcohol production by *Clostridium acetobutylicum* induced by methyl viologen. *Biotechnol. Lett.* 1986, *8*, 893–896.
[50] Rao, G., Mutharasan, R. Altered electron flow in continuous cultures of *Clostridium acetobutylicum* induced by viologen dyes. *Appl. Environ. Microbiol.* 1987, *53*, 1232–1235.
[51] Fischer, R. J., Helms, J., Dürre, P., Cloning, sequencing, and molecular analysis of the *sol* operon of *Clostridium acetobutylicum*, a chromosomal locus involved in solventogenesis. *J. Bacteriol.* 1993, *175*, 6959–6969.
[52] Nair, R. V., Bennett, G. N., Papoutsakis. E. T., Molecular characterization of an aldehyde/alcohol dehydrogenase gene from *Clostridium acetobutylicum* ATCC 824. *J. Bacteriol.* 1994, *176*, 871–885.
[53] Gerischer, U., Dürre, P., Cloning, sequencing, and molecular analysis of the acetoacetate decarboxylase gene region from *Clostridium acetobutylicum*. *J. Bacteriol.* 1990, *172*, 6907–6918.
[54] Petersen, D. J., Bennett, G. N., Purification of acetoacetate decarboxylase from *Clostridium acetobutylicum* ATCC 824 and cloning of the acetoacetate decarboxylase gene in *Escherichia coli*. *Appl. Environ. Microbiol.* 1990, *56*, 3491–3498.
[55] Gerischer, U., Dürre, P., mRNA analysis of the *adc* gene region of *Clostridium acetobutylicum* during the shift to solventogenesis. *J. Bacteriol.* 1992, *174*, 426 – 433.
[56] Chen, C.-K., Blaschek, H. P., Effect of acetate on molecular and physiological aspects of *Clostridium beijerinckii* NCIMB 8052 solvent production and strain degeneration. *Appl. Environ. Microbiol.* 1999, *65*, 499–505.
[57] Kosaka, T., Nakayama, S., Nakaya, K., Yoshino, S. *et al.,* Characterization of the *sol* operon in butanol-hyperproducing *Clostridium saccharoperbutylacetonicum* strain N1-4 and its degeneration mechanism. *Biosci. Biotechnol. Biochem.* 2007, *71*, 58–68.
[58] Thormann, K., Feustel, L., Lorenz, K., Nakotte, S. *et al.,* Control of butanol formation in *Clostridium acetobutylicum* by transcriptional activation. *J. Bacteriol.* 2002, *184*, 1966–1973.
[59] Ravagnani, A., Jennert, K. C. B., Steiner, E., Grünberg, R. *et al.,* Spo0A directly controls the switch from acid to solvent production in solvent-forming clostridia. *Mol. Microbiol.* 2000, *37*, 1172–1185.
[60] Nair, R. V., Green, E. M., Watson, D. E., Bennett, G. N. *et al.,* Regulation of the *sol* locus genes for butanol and acetone formation in *Clostridium acetobutylicum* ATCC 824 by a putative transcriptional repressor. *J. Bacteriol.* 1999, *181*, 319–330.
[61] Thormann, K., Dürre, P., Orf5/SolR: a transcriptional repressor of the *sol* operon of *Clostridium acetobutylicum*? *J. Ind. Microbiol. Biotechnol.* 2001, *27*, 307–313.
[62] Feustel, L., Nakotte, S., Dürre, P., Characterization and development of two reporter gene systems for *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 2004, *70*, 798–803.
[63] Sauer, U., Dürre, P., Differential induction of genes related to solvent formation during the shift from acidogenesis to solventogenesis in continuous culture of *Clostridium acetobutylicum*. *FEMS Microbiol. Lett.* 1995, *125*, 115–120.
[64] Walter, K. A., Bennett, G. N., Papoutsakis, E. T., Molecular characterization of two *Clostridium acetobutylicum* ATCC 824 butanol dehydrogenase isozyme genes. *J. Bacteriol.* 1992, *174*, 7149–7158.
[65] Winzer, K., Lorenz, K., Zickner, B., Dürre, P., Differential regulation of two thiolase genes from *Clostridium acetobutylicum* DSM 792. *J. Mol. Microbiol. Biotechnol.* 2000, *2*, 531–541.
[66] Sullivan, L., Bennett, G. N., Proteome analysis and comparison of *Clostridium acetobutylicum* ATCC 824 and Spo0A strain variants. *J. Ind. Microbiol. Biotechnol.* 2006, *33*, 298–308.
[67] Tomas, C.A., Alsaker, K. V., Bonarius, H. P., Hendriksen, W. T. *et al.,* DNA array-based transcriptional analysis of asporogenous, nonsolventogenic *Clostridium acetobutylicum* strains SKO1 and M5. *J. Bacteriol.* 2003, *185*, 4539–4547.
[68] Alsaker, K. V., Spitzer, T. R., Papoutsakis, E. T., Transcriptional analysis of *spo0A* overexpression in *Clostridium acetobutylicum* and its effect on the cell's response to butanol stress. *J. Bacteriol.* 2004, *186*, 1959–1971.

[69] Tummala, S. B., Junne, S. G., Paredes, C. J., Papoutsakis, E. T., Transcriptional analysis of product concentration-driven changes in cellular programs of recombinant *Clostridium acetobutylicum* strains. *Biotechnol. Bioeng.* 2003, *84*, 842–854.

[70] Tomas, C.A., Welker, N. E., Papoutsakis, E. T., Overexpression of *groESL* in *Clostridium acetobutylicum* results in increased solvent production and tolerance, prolonged metabolism, and changes in the cell's transcriptional program. *Appl. Environ. Microbiol.* 2003, *69*, 4951–4965.

[71] Tomas, C. A., Beamish, J. A., Papoutsakis, E. T., Transcriptional analysis of butanol stress and tolerance in *Clostridium acetobutylicum*. *J. Bacteriol.* 2004, *186*, 2006–2018.

[72] Borden, J. R., Papoutsakis, E. T., Dynamics of genomic-library enrichment and identification of solvent tolerance genes for *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 2007, *73*, 3061–3068.

[73] Alsaker, K. V., Papoutsakis, E. T., Transcriptional program of early sporulation and stationary-phase events in *Clostridium acetobutylicum*. *J. Bacteriol.* 2005, *187*, 7103–7118.

[74] Paredes, C. J., Alsaker, K. V., Papoutsakis, E. T., A comparative genomic view of clostridial sporulation and physiology. *Nat. Rev. Microbiol.* 2005, *3*, 969–978.

[75] Holt, R.A., Stephens, G. M., Morris, J. G., Production of solvents by *Clostridium acetobutylicum* cultures maintained at neutral pH. *Appl. Environ. Microbiol.* 1984, *48*, 1166–1170.

[76] Freier-Schröder, D., Wiegel, J., Gottschalk, G., Butanol formation by *Clostridium thermosaccharolyticum* at neutral pH. *Biotechnol. Lett.* 1989, *11*, 831–836.

[77] Dürre, P., Solventogenesis by clostridia, in: Nakano, M. M., Zuber, P. (Eds.), *Strict and Facultative Anaerobes: Medical and Environmental Aspects*, Horizon Bioscience, Wymondham, 2004, pp. 329–342.

[78] Ullmann, S., Kuhn, A., Dürre, P., DNA topology and gene expression in *Clostridium acetobutylicum*: Implications for the regulation of solventogenesis. *Biotechnol. Lett.* 1996, *18*, 1413–1418.

[79] Grupe, H., Gottschalk, G., Physiological events in *Clostridium acetobutylicum* during the shift from acidogenesis to solventogenesis in continuous culture and presentation of a model for shift induction. *Appl. Environ. Microbiol.* 1992, *58*, 3896–3907.

[80] Boynton, Z. L., Bennett, G. N., Rudolph, F. B., Intracellular concentrations of conenzyme A and its derivatives from *Clostridium acetobutylicum* ATCC 824 and their roles in enzyme regulation. *Appl. Environ. Microbiol.* 1994, *60*, 39–44.

[81] Zhao, Y., Tomas, C. A., Rudolph, F. B., Papoutsakis, E. T. *et al.,* Intracellular butyryl phosphate and acetyl phosphate concentrations in *Clostridium acetobutylicum* and their implications for solvent formation. *Appl. Environ. Microbiol.* 2005, *71*, 530–537.

[82] Schwarz, W. H., The cellulosome and cellulose degradation by anaerobic bacteria. *Appl. Microbiol. Biotechnol.* 2001, *56*, 634–649.

[83] Leschine, S., Degradation of polymers: cellulose, xylan, pectin, starch, in: Dürre, P. (Ed.), *Handbook on Clostridia*, CRC, Boca Raton 2005, pp. 101–131.

[84] Zhao, Y., Hindorff, L. A., Chuang, A., Monroe-Augustus, M. *et al.,* Expression of a cloned cyclopropane fatty acid synthase gene reduces solvent formation in *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* 2003, *69*, 2831–2841.

[85] Harris, L. M., Blank, L., Desai, R. P., Welker, N. E. *et al.,* Fermentation characterization and flux analysis of recombinant strains of *Clostridium acetobutylicum* with an inactivated *solR* gene. *J. Ind. Microbiol. Biotechnol.* 2001, *27*, 322–328.

[86] Santangelo, J. D., Dürre, P., Microbial production of acetone and butanol: Can history be repeated? *Chimica oggi/Chemistry today* 1996, *14*, 29–35.

[87] Dürre, P., New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. *Appl. Microbiol. Biotechnol.* 1998, *49*, 639–648.

[88] Ezeji, T. C., Qureshi, N., Blaschek, H. P., Butanol fermentation research: Upstream and downstream manipulations. *Chem. Record* 2004, *4*, 305–314.

[89] Ezeji, T. C., Qureshi, N., Blaschek, H. P., Bioproduction of butanol from biomass: from genes to bioreactors. *Curr. Opin. Biotechnol.* 2007, *18*, 220–227.

[90] Qureshi, N., Hughes, S., Maddox, I. S., Cotta, M. A., Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. *Bioprocess Biosyst. Eng.* 2005, *27*, 215–222.

[91] Ezeji, T. C., Karcher, P. M., Qureshi, N., Blaschek, H. P., Improving performance of a gas stripping-based recovery system to remove butanol from *Clostridium beijerinckii* fermentation. *Bioprocess Biosyst. Eng.* 2005, *27*, 207–214.

