Chapter 7

Biobutanol from Renewable Agricultural and Lignocellulose Resources and Its Perspectives as Alternative of Liquid Fuels

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1. Introduction

Biobutanol (n-C_4H_9OH, available as fermentation product of various carbohydrate derivatives obtained from different resources of agricultural production such as crops and wastes) is one of the most promising biofuels in the near future. It can be produced by the so-called ABE (acetone-butanol-ethanol) type anaerobic fermentation discovered by Pasteur [1, 2] and industrialized by Weizmann [3]. Main problems associated with industrial production of biobutanol include high energy demand for processing of dilute ferment liquors and high volume of wastewater. A bioreactor with a volume of 100 m^3 produces at 90% filling ratio 1053 kg of butanol, 526 kg of acetone and 175 kg of ethanol together with 2900 kg of carbon dioxide, 117 kg of hydrogen and 84150 kg of wastewater. Efforts to increase productivity and decrease production costs resulted in many new methods. This chapter summarizes some selected results on methods of biobutanol production.

2. History of industrial biobutanol production

During investigations aimed at discovering cheaper sources of acetone and butanol for chemical industry, Weizmann [3] isolated an organism which could ferment a fairly concentrated corn mash with good yields of acetone and butanol. In 1915 the British Admirality took over the research and carried out large-scale tests in an improvised apparatus but without

providing proper conditions for laboratory testing. Thus the experiment failed due to lack of strict sterility throughout the system. Later on, British Acetones Ltd. undertook the initiation to duplicate laboratory bacteriological conditions on a commercial scale using corn meal. Butanol and acetone were produced from April 1916 to November 1919 in a total of 3458 runs of 24,000 gals of mash each. There was no run unfit for distillation [4]. By modifying the raw materials and technological conditions an explosion-like development of acetone-butanol fermentation technologies took place. Beesch [5] collected the available knowledge about industrial acetone-butanol fermentation process details, including usability of raw materials, problems of contaminations, infections, treatment of the by-products and recovering the end-products. During World War II the ABE fermentation became the most voluminous industrial biochemical process. However, the cheap petrochemical-based butanol production withdrawn it almost completely in the USA and Europe later on. In China, however, the ABE fermentation industry started only in the early 1950s in Shanghai and expanded rapidly thereafter. At its peak, there were about 30 plants all over the country and the total annual production of solvents reached 170,000 tons [6]. The success of the ABE industry in China had special features like development of continuous fermentation technologies such as in Russia, where the AB plants were the only full-scale industrial plants which used hydrolyzates of lignocellosic wastes for butanol fermentation and the process was finally run in a continual mode [7]. In China, the main strategic considerations were as follows: maintaining maximal growth and acid production phase, adoption of multiple stages in the solvent phase to allow gradual adaptation to increasing solvent, and incorporation of stillage to offer enough nutrients to delay cell degeneration. A biorefinery concept for the use of all byproducts has been elaborated and was partially put into practice. Due to the tremendous national demand for solvents, China has begun a new round of ABE fermentation research. It is expected that a new era in the ABE industry is on the horizon [6].

3. Basic principles of biobutanol production

The ABE fermentation is a complicated multistage process with a series of consecutive and parallel reactions influenced by a series of technological factors. The presence or absence of natural constituents or contaminations in the used raw materials has important influence on the productivity and product distribution. The ABE fermentation is controlled by intracellular redox processes which is influenced by a variety of technological conditions.

3.1. General mechanism of ABE fermentation

The ABE fermentation is a two-stage process: first, an acid-producing and then a solvent producing process takes place, but the solvent producing metabolic pathway could be observed only above 20 g/L starting sugar concentration [8]. Key factors in starting of solventogenesis are the undissociated intracellular butyric acid concentration and the summarized amount of the undissociated butyric and acetic acids within the cells. These are in relationships with the pH and the concentration of butyric and acetic acids in the ferment mash of course, and a boundary condition is that glucose concentration should be above 15 g/L at the moment

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of the final consumption of butyric acid, because a high glucose flux is required to generate as much amount of ATP as is enough to supply the energy demand of the butyric acid-butanol transformation [9]. Hartmanis et al. studied the pathway for uptake of acids during the solvent formation phase of ABE fermentation by C. acetobutylicum using ^{13}C NMR [10]. Actively metabolizing cells showed that butyrate can be taken up from the medium and quantitatively converted to butanol without accumulation of intermediates. The activities of acetate phosphotransacetylase, acetate kinase, and phosphate butyryltransferase rapidly decreased to very low levels when the organism began to form solvents. This indicates that the uptake of acids does not occur via a reversal of these acid-forming enzymes. No short-chain acyl-CoA synthetase activity could be detected. Apparently, an acetoacetyl-CoA:acetate (butyrate) CoA-transferase is solely responsible for uptake and activation of acetate and butyrate in C. acetobutylicum. The transferase exhibits broad carboxylic acid specificity. The key enzyme in the uptake is acetoacetate decarboxylase which is induced late in the fermentation and pulls the transfer reaction towards formation of acetoacetate. The major implication is that it is not feasible to obtain a batch-wise BuOH fermentation without acetone formation and retention of a good yield of BuOH [10]. Ferredoxin enzymes also play important role in the ABE processes, thus the presence of iron in the appropriate form and concentration is essential factor in the appropriate solvent production. When Clostridium acetobutylicum was grown in batch culture under Fe limitation (0.2 mg/L) at pH 4.8, glucose was fermented to BuOH as the major fermentation end product, and small quantities of HOAc were produced. The final conversion yield of glucose into BuOH could be increased from 20% to 30% by Fe limitation. The BuOH-acetone ratio was changed from 3.7 (control) to 11.8. Hydrogenase specific activity was decreased by 40% and acetoacetate decarboxylase specific activity by 25% under Fe limitation. Thus, Fe limitation affects C and electron flow in addition to hydrogenase [11].

Terracciano and Kashket investigated the intracellular physiological conditions associated with the induction of butanol-producing enzymes in Clostridium acetobutylicum. During the acidogenic phase of growth, the internal pH decreased in parallel with decrease in the external pH, but the internal pH did not go below 5.5 throughout batch growth. Butanol was found to dissipate the proton motive force of fermenting C. acetobutylicum cells by decreasing the transmembrane pH gradient, whereas the membrane potential was affected only slightly. In growing cells, the switch from acid to solvent production occurred when the internal undissociated butyric acid concentration reached 13 mM and the total intracellular undissociated acid concentration (acetic plus butyric acids) was at least 40 to 45 mM [12]. C. acetobutylicum ATCC 824 cells harvested from a phosphate-limited chemostat culture maintained at pH 4.5 had intracellular concentrations of acetate, butyrate and butanol which were 13-, 7- and 1.3-fold higher, respectively, than the corresponding extracellular concentrations. Cells from a culture grown at pH 6.5 had intracellular concentrations of acetate and butyrate, which were only 2.2-fold higher than the respective external concentrations. The highest intracellular concentrations of these acids were attained at pH 5.5. When cells were suspended in anaerobic citrate-phosphate buffer at pH 4.5, exogenous acetate and butyrate caused a concentration-dependent decrease in the intracellular pH, while butanol had relatively little effect until the external concentration reached 150 mM. Acetone had no effect at concentrations ≤200 mM. These data demonstrate that acetate and butyrate are concentrated within the cell under acidic

conditions and thus tend to lower the intracellular pH. The high intracellular butyrate concentration presumably leads to induction of solvent production thereby circumventing a decrease in the intracellular pH great enough to be deleterious to the cell [13].

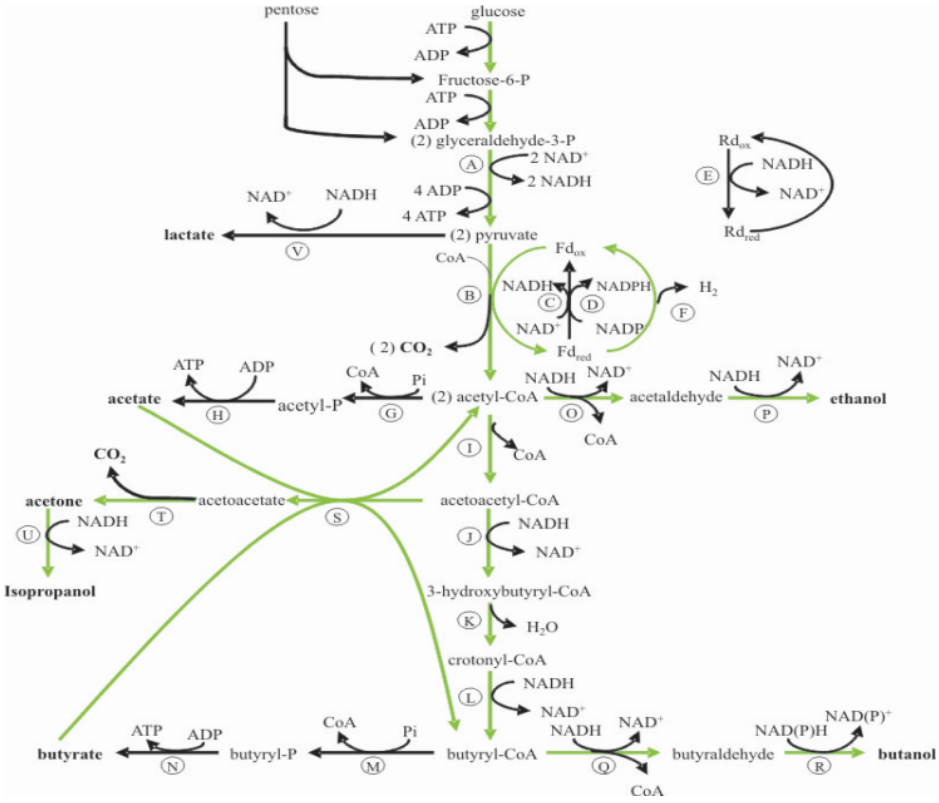

Figure 1. Mechanism of acetogenesis

Harris et al. suggested [14] that butyryl phosphate (BuP) is a regulator of solventogenesis in Clostridium acetobutylicum. Determination of BuP and acetyl phosphate (AcP) levels in various C. acetobutylicum strains (wild(WT), M5, a butyrate kinase (buk) and a phosphotransacetylase (pta) mutant) showed that the buk mutant had higher levels of BuP and AcP than the wild strain; the BuP levels were high during the early exponential phase, and there was a peak corresponding to solvent production [15]. Consistently with this, solvent formation was initiated significantly earlier and was much stronger in the buk mutant than in all other strains. For all strains, initiation of butanol formation corresponded to a BuP peak concentration that was more than 60 to 70 pmol/g (dry wt.), and higher and sustained levels corresponded to higher butanol formation fluxes. The BuP levels never exceeded 40 to 50 pmol/g (dry wt.) in strain M5, which produces no solvents. The BuP profiles were bimodal, and there was a second

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peak midway through solventogenesis that corresponded to carboxylic acid reutilization. AcP showed a delayed single peak during late solventogenesis corresponding to acetate reutilization. As expected, in the pta mutant AcP levels were very low, yet this strain exhibited strong butanol prodn. These data suggest that BuP is a regulatory mol. that may act as a phosphodonor of transcriptional factors. DNA array-based transcriptional anal. of the buk and M5 mutants demonstrated that high BuP levels corresponded to downregulation of flagellar genes and upregulation of solvent formation and stress genes [15].

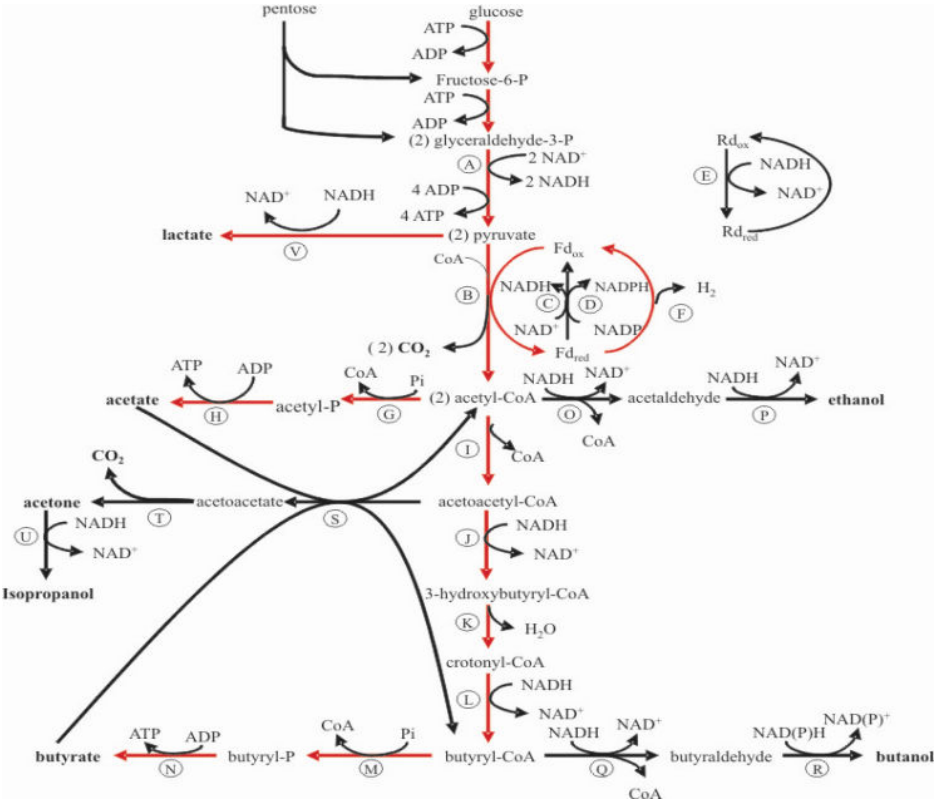

Figure 2. Mechanism of solventogenesis

3.2. Basic reasons for autoinhibition or butanol and intermediate acids toxicity

The toxicity of accumulated butanol and the intermediates is a very important feature of the ABE fermentation. Costa studied [16] the growth rates of Clostridium acetobutylicum in presence of BuOH, EtOH, Me_2CO, acetate and butyrate. Acetate and butyrate were the most toxic compounds, with concentrations of 5 and 8.5 g/L, respectively, stopped the cell growth. An EtOH concentration of 51 g/L or 11 g BuOH/L reduced cell growth by

50%. Acetone did not inhibit cell growth at 29 g/L, thus ethanol and acetone were non-toxic at a normal fermentation. Some mutant strains, however, more tolerant towards butanol, for example Lin and Bladchek [17] obtained a derivative of C. acetobutylicum ATCC 824 which grew at concentrations of BuOH that prevented growth of the wild-type strain at a rate which was 66% of the uninhibited control. This strain produced consistently higher concentrations of BuOH (5-14%) and lower concentrations of acetone (12.5-40%) than the wild-type strain in 4-20% extruded corn broth. Characterization of the wild-type and the mutant strain demonstrated the superiority of the latter in terms of growth rate, time of onset of BuOH production, carbohydrate utilization, pH resistance, and final BuOH concentration in the fermentation broth [17]. Moreira et al. [18] initiated a fundamental study attempting to elucidate the mechanism for BuOH toxicity in the acetone-BuOH fermentation by Clostridium acetobutylicum. Butanol as a hydrophobic compound inserted into the membrane increases the passive proton flux, forms a "hole" for proton on the membrane. This eliminates hydrogen ions form the cell and the intracellular pH increases. The strains which are able to decrease the membrane fluidity are more resistant towards butanol. The cells have deacidifying mechanism to keep the intracellular pH value at 6 when the pH value of the ferment liquor is located between 4 and 5 can reduce acids into alcohols, which increases their butanol producing ability. Lepage et al [19] studied the changes in membrane lipid composition of C. acetobutylicum during ABE fermentation. Large changes were found in phospholipid composition and in fatty acid composition, the latter characterized mainly by a decrease in the unsaturated/saturated fatty acid (U/S) ratio.

Compound	50 % inhibiton	100 % inhibition
Acetic acid	2.7	5.0
Butyric acid	4.1	8.5
Ethanol	51.0	69.0
Butanol	11.0	15.0

Table 1. Inhibitory concentrations (g L^{-1}) of ABE solvents and intermediates on fermentations carried out by C. acetobutylicum

Effects of the addition of alcohols (EtOH, BuOH, hexanol, and octanol) and of acetone were also studied. In all cases, large changes were observed in the U/S ratio, but with differences which were related to the chain length of the alcohols. The effect of solvents appears to account for a large part of changes in lipid composition observed during the fermentation. The pH was also important, a decrease in pH resulting in a decrease in the U/S ratio and in an increase in cyclopropane fatty acids. The effect of increasing temperature was mainly to increase fatty acid chain lengths [19].

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4. General conditions of the ABE fermentation

Optimal conditions of ABE fermentation strongly depend on many factors such as the selected raw materials or their composition, and are essentially influenced by the selected strain as well. Furthermore, a series of important factors can decrease or increase the yield and changes the distribution of the ABE solvents even with the same raw material or bacterium strain. Some selected pieces of information are summarized below.

4.1. Selection of raw materials

A wide scale of monosaccharide or disaccharide or other sugar-based oligomeric or polymeric substrates can be used as starting material in ABE fermentations. Compere and Griffith [20] studied the effect of substrate types on the yield and distribution of valuable ABE products with different strains of Clostridia. Different concentrations (2.5-10%) of arabinose, ribose, xylose, xylane, fructose, glucose, cellobiose, lactose, sucrose, dextrin and cellulose were tested and the amount and the distribution of acetone, ethanol, butanol and the H_2/CO_2 ratio strongly depended on the type of strain, the type of sugar and their concentrations as well. Ounine et al. [21] studied the fermentation of glucose, arabinose and xylose with C. Acetobutylicum and found conversion into solvents in 32, 29, and 28%, respectively. Growth yields were similar on the all sugars, but glucose or arabinose was consumed in preference to xylose and with faster growth. It means that not only corn, cereals, molasses, but wastes of dairy products [22,23] or agricultural byproducts as corn stalk, corncob, cellulose wastes and other raw materials can also be utilized [24,25]. By using wastes, however, it is an important key factor that the contaminants can prevent the ABE fermentation. For example, when alkaline peroxide pretreated wheat straw was hydrolyzed using cellulolytic and xylanolytic enzymes, and the hydrolyzate was used to produce butanol using Clostridium beijerinckii P260, the culture produced less than 2.59 g L-1 ABE solvents, but after removal of the formed inhibitor salts with electrodialysis, 22.17 g/L of ABE solvents were formed. This was higher value than the ABE solvent concentration (21.37 g/L) given from glucose. A comparison of use of different substrates (corn fiber, wheat straw) and different pretreatment techniques (dilute sulfuric acid, alkaline peroxide) suggested that generation of inhibitors was substrate and pretreatment specific [26]. Selection of raw material for ABE process cannot be independent from the selection of the bacteria strain. For example, cassava, due to its high starch content and low cost, is a promising candidate substrate for large-scale ABE fermentation processes. However, the solvent yield from the fermentation of cassava reaches only 60% of that achieved by fermenting corn. Addition of ammonium acetate (CH_3COONH_4) to the cassava medium significantly promotes solvent production with a high butanol ratio C. Acetobutylicum mutant (EA 2018). When cassava medium was supplemented with 30 mM ammonium acetate, the acetone, butanol and total solvent prodn. reached 5.0, 13.0 and 19.4 g/l, respectively, after 48 h of fermentation which level of solvent production is comparable to that obtained from corn medium. Both ammonium (NH_4^+) and acetate (CH_3COO-) were required for increased solvent synthesis [27]

4.2. Fermenting microorganisms

Depending on the composition and properties of raw materials, the selection and conditioning of the appropriate bacterium strain are essential. In order to improve the economic efficacy of ABE fermentation, the butanol ratio is to be increased by eliminating the production of other byproducts such as acetone and specific mutants are to be developed which show high butanol tolerance, high productivity or other advantageous properties.

Harada [28, 29] isolated a new strain of Clostridium (Cl. Madisonii) which produced BuOH amounting to 28.7% of the initial total sugar and the fermented broth included 1.38% BuOH. The age of the culture also plays important role in the productivity. By using older inoculated bacteria, the production of acetone increased and the ratio of BuOH to Me_2CO decreased from 2.24 to 1.88 [30]. Harada [31] concluded that the seed culture at the last stage of the acid-decreasing phase gave the best yield as inoculum in the main fermentation. Butanol-resistant mutants have been isolated by Hermann from soil which produced significantly higher solvent concentrations (about 30%) than the wild-type strain [32]. The sporulation-deficient (spo) early-sporulation Clostridium acetobutylicum P262 mutants produced higher solvent yields than did the spoB mutant which was a late-sporulation one. In conventional batch fermentation, the wild-type strain produced 15.44 g L^{-1} of solvents after 50 h at a productivity of 7.41 g L^{-1} d^{-1} of solvents. The spoA2 mutant produced 15.42 g L^{-1} of solvents at a productivity of 72.4 g L^{-1} d^{-1} of solvents with a retention time of 2.4 h in a continuous immobilized cell system employing a fluidized bed reactor [33].

Using two different types of Clostridia to improve the productivity of each (acidogenic and solventogenic) phase is also known. Bergstroem and Foutch [34] improved the BuOH production from sugars by combining two cultures of Clostridium: one that produces butyric acid, and another that converts butyrate to BuOH. Thus, C. butylicum NRRL B592 and C. pasteurianum NRRL B598 were cultured together in thioglycolate medium containing 2.5% added glucose and a $CaCO_3$ chip to maintain pH, at 37 °C under anaerobic conditions. The yield of BuOH was 20 % more as compared to the value when C. butylicum was cultured alone.

Initiation of gene-structure changes by destructive methods such as irradiations or chemicals followed by selection is a well known method in the production of highly effective Cl. Acetobutylicum strains. Yasuda [35] heated ABE producing microorganisms at 100 °C to destroy all vegetative forms except spores which were kept at –10 °C, then treated with electric discharge in vacuum by using 50,000 V and 0.002 A DC for stimulation. High-yield butanol producing Clostridium strain was prepared through irradiation of the wild strain with ^{60}Co γ-rays at an irradiation dosage of 100-1,000 Gy and a dosage rate of 3-5 Gy/min [36].

Chemical mutation with N-methyl-N′-nitrosoguanidine is one of the most frequently used method to produce excellent ABE fermenting strains. Hermann et al [37] prepared a strain of C. acetobutylicum that hyperproduces acetone and BuOH by mutation of C. acetobutylicum IFP903. A new mutant (CA101) of C. pasteurianum prepared in this way could produce 2.1 g BuOH/L in 2 days. By using the parental strain, the production of BuOH was only 0.6 g/L [38]. The C. acetobutylicum strain 77 was isolated from the parent strain ATCC 824 with the abovementioned method in the presence of butanol. The mutant grew more rapidly (μ = 0 69

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h^{-1}) than the parent strain (μ = 0 27 h^{-1}) and, at the stationary phase, the cell dry weight of mutant strain was about 50% higher than that of the parent strain. Strain 77 metabolised glucose faster than wild strain and solvent production started earlier with higher specific production rates than the parent strain. From 65 g of glucose, 20 g L^{-1} of solvents (butanol, 14 5 g; acetone, 3 5 g; ethanol, 2 g) were formed by the wild strain in 53 h, whereas the mutant used 75 g of glucose and excreted nearly 24 g L^{-1} of solvents (butanol, 15.6 g; acetone, 4.5 g; ethanol, 3.7 g) in 44 h [39]. A frequently used chemical to inititate mutation in C. Acetobutylicum strains is methanesulfonic acid ethyl ester (EMS). EMS is effective in inducing mutants resistant to ampicillin, erythromycin, and butanol (15 g/l). Optimal mutagenesis occurs at 85–90% kill corresponding to a 15 minute exposure to 1.0% (v/v) EMS at 35 C. At optimal conditions, the frequency of resistant mutant CFU/ total CFU plated increases 100–200 fold [40].

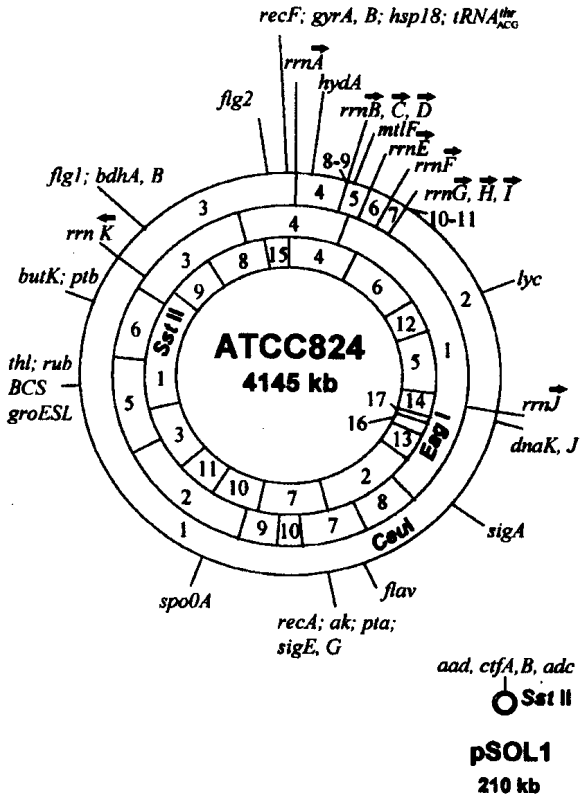

Figure 3. Physical and genetic map of the C. acetobutylicum ATCC 824 genome [242].

Genetical engineering opened unlimited perspectives in the preparation of ABE fermenting microorganisms. Genetically modified C. Acetobutylicum, E. Coli and S. Cereviase

and other microorganisms play important role in the future production of ABE solvents under more convenient conditions than in classical ABE fermentation. The acetoacetate decarboxylase gene (adc) in the hyperbutanol-producing industrial strain Clostridium acetobutylicum EA 2018 was disrupted when the butanol ratio was increased from 70 to 80.05%, while acetone production decreased to approx. 0.21 g/L in the adc-disrupted mutant (2018adc). Regulation of the electron flow by addition of methylviologen altered the carbon flux from acetic acid production to butanol production in strain 2018adc, which resulted in an increased butanol ratio of 82% and a corresponding improvement in the overall yield of butanol from 57 to 70.8% [41].

Larossa and Smulski found genes involved in a complex that is a three-component proton motive force-dependent multidrug efflux system to be involved in E. coli cell response to butanol by screening of transposon random insertion mutants. Reduced production of the AcrA and/or AcrB proteins of the complex confers increased butanol tolerance [42]. Green and Bennett subcloned the genes coding for enzymes involved in butanol or butyrate formation into a novel Escherichia coli-Clostridium acetobutylicum shuttle vector constructed from pIMP1 and a chloramphenicol acetyl transferase gene [43]. The resulting replicative plasmids, referred to as pTHAAD (aldehyde/alcohol dehydrogenase) and pTHBUT (butyrate operon), were used to complement C. acetobutylicum mutant strains, in which genes encoding aldehyde/alcohol dehydrogenase (aad) or butyrate kinase (buk) had been inactivated by recombination with Emr constructs. Complementation of strain PJC4BK (buk mutant) with pTHBUT restored butyrate kinase activity and butyrate production during exponential growth. Complementation of strain PJC4AAD (aad mutant) with pTHAAD restored NAD(H)-dependent butanol dehydrogenase activity, NAD(H)-dependent butyraldehyde dehydrogenase activity and butanol production during solventogenic growth [43]. Shen and Liao constructed an Escherichia coli strain that produces 1-butanol and 1-propanol from glucose [44]. First, the strain converts glucose to 2-ketobutyrate, a common keto-acid intermediate for isoleucine biosynthesis. Then, 2-ketobutyrate is converted to 1-butanol via chemicals involved in the synthesis of the unnatural amino acid norvaline. The synthesis of 1-butanol is improved through deregulation of amino-acid biosynthesis and elimination of competing pathways. The final strain demonstrated a production titre of 2 g/L with nearly 1:1 ratio of butanol and propanol [44]. Green et al [45] made recombinant thermophilic bacteria of the family Bacillaceae which have been engineered to produce butanol and/or butyrate. The Bacillaceae is preferably of the genus Geobacillus or Ureibacillus [45]. Young et al described a method of modifying prokaryotic and eukaryotic hosts for the fermentation production of aliphatic alcohols. Elements of the gene for a CAAX proteinase (prenylated protein-processing C-terminal proteinase) are used to increase alcohol tolerance. This can be used in combination with other changes to increase alcohol tolerance [46]. Fermenting with modified eukaryotic cells in a suitable fermentation broth, wherein butanol and ethanol are produced at a ratio between 1:2 to 1:100, is described by Dijk et al. [47]. Since fermentations with yeasts do not require sterile environment, genetically modified yeasts are very prosperous microrganisms in ABE fermentation. Yeast cells capable of producing butanol and comprising a nucleotide sequence encoding a butyryl-CoA dehydrogenase and at least one nucleotide sequence encoding an electron transfer flavoprotein were described by Mueller et al. [48].

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4.3. Effect of medium composition, temperature and nitrogen sources

The appropriate temperature for optimal fermentation ability of C. Acetobutylicum strains strongly depends not only on the type of strain, but on the composition of the medium and raw materials as well, and is strongly influenced by a series of factors such as presence or absence of additives, sugar concentration, pH, and others. McNeil and Christiahsen studied the effect of temperature on the solvent production by C. acetobutylicum in the range 25 to 40°C [49]. It was found that the solvent yield decreased with increasing temperature. Considering total solvent yield and productivity only, the optimum fermentation temperature was found to be 35°C [49]. Comparison of the solvent production by using strains of C. acetobutylicum and C. butylicum from whey showed that higher yields of solvents were observed at 37 °C or 30 °C, respectively [50]. Oda and Yamaguchi [51] concluded that temperature control played important role in the solvent yield and the optimal temperatures were not found to be the same during different stages of the process. Harada [52] concluded that the yield of BuOH was increased from 18.4-18.7% to 19.1-21.2% by lowering the temperature from 30 °C to 28 °C when the growth of the bacteria reached a maximal rate.

Fouad et al. [53] studied fourteen different media in the fermentative production of acetone and butanol. The highest total yields were achieved in medium containing potato starch and soluble starch as C sources. Compositon and pH of the medium have important influence on ABE fermentation. The contaminants in the media have decisive effect on the ABE fermentation. For example, hydrolysates obtained by enzymatic saccharification of wheat straw or cornstover pretreated by steam explosion in classical or acidic conditions, were found non-fermentable into acetone-butanol. A simple treatment involving heating the hydrolysates in presence of calcium or magnesium compounds such as $Ca(OH)_2$ or $MgCO_3$ at neutral pH values restored normal fermentability to these hydrolysates [54]. Sugar concentration of the media also influences the ABE fermentation. Fond et al. [55] studied growing of C. acetobutylicum in fed-batch cultures at different feeding rates of glucose. The sugar conversion to BuOH and Me_2CO increased with increasing the glucose flow whereas, on the contrary, conversion to butyric acid was highest at slow glucose feeding rate. The AcOH concentration was constant at different flows of glucose and the solventogenesis was not inhibited at high flow of sugar [55].

The amount and chemical form of inorganic and organic nitrogen sources basically affect on the ABE process. They influence also strongly depends on presence or absence of other important additives. Among studied inorganic nitrogen compounds, ammonium nitrate and urea could stop the fermentation in the middle, $(NH_4)HSO_4$, NH_4Cl, and $(NH_4)_2HPO_4$ resulted acetone-rich fermentation, while $(NH_4)_2CO_3$ and NH_4OH gave BuOH-rich fermentation [56]. Baghlaf et al [57] studied the effect of different concentrations of corn steep liquor, fodder yeast, soybean meal, corn bran, rice bran, and KH_2PO_4 in the ABE fermenatation, and the organism preferred utilization of natural organic sources. The best concentration of KH_2PO_4, favouring the ABE production was found to be 2 g/L. Oda [58,59] occurred a little effect of adding $(NH_4)_2SO_4$ to EtOH-extracted soybean meal in the yield of solvents, however, cane molasses and dried yeasts were good supplements to the same soybean meal. Addition of asparagine retarded the fermentation. When used as the sole N source, soybean press cake and egg white were good; the others tested were, in the order of decreasing suitability, EtOH-extracted

soybean meal, casein, fish protein, zein, gluten, yeast protein, and gelatine. With peanut cake as the N source, Ca salts were not desirable. The stimulants tested were mostly effective: they were, in the order of decreasing effect, liver (best), rice bran-clay, α-alanine, α-methylphenethylamine-H_2SO_4, β-alanine, p-aminobenzoic acid, naphthaleneacetic acid, and cane molasses-clay (the last two were slightly worse than the control without stimulant). Doi et al. [60] could occur that growth-promoting amino acids in the casein acid-hydrolyzate can be divided into three groups: the bacteria required isoleucine, valine, and glutamic acid; asparagine, serine, threonine, alanine, and glycine accelerated fermentation. Leucine, phenylalanine, methionine, tryptophan, proline, lysine, histidine, and arginine were not required for growth and cystine and tyrosine inhibited fermentation.

4.4. Acetate and butyrate additives

Since both acetic and butyric acids are intermediate products of ABE fermentation, and butyrate is almost completely consumed during the solventogenic phase, addition of these intermediates to increase the yield of butanol has already been studied in detail. Beneficial effects were observed with addition of AcOH (completely reduced to Me_2CO), butyric acid (50-80% recovery as BuOH), and sodium acetate NaOAc (60% recovery as Me_2CO), while bad results were obtained with addition of formic acid and calcium acetate [61,62]. Nakhmanocivh and Shcheblikina [63] used a 4% glucose medium, with corn gluten or flour mash, and additon of 0.1N $Ca(OAc)_2$ raised acetone yield by 20-24% and 0.1 N calcium butyrate raised BuOH yield by 45-60% in C. acetobutylicum fermentations. Though $Ca(OAc)_2$ accelerated the fermentation, it was only 40-50% fermented itself. Utilization of $Ca(OOCPr)_2$ goes further (above 70%), mostly by conversion to $Ca(OAc)_2$.

Tang concluded [64] that addition of 1.5 g/L acetic acid increased the cell growth and enhanced acetone production in ABE fermentation. The final concentration of acetone was 21.05%, and the butanol production was not improved. Similarly, addition of 1.0 g/L butyric acid increased the cell growth and enhanced butanol production, the final concentration of butanol was 24.32% while the acetone production was not improved. Additon of acetic acid and butyric acid together (10 mM each) to C. acetobutylicum grown on glucose (2%) in a pH-controlled minimal medium caused rapid induction of acetone and butanol synthesis (within 2 h) [65]. The specific growth rate of the culture and the rate of H_2 production decreased gradually from the onset of the experiment, whereas the rate of CO_2 production remained unchanged. No correlation was found between solvent production and sporulation of the culture [65]. A 32 % conversion rate of the glucose into solvents took place when the same fermentation was carried out on a synthetic medium (BuOH:acetone:EtOH was 0.6:1.9:6). This was changed to 34 and 35 % (BuOH:acetone:EtOH was 5:3:6 or 0.8:2.4:6) by adding HOAc or butyric acid, respectively [66].

Fond et al. [67] studied the effect of HOAc and butyric acid additon in the fermentation of various kinds of carbohydrates using fed-batch fermentations. Different specific rates of carbohydrate utilisation were obtained by variations in feeding rates of sugar. At low catabolic rates of sugar addition of acetic acid or butyric acid, alone or together, increased the rate of metabolic transition by a factor 10 to 20, the amount of solvents by a factor 6 and the percentage of fermented glucose to solvents by a factor 3. The same results were obtained with both glucose and xy-

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lose fermentations. Depending on the rates of growth, butanol production began at acid levels of 3–4 g L^{-1} for fast metabolism and at acid levels of 8–10 g L^{-1} for slow metabolism. Associated with slow metabolism, reassimilation of acids required values as high as 6.5 g L^{-1} of acetic acid and 7.5 g L^{-1} of butyric acid. At a high rate of metabolism, acetic and butyric acids were reassimilated at concentrations of 4.5 g L^{-1} [67]. Significant increases in acetone and BuOH production could be observed by Yu and Saddler [68] by growing C. acetobutylicum on xylose in presence of added HOAc or butyric acid. Increased yields could not be accounted for by conversion of the low amounts of acetic or butyric acid added. The effect was greater when the acid was added before, rather than during fermentation, so pH change alone is probably not responsible and enzyme induction may be involved in this process. Addition of acetate or butyrate ensures fermentation at neutral pH conditions as well. Holt et al. used C. acetobutylicum NCIB 8052 (ATCC 824) and monitored a batch culture at 35 °C in a glucose (2%) minimal medium. At pH 5, good solvent production was obtained in the unsupplemented medium, although addition of acetate plus butyrate (10 mM each) caused solvent production to be initiated at a lower biomass concentration. At pH 7, although a purely acidogenic fermentation was maintained in the unsupplemented medium, low concentrations of acetone and n-butanol were produced when the glucose content of the medium was increased (to 4% [wt./vol.]). Substantial solvent concentrations obtained at pH 7 in a 2% glucose medium supplemented with high concentrations of acetate plus butyrate (100 mM each, supplied as their K salts). Thus, C. acetobutylicum NCIB 8052, like C. beijerinckii VPI 13436, are able to produce solvents at neutral pH, although good yields are obtained only when adequately high concentrations of acetate and butyrate are supplied. Supplementation of the glucose minimal medium with propionate (20 mM) at pH 5 led to production of some n-propanol as well as acetone and n-butanol; the final culture medium was virtually acid free. At pH 7, supplementation with propionate (150 mM) again led to formation of n-propanol but also provoked production of some acetone and n-butanol, although in considerably smaller amounts than those obtained when the same basal medium had been fortified with acetate and butyrate at pH 7 [69].

4.5. Effect of carbon monoxide, carbon dioxide and hydrogen

From technological viewpoint, fermentation can be divided into two well separable phases: acid formation phase and, after reaching an autoinhibition limit value of the acids, solvent formation phase. These steps can be performed in separated technological environments as well [70].

Hydrogen formation takes place in the acidogenic phase, so the composition of the gases (CO_2, H_2) changes during the fermentation process. The larger part of the carbon dioxide is formed in the pathway of acetone formation. Presence of hydrogen and carbon dioxide has large influence on each metabolic step. The effect of H_2 and CO_2 as product gases on solvent production was studied in a continuous culture of alginate-immobilized C. acetobutylicum. Fermentations were carried out at various dilution rates. With 10% H_2 and 10% CO_2 in the sparging gas, a dilution rate of 0.07 h^{-1} was found to maximize volumetric productivity (0.58 $g \times L^{-1} \times h^{-1}$), while maximal specific productivity of 0.27 $g^{-1} \times h^{-1}$ occurred at 0.12 h^{-1}. Continuous cultures with vigorous sparging of N_2 produced only acids. It was concluded that in the case of continuous fermentation H_2 is essential for good solvent production, although good solvent production is possible in

an H_2-absent environment in case of batch fermentations. When the fermentation was carried out at atmospheric pressure under H_2-enriched conditions, presence of CO_2 in the sparging gas did not slow down glucose metabolism; rather it changed the direction of the phosphoroclastic reaction and, as a result, increased the butanol/acetone ratio [71].

Klei et al. [72] studied the effect of pure CO_2 on the second phase of ABE fermentation. CO_2 pressures up to 100 psig were used in a batch fermentor using glucose as substrate. Maximal solvent production occurred near 25 psig CO_2 at the expense of cell growth. In addition, the BuOH:Me_2CO ratio changed sharply at 40 psig from 5:1 to 20:1 and EtOH production was eliminated at >50 psig. As the pressure increased, both conversion rates of organic acids to solvents and the utilization rate of substrate glucose decreased.

Pressurization of the fermentation vessel with H_2 appeared to decrease, rather than increase, the formation of neutral solvents in batch fermentations [73]. However, increasing H_2 partial pressure increased BuOH and EtOH yields from glucose by an average of 18% and 13%, respectively, and the yields of acetone and of endogenous H_2 decreased by an average of 40% and 30%, respectively, and almost no effect was observed on the growth of the culture. The BuOH-to-acetone ratio and the fraction of BuOH in the total solvents also increased with H_2 partial pressure. There were no major differences in the observed pattern of change with pressurization at either t = 0 or t = 18 h [74].

Redox active additives such as carbon monoxide have important influence on the ABE fermentation processes. Addition of CO inhibited the hydrogenase activity of cell extracts and viable metabolizing cells. Increasing the partial pressure of CO (2 to 10%) in unshaken anaerobic culture tube headspaces significantly inhibited (90% inhibition at 10% CO) both growth and H_2 production. The growth was not sensitive to low partial pressures of CO (~15%) in pH-controlled fermentors (pH 4.5). CO addition dramatically altered the glucose fermentation balance of C. acetobutylicum by diverting carbon and electrons away from H_2, CO_2, acetate and butyrate production and towards production of EtOH and BuOH. The BuOH concentration increased from 65 to 106 mM and the BuOH productivity (the ratio of BuOH produced/total acids and solvents produced) increased by 31% when glucose fermentation was maintained at pH 4.5 in presence of 85% N_2-15% CO vs. N_2 alone [75]. Carbon monoxide sparged into batch fermentations of C. acetobutylicum inhibited production of H_2 and enhanced production of solvents by making available larger amounts of NAD(P)H_2 to the cells. CO also inhibited biomass growth and acid formation as well. Its effect was mostly pronounced under fermentation conditions of excess carbon- and nitrogen-source supply [76]. When continuous, steady-state, glucose-limited cultures of Clostridium acetobutylicum were sparged with CO, complete or almost complete acidogenic fermentations became solventogenic. Alcohol (butanol and ethanol) and lactate production at very high specific production rates were initiated and sustained without acetone, and little or no acetate and butyrate formation. In one fermentation strong butyrate uptake without acetone formation was observed. Growth could be sustained even with 100% inhibition of H_2 formation. Although CO gasing inhibited growth up to 50%, and H_2 formation up to 100%, it enhanced the rate of glucose uptake up to 300%. These results support the hypothesis that solvent formation is triggered by an altered electron flow [77]. The metabolic modulation by CO was particularly

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effective when organic acids such as acetic and butyric acid were added to the fermentation as electron sinks. The uptake of organic acids was enhanced, and increase in butyric acid uptake by 50-200% over control was observed. H_2 production could be reduced by 50% and the ratio of solvent could be controlled by CO modulation and organic acid addition. Acetone production could be eliminated if desired. BuOH yield could be increased by 10-15%. Total solvent yield could be increased by 1-3% and the electron efficiency to acetone-BuOH-EtOH solvents could be increased from 73% for controls to 80-85% for CO- and organic acid-modulated fermentations. The dynamic nature of electron flow in this fermentation was elucidated and mechanisms for metabolic control were hypothesized [78].

4.6. Other factors

Wyne [79] studied the inhibition of ABE fermentation of maize mash by C. acetobutylicum influenced by 30 representative inorganic and organic acids. Several acids caused complete inhibition when the initial reaction was between pH 3.90 and 3.65, the following being included: HCl, HNO_3, H_2SO_4, H_3PO_4, succinic, maleic, malonic, levulinic, crotonic, glycolic, p-hydroxybutyric, formic, acetic, propionic, butyric and isobutyric. The toxic effects are probably associated with a critical C_{H+} in the cell interior, closely approximating the observed extracellular C_{H+} associated with an inhibitory effect. All three chloroacetic acids are much more toxic than acetic acid, but hydroxy derivatives of the lower fatty acids are not more toxic than the corresponding normal acids. Pyruvic, lactic and glyceric acids are tolerated at higher C_{H+} levels. In the lower fatty acids the inhibiting C_{H+} was appreciably lower with each successive higher homolog. On the basis of molar concentration the order of effectiveness of inhibition was as follows: nonylic > caprylic > heptylic > formic > caproic = isocaproic > valeric = isovaleric > isobutyric = butyric ≥ propionic = acetic. Capillary activity has relatively little effect with formic, acetic, propionic and butric acids, but was very marked with higher homologs [79]. Inhibitory effect of these acids can easily be removed by neutralization [80]. When the ABE fermentation is over, the culture medium may be treated by blowing NH_3 to neutralize most of organic acids and, after distilling out the solvents, the residue can be treated with non-N-containing nutrients, e.g. dried sweet potatoes, and the fermentation may be repeated in the same way in order to save the quantity of nutrient and to increase the yield [81].

The effect of agitation speed and pressure was studied by Doremus et al [82]. Batch fermentations were run at varying agitation rates and were either pressurized to 1 bar or nonpressurized. Agitation and pressure both affect the level of dissolved H_2 in the media which, in turn, influence solvent production. In nonpressurized fermentations volumetric productivity of BuOH increased as the agitation rate decreased. While agitation had no significant effect on BuOH productivity under pressurized conditions, overall BuOH productivity increased over that obtained in nonpressurized runs. Maximal butyric acid productivity, however, occurred earlier and increased as agitation increased. Peak H_2 productivity occurred simultaneously with peak butyric acid productivity. The proportion of reducing equivalents used in forming the above products was determined using a redox balance based on the fermentation stoichiometry. An inverse relationship between the final concentrations of acetone and acetoin was found in all fermentations studied [82]. Using shear activation of C. acetobutylicum by

pumping the cells through capillaries, the cell growth, glucose consumption and product formation rates are considerably increased. Shear-activated continuous cell culture can be used as an inoculum with a well-defined fermentation activity for batch cultures. Different runs of such batch cultivation yield well-reproducible results which could not be obtained from inocula of other cultures or even of heat-shocked spores. The cells can attain a growth rate higher than 1.6 h^{-1}. The shear-activated continous culture growth is affected already at a butanol concentration lower than 1.6 g L^{-1}[83], Afschar et al (1986) [80]. The effect of viscosity on the ABE fermentation was studied by Korneeva et al. [84]. Viscosity of the medium was a limiting factor in ABE production by C. acetobutylicum during fermentation with starch and grains such as wheat and rye flour. Various concentrations of agar-agar (0.1, 0.5 and 0.8%) were added to the medium which showed that elevation of viscosity reduces saccharification, increases the concentration of nonfermented sugars, and decreases the yield of solvents. Prior treatment of the substrate with α-amylase reduced the viscosity of the medium and improved fermentation and solvent yields [84].

Although the ABE fermentation is a strictly anaerobic process, [2] Nakhmanovich and Kochkina [85] could increase the BuOH yield by 3.4-9.1% by short periodical aeration of the medium. Redox potential was measured before and after bubbling and decreased sharply by aeration. In batch and continuous cultivations of C. acetobutylicum ATCC 824 on lactose, a strong relationship was observed between redox potential of broth and cellular metabolism [86]. The specific productivity of BuOH and of butyric acid was maximal at a redox potential of -250 mV. The specific production rate of butyric acid decreased rapidly at higher and lower redox potentials. For BuOH, however, it achieved a lower but stable value. This was true for both dynamic and steady states. Continuous fermentations involving lactose exhibited sustained oscillation at low dilution rates. Such oscillation appears to be related to BuOH toxicity to the growth of cells. At higher dilution rates, where BuOH concentrations were relatively low, no such oscillation was observed. Broth redox potential apparently is an excellent indicator of the resulting fermentation product partitioning [86]. Some selected examples are given in Table 2 and 3.

5. General considerations on developments of ABE fermentation

5.1. Immobilization

Immobilization of C. Acetobutylicum strains prevents bacteria from existing in the ferment mash and is a very essential facility in a variety of integrated solvent recovery methods. Haeggstroem and Molin [87] concluded that immobilized vegetative cells of C. Acetobutylicum have a similar product formation pattern when incubated in a simple glucose-salts solution as ordinary growing cells. If vegetative cells of the organism are immobilized in the solvent production phase, solvents are continuously produced on extended incubation. By immobi1izing spores of the organism, the disturbance of the cells metabolic activity during the immobilization procedure was avoided. After the outgrowth of viable cells within the gel, the washed gel preparation retained at a high production capacity in the non-growth stage

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and the results indicate that continuous production might be fully possible. The butanol productivity was also found to be higher with immobilized cells than in a normal batch process. Haeggstroem [88] used immobilized spores of Clostridium acetobutylicum in a calcium alginate gel. The productivity of the system was 67 g BuOH/L-day and with immobilized cells it was possible to achieve continuous BuOH production for 1000 h. Foerberg et al. [89] developed a technique for maintaining constant activity during continuous production with immobilized, non-growing cells. A single stage continuous system with alginate-immobilized C. Acetobutylicum, was mainly fed with a glucose medium that supported fermentation of acetone-BuOH but did not permit microbial growth. The inactivation that occurred during these conditions was prevented by pulse-wise addition of nutrients to the reactor. By using this technique, the ratio of biomass to BuOH was reduced to 2% compared to 34% in a traditional batch culture. At steady state conditions BuOH was the major end product with yield coefficients of 0.20 (g/g glucose). The productivity of BuOH was 16.8 g L^{-1} d^{-1} during these conditions. In a corresponding system with immobilized growing cells the ratio of biomass to BuOH was 52-76% and the formation of butyric and acetic acid increased thereby reducing the yield coefficients for BuOH to 0.11 (g g^{-1}). With the intermittent nutrient dosing technique, const. activity from immobilized non-growing cells has been achieved for 8 weeks.

Characteristics of the process	Yield	Content Productivity		Ref.
	g g^{-1}	g L^{-1}	g $L^{-1}h^{-1}$	
Complex medium, yeast extract, glucose, *Cl.Acetobutylicum ATCC 824,* continuous	0.26	12.0	2.50	[244]
Synthetic P-limited medium, two-stage reactorglucose, continuous, *Cl. AcetobutylicumATCC 824*	0.42	18.0	0.54	[70]
Complex medium, continuous, glucose*Cl. Acetobutylicum ATCC 824*	0.32	13.0	0.75	[245]
Synthetic medium, yeast extract, two-stage, cell recycling, *Cl. Acetobutylicum ATCC 824*glucose, continuous	0.30	7.0	4.50	[108]
Synthetic medium, glucose, cell recycling, *Cl. Acetobutylicum ATCC 824,* continuous	0.29	13.0	6.50	[111]
Complex medium, yeast extract, immbolized, intermittent feeding, glucose, continuous,*Cl. Acetobutylicum*	0.20	1.0	0.70	[89]
Complex medium, yeast extract, two-stage, Immobilized *Cl. Acetobutylicum DSM 792,* Glucose, continuous	0.21	3.9	4.02	[94]
Complex medium, glucose, yeast extract, two-stage, *Cl. Acetobutylicum DSM 792,* continuous	0.25	15.4	1.93	[94]

Table 2. Comparison of maximum solvent productivities, yields and concentrations with glucose as sugar source

Several carriers have been tested for production of ABE solvents by immobilized local strain of C. acetobutylicum. Thus, both batch and continuous fermentations were performed by using sodium alginate, polyacrylamide, activated carbon, and silica gel carriers. Calcium alginate was found to be the most suitable with batch culture techniques where the total solvent production was 19.55 g L^{-1} after 4 days. On the other hand, higher solvent yields with continuous fermentation was noticed with silica gel G-60 (0.063-0.2 mm) with 13.06 g L^{-1} solvent production. In all cases, the tested solid supports were of inferior effect for solvent production under the exptl. conditions used as compared with Ca-alginate [90]. High–strength carriers were also tested for C. acetobutylicum ATCC 824 in batch fermentation. Coke, kaolinite and montmorillonite clay appeared to have a beneficial effect on the fermentation, although the effectiveness appeared to be dependent on the medium used. One of the least expensive materials, coke, was suitable for use in continuous culture. Steady state conditions could be maintained for more than 30 days with total solvent productivity and a yield of 12 g L^{-1}, 1.12 g L^{-1} h^{-1} and 0.3 g total solvent/g glucose used, respectively [91]. Entrapment of C.acetobutylicum AS 1.70 with PVA as the base and by means of absorption in the corncob as the carrier is recommended. Experiments have been done to produce acetone and butanol in a statical way in batches and by changing the corn as medium circulatingly [92]. The vegetative cells of C. acetobutylicum AS 1.70 were also immobilized onto CR (ceramic ring) carriers by adsorption. The continuous production of acetone-BuOH from 8% corn mash concentration was carried out for 90 days in a system of 3-stage packed column reactor (total vol. 5.18 L). The maximal concentration of solvent (acetone, BuOH, and EtOH) was 21.9 g L^{-1} and the productivity of the column was 24.73 g L^{-1} d^{-1}. The residual starch concentration was 0.43% and the conversion efficiency of starch was 40.5% [93]. ABE solvent production was also carried out with C. acetobutylicum DSM 792 (ATCC 824) in a two-stage stirred tank cascade using free and immobilized cells. The cells were immobilized by alginate, κ-carrageenan or chitosan. The cell-containing pellets were dried or chemically treated to improve their long-term stability. Dried calcium alginate yielded the best matrix system. It remained stable after a fermentation time of 727 h in stirred tank reactors. The solvent (sum of acetone, butanol and ethanol) productivity of 1.93 g L^{-1} h^{-1} at a solvent concentration of 15.4 g L^{-1} with free cells was increased to 4.02 g L^{-1} h^{-1} at a solvent concentration of 4.0 g L^{-1} h^{-1} with calcium alginate-immobilized cells (25% cell loading, 12 g L^{-1} pellet concentration, 3 g L^{-1} wet cell mass concentration). With pellet diameter of 0.5 mm, the biocatalyst efficiency was <50% [94]. Immobilized cells of C. saccharoperbutylacetonicum N1-4 (ATCC 13564) were tested in an anaerobic batch culture system. Two different methods of immobilization, active immobilization in alginate and passive immobilization by employing stainless steel scrubber, nylon scrubber, polyurethane with uniform pore's size, polyurethane with different pore's size and palm oil empty fruit bunch fiber were studied. Immobilization in alginate was carried out on the effect of cell's age, initial culture pH and temperature on the production of ABE. Immobilized solventogenic cells (18 h) produced the highest total solvents concentration as compared to other phases with productivity of 0.325 g L^{-1} h^{-1}. The highest solvents production by active immobilization of cells was obtained at pH 6.0 with 30 °C with productivity of 0.336 g L^{-1} h^{-1}. Polyurethane with different pore's size is significantly better than other materials tested for solvents productivity and YP/S at 3.2 times and 1.9 times, respectively, compared to free cells after 24 h fermentation. We concluded that passive immobilization technique increases the productivity (215.12 %) and

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YP/S (88.37 %) of solvents by C. saccharoperbutyl-acetonicum N1-4 [95]. C.beijerinckii was immobilized in calcium alginate to produce BuOH continuously from glucose. Two different alginate geometries (beads and coated wire-netting) were used for continuous experiments and two mathematical models (sphere and flat plate) were developed. Calculations revealed that no glucose limitation was present in both cases. Furthermore, the biomass build-up in the alginate was probably a surface process [96].

Cells of C.acetobutylicum immobilized on bonechar were used for the production of ABE solvents from whey permeate. When the process was performed in packed bed reactors operated in a vertical or inclined mode, solvent productivities up to 6 kg m^{-3} h^{-1} were obtained. However, the systems suffered from blockage due to excess biomass production and gas hold-up. These problems were less apparent when a partially-packed bed reactor was operated in horizontal mode. A fluidized bed reactor was the most stable of the systems investigated, and a productivity of 4.8 kg m^{-3} h^{-1} was maintained for 2000 h of operation. The results demonstrate that this type of reactor may have a useful future role in the ABE fermentation [97]. Schoutens determined the optimal conditions necessary for the continuous BuOH production from whey permeate with C. beyerinckii LMD 27.6 immobilized in calcium alginate beads. The influence of three parameters on the BuOH production was investigated: fermentation temperature, dilution rate (during start-up and at steady state) and concentration of Ca^{2+} in the fermentation broth. Both a fermentation temperature of 30 °C and a dilution rate of ≤0.1 h-1 during the start-up phase are required to achieve continuous BuOH production from whey permeate. BuOH can be produced continuously from whey permeate in reactor productivities 16-fold higher than those found in batch cultures with free C. beyerinckii cells on whey media [98]. Fermentation of cane sugar molasses by immobilized C. acetobutylicum cells was greatly affected by inoculum size, calcium alginate concentration and molar ammonium nitrogen to molasses ratios. The pH value of the medium and incubation temperature both influenced the ABE production. The maximum total solvent content reached 22.54 g L^{-1} at inoculum size 6% (w/w), molasses concentration 140 g/l, sodium alginate amount 3 %, and molar ammonium nitrogen to molasses ratios 0.48, pH 5.5. Attempts to recyclize the fermentation process by using immobilized spores of C. acetobutylicum afforded total solvent contents of 22.54, 20.64, 19.31 g L^{-1} during the first 3 runs, respectively [99].

5.2. Continuous fermentation

Continuous fermentation is a preferred operational mode to decrease cost of production and increase efficiency. It can easily be performed with using cascade reactors with suppressing butanol concentration below the inhibition limit. The butanol concentration supressing can be performed by dilution or with various methods of recovery with adsorption, extraction, stripping, membrane techniques or with combination of these methods. Increase of the active biomass amount in the mash by cell recycling plays key role in continuous ABE fermentation processes, as well.

Dyr et al. [100] observed formation of neutral solvents in continuous ABE fermentation process by means of C. acetobutylicum without morphological adaptation due to the altered way of cultivation. The results obtained leave no doubt as to the possibility of employing the continuous method for acetone-butanol fermentation [101]. A cascade type continuous ABE fermentation

method was developed from soluble starch by building an equipment consistsing a battery of 11 fermenting tanks [102]. The first tank is used as an incubator and an activator for the culture. In the remaining tanks, the actual fermentation is carried out. The feed liquor is continuously supplied. The continuous fermentation process for Me2CO-BuOH production is a 1st-order reaction. A continuous ABE fermentation process was developed and adopted in plants using starch raw materials by Yarovenko [103]. The basis for a continuous process is knowledge of laws of continuous mixing of liquids in batteries of connected vessels which are discussed by Yarovenko [104]. The length of fermentation considerably influences the acidity of the fermented mixture at the end of the process. Owing to differences in mash composition and duration of process, acid level is mostly higher in continuous fermentation than in a discontinuous one. With continuous acetone-butanol process fermentation, speed could be raised 1.58 times compared to the semicontinuous method. In the continuous fermentation, it is useful to operate with 2-5 parallel batteries and to cultivate bacteria in separated vessels. The carbohydrates produced by saccharification under different conditions were studied as they were of great importance on length and course of fermentation. Operation of the battery's head fermentor has a great influence on the whole process, the amount of inoculum, acid production, and fermentation speed. To provide an adequate microorganism concentration and to reduce the risk of infection in the battery's head fermentor, mash from the 2nd vessel is recycled. The acidity increase was evident primarily in the last tank. Optimum concentration of the cells to be inoculated at the start of the fermentation 7×10^9/ml for C. acetobutylicum and physiologically mature cells should comprise about 80% of the total inoculum. The flow rate into the main fermentor should be harmonized with the utilization rate of carbohydrate in the battery. Bacteria in the main vessel must be maintained at their respective stationary phase of growth. The continuous ABE fermentation increased productivity efficiency 20%. The carbohydrate utilization was improved by 2.4%, along with the characteristics of the beer [103,104]. The Japanese K.F. Engineering [105] described an apparatus for production of Me_2CO and BuOH by immobilized ABE-producing microorganisms, where the immobilized microorganisms are first exposed to a batch process until active gas formation is observed, and then, a continuous production process was performed.

The availability and demand of biosynthetic energy (ATP) is an important factor in the regulation of solvent production in steady state continuous cultures of C. acetobutylicum. The effect of biomass recycle at a variety of dilution rates and recycle ratios on product yields and selectivities was determined. Under conditions of non-glucose limitation, when the ATP supply is not growth-limiting, a lower growth rate imposed by biomass recycle leads to a reduced demand for ATP and substantially higher acetone and butanol yields. When the culture is glucose limited, however, biomass recycle results in lower solvent and higher acid yields [106]. Wijjeswarapu et al. studied continuous BuOH fermentation by C. acetobutylicum in a stirred tank reactor. The results of glucose fermentation with cell recycling revealed the formation of small amounts of EtOH, moderate amounts of Me_2CO and BuOH, and large amounts of AcOH and butyric acid. Without cell recycling overall BuOH production was decreased by a factor of 3.5 [107]. Afshar et al. used a cascade system and cell recycling. At a dry cell mass concentration of 8 g/L and a dilution rate of $D=0.64\ h^{-1}$, a solvent productivity of $5.4\ g/L^{-1}\ h^{-1}$ could be attained. To avoid degeneration of the culture which occurs with high concentrations of ABE solvents a 2-stage cascade with cell recycling and turbidostatic cell concentration control was used as optimal

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solution, the 1st stage of which was kept at relatively low cell and product concentrations. A solvent productivity of 3 and 2.3 g L^{-1} h^{-1}, respectively, was achieved at solvent concentrations of 12 and 15 g L^{-1} [108]. Huang and Ramey [109] determined the influence of dilution rate and pH in continuous cultures of Clostridium acetobutylicum in a fibrous bed bioreactor with high cell density and butyrate concentrations at pH 5.4 and 35°C. By feeding glucose and butyrate as co-substrates, the fermentation was maintained in the solventogenesis phase, and the optimal butanol productivity of 4.6 g L^{-1} h^{-1} and a yield of 0.42 g g^{-1} were obtained at a dilution rate of 0.9 h^{-1} and pH 4.3. Eight Clostridium acetobutylicum strains were examined for α-amylase and strains B-591, B-594 and P-262 had the highest activities. Defibered-sweet-potato-slurry containing starch supplemented with potassium phosphate, cysteine-HCl, and polypropylene glycol was used as continuous feedstock to a multistage bioreactor system. The system consisted of four columns (three vertical and one near horizontal) packed with beads containing immobilized cells of C. acetobutylicum P-262. The effluent contained 7.73 g solvents L^{-1} (1.56 g acetone; 0.65 ethanol; 5.52 g butanol) and no starch. Productivity of total solvents synthesized during continuous operation was 1.0 g L^{-1} h^{-1} and 19.5% yield compared to 0.12 g L^{-1} h^{-1} with 29% yield in the batch system [110]. Pierrot et al. introduced a hollow-fiber ultrafiltration to separate and recycle cells in continuous ABE fermentation. Under partial cell recycling and at a dilution rate of 0.5 h^{-1}, a cellular concentration of 20 g L^{-1} and a solvent productivity of 6.5 g L^{-1} h^{-1} is maintained for several days at a total solvent concentration of 13 g L^{-1} [111]. The device developed was sterilizable by steam and permitted drastic cleaning of the ultrafiltration membrane without interrupting continuous fermentation. With total recycle of biomass, a dry weight concentration of 125 g L^{-1} was attained, which greatly enhanced the volumetric solvent productivity averaging 4.5 g L^{-1} h^{-1} for significant periods of time (>70 h) and maintaining solvent concentration and yield at acceptable levels [112].

A stable continuous production system with nongrowing cells of C. acetobutylicum adsorbed to beechwood shavings was obtained by different types of adsorption procedures for production of ABE solvents by Foerberg and Haegsstroem [113]. The system was started with continuous flow of a complete nutrient medium. A thick cell layer was formed on the wood shavings during the 1st day but it disappeared rapidly. Under glucose limitation, a new cell layer developed during the following period (2-5 days). After this phase, a continuous flow of nongrowth medium with nutrient dosing (8 h dosing interval) was started. This led to a washout of most adsorbed cells and ~85% of suspended cells. Another cell layer was formed during this period and the system was controlled by the nutrient dosing technique. The system was stable with no cell leakage for weeks. The maximal productivity of butanol, acetone, and EtOH was 36 g L^{-1} d^{-1} with a product ratio of 6:3:1 [113].

A continuous ABE production system with high cell density obtained by cell-recycling of Clostridium Saccharoperbutylacetonicum N1-4 was also studied. In a conventional continuous ABE culture without cell-recycling, the cell concentration was below 5.2 g L^{-1} and the maximal ABE productivity was only 1.85 g L^{-1} h^{-1} at a dilution rate of 0.20 h^{-1}. To obtain a high cell density at a faster rate, we concentrated the solventogenic cells of the broth 10 times by membrane filtration and were able to obtain ~20 g L^{-1} of active cells after only 12 h of cultivation. Continuous culture with cell recycling was then started, and the cell concentration increased gradually through

cultivation to a value greater than 100 g L^{-1}. The maximum ABE productivity of 11.0 g L^{-1} h^{-1} was obtained at a dilution rate of 0.85 h^{-1}. However, a cell concentration >100 g L^{-1} resulted in heavy bubbling and broth outflow, which made it impossible to carry out continuous culture. Therefore, to maintain a stable cell concentration, cell bleeding and cell recycling were performed. At dilution rates of 0.11 h^{-1} and above for cell bleeding, continuous culture with cell recycling could be operated for more than 200 h without strain degeneration and an overall volumetric ABE productivity of 7.55 g L^{-1} h^{-1} was achieved at an ABE concentration of 8.58 g L^{-1} [114].

Characteristics of the process	Yield	Content	Productivity	Ref.
	g g^{-1}	g L^{-1}	g $L^{-1}h^{-1}$	
Aspen hydrolysate (SO_2 and enzymatic), *Cl. Acetobutylicum P262,* extractive ferm., (dibutyl phthalate), cell recycling	0.36	17.7	0.73	[246]
Pine hydrolysate (SO_2 and enzymatic), *Cl. Acetobutylicum P262*, extractive ferm. (dibutyl phthalate), cell recycling	0.32	22.9	0.95	[246]
Corn stove hydrolysate (SO_2 and enzymatic), *Cl. Acetobutylicum P262*, extractive ferm., (dibutyl phthalate), cell recycling	0.34	25.7	1.07	[246]
Bagasse, alkali and enzymatic hydrolsis, C. saccharoperbutylacetonicum ATCC 27022 Simultaneous ferm., active C	0.33	18.1	0.30	[247]
Rice straw, alkali and enzymatic hydrolsis, C. saccharoperbutylacetonicum ATCC 27022 Simultaneous ferm., active C	0.28	13.0	0.15	[247]
Wheat straw, *Cl. Acetobutylicum IFP 921,* alkali-enzymatic hydrolysis and simultaneous fermentation	0.18	17.7	0.47	[248]
Corn fiber, sulphuric acid hydrolysis, XAD-4 resin purifn., *C. Beijerinckii BA101*	0.39	9.3	0.10	[249]
Corn fiber, enzymatic hydrolysis, *C. Beijerinckii BA101*	0.35	8.6	0.10	[249]
Wheat straw, *Cl. Beijerinckii P260*, simultaneous saccharification and fermentation, gas stripping	0.41	21.42	0.31	[250]
Rice straw, enzymatic simultanenous Hydrolysis and -fermentation, C. Acetobutylicum C375	0.30	12.8	0.21	[251]
Cornstalk stover, enzymetic hydrolysis, membrane reactor, steam exploding, C. Acetobutylicum ASI 132	0.21	-	0.31	[252]
Wheat straw, fed-batch, *Cl. Beijerinckii P 260*, simultaneous saccharification and fermentation, gas stripping	0.44	192.0	0.36	[253]

Table 3. Comparison of maximum solvent productivities, yields and concentrations with lignocellulose based sugar sources

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6. Removal methods of ABE solvents from ferment liquors

The complexity and chemical interactions of the aqueous mixture of BuOH, 2-PrOH, acetone, and EtOH produced by the bacterial fermentation of various carbohydrate launched development of numerous innovative separation processes. Belafi-Bako et al. [115] reviewed with 246 reference results and developments on simultaneous product removal in ethanol and acetone/butanol/ethanol fermentation regarding thermal processes (e.g., evaporation, distillation), physical and chemical methods (e.g., extraction, adsorption as well as catalytic reactions), and different membrane separation techniques (e.g., perstraction, reversed osmosis, pervaporation, dialysis).

6.1. Removal by vacuum

The simplest recovering method is removal of ABE solvent during fermentation by using vacuum, because the relative volatility of ethanol, acetone or butanol is much higher than the volatility of water. The I.G.Farbenindustrie] [116] used this method periodically, and removed the butanol in vacuum before completion of the fermentation, fresh wort was added and the fermentation was continued. Dreyfus [117] removed the ABE solvents during fermentation by vaporization with the aid of cyclohexane forming an azeotropic mixture therewith and, passing an oxygen-free gas through the liquor. The cyclohexane may be added continuously or intermittently and may be carried as vapor by the gas stream. Removal of ABE solvents in vacuum-assisted in-situ pervaporation techniques at the temperature of fermentation is discussed in chapter 6.6.

6.2. Removal by gas stripping

Qureshi reviewed the ABE fermentation in various types of reactor systems and recovery by gas stripping with 13 references. Gas stripping is a simple technique which does not require expensive apparatus, does not harm the culture, does not remove nutrients and reaction intermediates and reduces butanol toxicity (inhibition). As a result of butanol removal by gas stripping, concentrated sugar solutions can be used to produce ABE solvents. Compared to sugar utilization of 30 g L^{-1} in a control batch reactor, sugar utilization of 199 g L^{-1} has been reported with 69.7 g L^{-1} solvent production. In fed-batch reactors concentrated sugar solutions (350 g L^{-1}) have been used. Additionally, the process of ABE production results in concentrated product streams containing 9.1-120 g L^{-1} ABE solvent. In the integrated ABE production and recovery systems, selectivity figures of 4-30.5 have been reported [118]. The effect of factors such as gas recycle rate, bubble size, presence of acetone, and ethanol in the solutions or broth were investigated in order to remove butanol from model solution or fermentation broth. Butanol stripping rate was found to be proportional to the gas recycle rate. In the bubble size range attempted (< 0.5 and 0.5-5.0 mm), the bubble size did not have any effect on butanol removal rate. In C. beijerinckii fermentation, ABE productivity was reduced from 0.47 g L^{-1} h^{-1} to 0.25 g L^{-1} h^{-1} when smaller (< 0.5 mm) bubble size and an excessive amount of antifoam (to inhibit production of foam caused by smaller bubbles) were used. This suggested that fermentation was negatively affected by antifoam [119].

Gas stripping can be performed by using fermentation gases (H_2 and CO_2) formed during fermentation. Concentrated sugar solutions (250-500 g/L) were used in continuous fermentation of Clostridium beijerinckii BA101, which operated for 21 d (505 h), producing 460 g acetone-BuOH/L [120]. In the integrated fed-batch fermentation and product recovery system, solvent productivities were improved to 400% of the control batch fermentation productivities. In a control batch reactor, the culture used 45.4 g glucose L^{-1} and produced 17.6 g total solvents L^{-1} (yield 0.39 g g^{-1}, productivity 0.29 g L^{-1} h^{-1}). Using integrated fermentation-gas stripping product recovery system with CO_2 and H_2 as carrier gases, the fed-batch reactor was operated for 201 h. At the end of fermentation, an unusually high concentration of total acids (8.5 g L^{-1}) was observed. A total of 500 g glucose was used to produce 232.8 g solvents (77.7 g acetone, 151.7 g butanol, 3.4 g ethanol) in 1 L culture broth. The average solvent yield and productivity were 0.47 g g^{-1} and 1.16 g L^{-1} h^{-1}, respectively [121]. Using a potential industrial substrate (liquefied corn starch, 60 g L^{-1}) in a batch process integrated with gas stripping resulted in the production of 18.4 g L^{-1} ABE solvents, with 92% utilization of sugars present in the feed. In a fed-batch reactor fed with saccharified liquefied corn starch, 81.3 g L^{-1} ABE was produced as compared to 18.6 g L^{-1} in the control. In this integrated system, 225.8 g L^{-1} corn starch sugar (487% of control) was consumed. In absence of product removal, it is not possible for C. beijerinckii BA101 to utilize more than 46 g L^{-1} glucose [122].

6.3. Removal by adsorption

Tomota and Fujiki [123] observed that the presence of a small amount of activated carbon promotes BuOH fermentation of corn. Oda [124] compared various activated carbons for the removal of BuOH in order to avoid its toxicity. The commercial supernorite proved to be the most effective with intermittent additions. Oda [125] studied the effect of pre-treatment of carbons on BuOH removing capacity, but similar beneficial results were obtained by adding commercial active C to the mash and the acid or alkali treated activated carbons. Yamazaki et al. [126-128] packed activated carbon into a column, and after saturation with ABE solvents it was heated at 150 °C, and steamed to recover solvents, when 98% of the BuOH and 99% of the Me_2CO could be recovered. Activated carbon could be used repeatedly without refreshing. The efficiency of carbon was a little reduced by repeated sorption with soft carbon but hardly reduced with hard carbon. Freundlich's adsorption isotherm by some commercial carbons was, respectively, for Me_2CO and BuOH at 37º, $x/m=0.151C^{0.52}$ and $x/m=0.275C^{0.57}$, where x was the amount of solvent (millimole L^{-1}) adsorbed by a mg of adsorbent, and C the concentration, (millimole L^{-1}) of solvent remaining after equilibrium was reached. The amount of BuOH absorbed by carbon was ≥4 times as large as that of Me_2CO, and this selective sorption was more marked with increasing concentration of solvents. The sorption of BuOH was slower than that of Me_2CO, and >48 h was necessary for reaching equilibrium. Smaller granules of carbon were more effective, and carbon packed in a bag suspended in fermenting mash was convenient. Fermentation experiments with 12-18 g sugar and 5-6 g C/100 ml proved to be the optimal. Carbon granules of the size 2-4 mm^3 were most adequate. Addition of carbon after the growth phase or the maximal acidity phase gave best results. A sugar mash (12 g/100 ml) was fermented with 6 g/100 ml. active C in 3 days by C. acetobutylicum to give a solvent yield of 36% (based on added sugar). The ratio of produced Me_2CO and BuOH was 1:2.

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Urbas [129] developed a method for adsorption of ABE components from ferment mash produced by C. acetobutylicum on activated carbons with elution by a volatile solvent. Elution was carried out by feeding the solvent vapor to the carbon bed that is maintained at, or slightly less than, the solvent condensation temperature at a rate of ~1/2 bed vol h^{-1} until the volatile solvent is detected in the eluate and continuing until ~1/2 additional bed volume of eluate is collected. The 1st fraction was mainly water (up to ~96% of the initial amount) and the 2nd a concentrated aqueous solution of the organic compound in the volatile solvent. The solvent is distilled off. The concentration of the final aq. solution is ~30%. The volatile solvents were Me_2CO, 2-butanone, EtOAc, i-PrOH, MeOH, and Et_2O.

A series of adsorbents such as bone charcoal, activated charcoal, silicalite, polymeric resins (XAD series), bonopore, and polyvinylpyridine were tested in the separation of butanol from aqueous solutions and/or fermentation broth by adsorption. Usage of silicalite appeared to be the more attractive as it could be used to concentrate butanol from dilute solutions (5 to 790-810 g L^{-1}) and resulted in complete desorption of ABE solvents. In addition, silicalite could be regenerated by heat treatment. The energy requirement for butanol recovery by adsorption-desorption processes was 1,948 kcal kg^{-1} butanol as compared to 5,789 kcal kg^{-1} butanol during steam stripping distillation. Other techniques such as gas stripping and pervaporation required 5,220 and 3,295 kcal kg^{-1} butanol, respectively [130]. Milestone and Bibby [131] studied the usability of silicalite, which provided a possible economic route for the separation of alcohols from dilute solutions. Thus, EtOH was concentrated from a 2% (wt/vol) solution to 35% and BuOH from 0.5 to 98% (wt/vol) by adsorption on silicalite and subsequent thermal desorption. Maddox [132] found that 85 mg BuOH/g silicalite can be adsorbed from ferment liquors.

Polymeric resins with high n-butanol adsorption affinities were identified from a candidate pool of commercially available materials representing a wide array of physical and chemical properties. Resin hydrophobicity, which was dictated by the chemical structure of its constituent monomer units, most greatly influenced the resin-aqueous equilibrium partitioning of n-butanol, whereas ionic functionalization appeared to have no effect. In general, those materials derived from poly(styrene-co-divinylbenzene) possessed the greatest n-butanol affinity, while the adsorption potential of these resins was limited by their specific surface area. Resins were tested for their ability to serve as effective *in situ* product recovery devices in the n-butanol fermentation by C. acetobutylicum ATCC 824 [133]. In small-scale batch fermentation, addition of 0.05 kg L^{-1} Dowex Optipore SD-2 facilitated achievement of effective n-butanol titers as high as 2.22% (w/v), well above the inhibitory threshold of C. acetobutylicum ATCC 824, and nearly twice that of traditional, single-phase fermentation. Retrieval of n-butanol from resins via thermal treatment was demonstrated with high efficiency and predicted to be economically favorable [133]. Testing performed on four different polymeric resins in the fermentation by C. acetobutylicum showed that the pH increasing could prevent adsorption of intermediates such as acetic and butyric acids. Bonopore, the polymer giving the best adsorption pattern for butanol with no undesirable effects.The adsorption characteristic of butanol from aqueous fermentation broth were also determined on RA, GDX-105, and PVP resins. The adsorption order is GDX-105>RA>PVP and the isotherms could be represented by the Langmuir equation. The adsorption increases with increasing temperature excepting very low concentrations of

butanol. The ΔG^0, ΔH^0 and ΔS^0 values for the butanol adsorption processes from aqueous solutions on GDX-105 showed that the enthalpy decreased and the entropy increased [134].

In butanol/isopropanol batch fermentation, adsorption of alcohols can increase the substrate conversion. The fouling of adsorbents by cell and medium components is severe, but this has no measurable effect on the adsorption capacity of butanol in at least three successive fermentations. With the addition of some adsorbents it was found that the fermentation was drawn towards production of butyric and acetic acids [135].

6.4. Basic considerations of solvent extraction

Solvent extraction techniques have the potential for tremendous energy savings in the recovery of fermentation products such as ABE solvents. Such savings will have a direct impact on the economics for the entire fermentation. In order to find the optimal conditions of extractive butanol recovery, however, numerous conditions and factors have to be taken into consideration. A special case of the extractive recovery is the so-called in-situ extractive fermentation (see in chapter 6.5), where extraction is performed during, and together with fermentation. In this case, not only the separation and recovering characteristics play key role in the process, but also the toxicity of the non-miscible solvents basically determines the applicability of the method. In presence of an extractant solvent, however, due to distribution equilibriums, the concentration of each component of the fermentation broth (acids used in decreasing the pH to initiate the solventogenic stage, substrates or intermediates such as glucose, acetate, butyrate) changes. Being aware of these concentration relationships is essential to be able to control the process. Mass and energy balances of side-stream and countercurrent extraction were compared with the appropriate parameters of a classic distillation procedure in recovery of ABE solvents from fermentation broth [136]

A general mathematical model for performance evaluation of acetone-butanol continuous flash extractive fermentation system was formulated in terms of productivity, energy requirement (energy utilization efficiency) and product purity. Simulation results based on experimental data showed that the most pronounced performance improvement could be achieved by using a highly concentrated substrate as feed and the increase in solvent dilution rate could only improve the total productivity at the expense of energy utilization efficiency. A two-vessel partial flash system, with the first vessel of two to three plates and the second vessel as a complete flash vessel, is required to ensure high product purity [137]. Extraction with solvents having distribution coefficients above one appears to have a more favourable energy balance than in case of distillation [136].

Distribution coefficients of ABE solvents between water and the selected extractant and biocompatibility of the extractant are crucial parameters. A solvent screening criterion was developed based on the maximum product concentartion attainable for the assessment of batch and semicontinuous multicomponent extractive fermentations [138]. Dadgar and Foutsch evaluated 47 solvents for the ability to recover Clostridium fermentatiuon products. Equilibrium distribution coefficients and separation factors from water for ethanol, butanol, and acetone were determined [139]. Griffith et al. [140] measured the organic/aqueous distribution coefficients of numerous potential BuOH extractants and simultaneously tested several in

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bacterial culture. The most effective appeared to be polyoxyalkylene ethers which had distribution coefficients in the range of 1.5-3 and showed little or no toxicity toward the fermentation. The esters and alcohols tested generally had better distribution coefficients but higher biotoxicity. Barton and Daugulis performed biocompatibility tests on 63 organic solvents, including alkanes, alcohols, aldehydes, acids, and esters. Thirty-one of these solvents were further tested to determine their partition coefficients for butanol in fermentation medium of C. acetobutylicum. The biocompatible solvent with the highest partition coefficient for BuOH (4.8) was poly(propylene glycol) 1200 which was selected for fermentation experiments F141G [141]. Thirty-six chemicals were tested for the distribution coefficients for BuOH, the selectivity of alcohol/water separation and the toxicity towards Clostridia. Convenient extractants were found in the group of esters with high molar mass. Liquid-liquid extraction was carried out in a stirred fermentor and a spray column. Formation of emulsions and fouling of the solvent in fermentation broth causes problems with the operation of this type of equipment [142].

Based on the solvent screening criterion and practical experience, one of the best solvents proved to be oleyl alcohol [143]. Oleyl alcohol was used in 40% that of the culture medium to extract BuOH and acetone from the fermentation broth produced from glucose by C. Acetobutylicum and fermentation of the raffinate was continued after the extraction [144]. With a known biocompatibility of extractants such as oleyl alcohol, 1-decanol, 1-octanol, 1-heptanol and ethyl acetate, considering economic viewpoints as well, a mixed extractant of oleyl-alkohol and decanol was chosen for extraction at phase rate of 1:5 [145].

Both butyric acid and butanol could readily be extracted from microbial fermentation broth with vinyl bromide. The vinyl bromide fraction was separated from the aqueous broth and evaporated to give substantially pure butyric acid and (or) BuOH. Three passes of broth through separation columns of vinyl bromide at 4° enabled to isolate ~65% of total butyric acid and ~60% of BuOH in the broth [146]. The methyl, ethyl, propyl and butyl esters of vegetable oils are effective extractants for butanol from aqueous solutions. The effect of four salts, three alcohols and a ketone could be expected to affect the extraction of BuOH from industrial fermentation systems were evaluated. Variations in NaCl, Na_2SO_4, Na_2SO_3 and KH_2PO_4 from 0 to 0.15 M on the extraction of 0.1-4.1% BuOH from aqueous solutions at 25, 40, and 55° gave small changes in distribution coefficients. Mild increases occurred with increasing temperature and increasing NaCl, Na_2SO_4, and KH_2PO_4. Mild decreases in BuOH extraction occurred with increasing Na_2SO_3. Variations in acetone, EtOH, and 2-PrOH concentration ranging between 0 and 4% at 25, 40, and 55° gave small changes in distribution coefficients at BuOH concentrations of 0.1-4.1%. A slight increase in BuOH extraction was observed with increasing 1-pentanol under similar conditions [147]. Extraction of ABE solvents with long-chain fatty acid esters/using the extracts without separation as diesel fuel is discussed in chapter 8.

Ionic liquids are novel green solvents that have the potential to be employed as extraction agents to remove butanol from aqueous fermentation media. An extraction procedure used 1-butyl-3-methylimidazolium-bis(trifluoromethylsulfonyl)imide or 1-butyl-3-methylimidazolium hexafluoro-phosphate ionic liquids was developed by Eom et al. [148]. Knowledge of phase behaviour of ionic-liquid-butanol-water systems is essential in selection the appropriate

solvent [149,150]. The 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide exhibits Type 2 liquid-liquid equilibrium behavior toward butanol-water system, thus this ionic liquid can easily separate 1-butanol from water [150].

6.5. *In situ* extractive fermentation

End product inhibition can be reduced by *in situ* removal of inhibitory fermentation products as they form. The first experiments were performed by Bekhtereva who studied the effect of BuOH on the ABE fermenting process and on the development of Clostridium acetobutylicum in concentrated mash. Experimental removal of neutral products from the substrate during fermentation was tested by continuous extraction with castor oil. This oil could extract acetone 13-60, EtOH 5-20 and BuOH 50-88% from the wort. By adding the oil to the medium in varying amounts depending on the carbohydrate content, it was possible to ferment corn mash of 3-5 times the usual concentrations. The yield of acetone was 20-37 g L^{-1} of wort, that of all neutral products 60-100 g L^{-1}. Their concentrations in the wort under the oil layer was usually lower than in control vessels, e. g., total products 1.4-2.3%, BuOH 0.4% against 1.2-1.3% in usual fermentation. The extraction was beneficial to the development of the bacteria [151]. Other starch-containing materials could also be fermented in the usual manner, and BuOH and Me_2CO were continuously removed by means of a solvent immiscible with H_2O, e.g. castor oil [152]. The extraction processes were coupled to batch, fed-batch, and continuous BuOH fermentation to affirm the applicability of recovery techniques in the actual process. In batch and fed-batch fermentation, a 3-fold increase in the substrate consumption, in continuous fermentation ~30% increase could be achieved. [142].

Toxicity and selectivity of 13 organic compounds were tested in extractive batch fermentations performed with C. acetobutylicum. Among them, oleyl alcohol and mixed alcohol (the mixture of oleyl alcohol and C_{18} alcohol) were the best for acetone-BuOH fermentation. The orthogonal-cross-test method with 3 elements and 3 levels was used to evaluate effects of fermentation temperature, inital glucose concentration, and solvent/water ratio on extractive batch ABE fermentation. Extractive batch ABE fermentation in a stirred fermentor was studied at different initial glucose concentrations at 41/35° and at solvent/water ratio 1:2. When initial glucose concentration was 110 g L^{-1}, at the end of extractive fermentation the BuOH concentration in the broth and in the solvent was 5.12 and 22.3 g L^{-1}, respectively. The total BuOH and ABE concentrations based on the broth volume were ~16.27 and 33.63 g L^{-1}, the conversion ratio of glucose was 98% and the total ABE yield was 0.312. *In situ* extractive fermentation could eliminate the inhibition of BuOH on microbial growth, increased the initial glucose concentration and reduced the wastewater amount, thus the consumption of energy could be reduced for the separation and purification of the products [153]. Roefler et al [154] studied the effect of six extractants in batch extractive fermentation: kerosene, 30 wt.% tetradecanol in kerosene, 50 wt.% dodecanol in kerosene, oleyl alcohol, 50 wt.% oleyl alcohol in a decane fraction, and 50 wt.% oleyl alcohol in benzyl benzoate. Best results were obtained with oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate. In normal batch fermentation of C.acetobutylicum, glucose consumption is limited to ~80 kg m^{-3} due to accumulation of BuOH in the broth. In extractive fermentation using oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate,

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>100 kg m^{-3} of glucose can be fermented. Maximal volumetric BuOH productivity was increased by ~60% in extractive fermentation compared to batch fermentation. BuOH productivities obtained in extractive fermentation compare favorably with other *in situ* product removal fermentations [154].

A medium for ABE fermentation by C. acetobutylicum was mixed with 0.2-5.0% 1-octanol or 2-ethylhexanol and various parameters of fermentation were studied. Glucose consumption, cell growth, ABE formation, and acetate and butyrate formation were inhibited, especially at higher solvent concentrations. Octanol was more toxic than 2-ethylhexanol [155]. A mathematical model for simultaneous fermentation and extraction of the products was derived for ABE production by immobilized C.acetobutylicum cells in a microporous hollow fiber based tubular fermentor-extractor. The solvent, 2-ethyl-1-hexanol, is used for *in situ* dispersion-free extraction of products. Both predicted and experimental data follow the same trend. The experimentally observed value of total solvent productivity increased by >40% as a result of *in situ* solvent extraction [156]. Unfortunately, good extractants for BuOH, such as decanol, are toxic to C. acetobutylicum. The use of mixed extractants, namely, mixtures of toxic and nontoxic coextractants, was tested to circumvent this toxicity. Decanol appeared to inhibit BuOH formation by C. acetobutylicum when present in a mixed extractant that also contained oleyl alcohol, however, maintenance of the pH at 4.5 alleviated the inhibition of BuOH production and the consumption of butyrate during solventogenesis. A mixed extractant that contained 20% decanol in oleyl alcohol enhanced BuOH formation by 72% under pH-controlled conditions. A mechanism for the effects of decanol on product formation is proposed [157]. The same mixed extractant that contained 20% decanol in oleyl alcohol were combined by Wang et al. to carry out in-situ extractive acetone-butanol fermentation, resulting 19.21 g/L of butanol concentration. Butanol productivity could be 62.8% higher than that of control; meanwhile, total organic solvent productivity increases by 42.3% as compared to the control [145].

BuOH fermentation was carried out by contact with solvent containing C_{10-14} alcohols, as well. A seed culture of C. acetobutylicum IAM19013 was inoculated and mixed with tridecanol. The broth was anaerobically fermented, with stirring, at 37 °C for 60 h. The solvent layer at the top of the fermentor was circulated to the bottom. The concentrations of BuOH in the solvent and vapor were 41.6 g L^{-1} and 66%, respectively [158]. Higher alcohols, e.g. C_{16-18} unsaturated alcohols and C_{16-20} branched alcohols were also tested for continuous extraction of BuOH from the medium during the fermentation period. Extraction of the BuOH from the medium by using unsaturated or branched alcohols innoxious to the microorganism markedly increased BuOH yield. Thus, C. acetobutylicum was anaerobically cultivated at 37°C on a medium containing 10% glucose and, after 30 h, 40% oleyl alcohol was added to the broth to remove the BuOH from the aqueous phase and thereby reactivate the fermentation.This increased the total BuOH concentration to 2.5-fold in an additional 70 h [159]. Oleyl alcohol was found to be one of the best solvents for in-situ extractive ABE fermentation. Its butanol partition coefficents value was varied between 3.0 and 3.7 depending on the composition of the broth, nontoxic, nonmiscible and its boiling point is high as compared to ABE solvents. Batch and fed-batch extractive fermentation by C. acetobutylicum was studied with oleyl alcohol as extractant. Extractive fermentation could reduce the product inhibition, increase the initial glucose

concentration and increase the fermentation rate. A mathematical model was suggested to describe batch fermentation processes. The proposed model could simulate the experimental data fairly well [160].

In situ removal of inhibitory products from C. acetobutylicum resulted in increased reactor productivity; volumetric butanol productivity increased from 0.58 kg m^{-3} h^{-1} in batch fermentation to 1.5 kg m^{-3} h^{-1} in fed-batch extractive fermentation using oleyl alcohol as the extraction solvent. The use of fed-batch operation allowed glucose solutions of up to 500 kg m^{-3} to be fermented, resulting in a 3.5-5-fold decrease in waste water vol. Butanol reached a concentration of 30-35 kg m^{-3} in the oleyl alcohol extractant at the end of fermentation, a concentration that is 2-3 times higher than is possible in regular batch or fed-batch fermentation. Butanol productivity and glucose conversion in fed-batch extractive fermentation was compared with continuous fermentation and *in situ* product removal fermentation [161].

In ABE fermentation using C. acetobutylicum IAM 19012, it was necessary not only to keep BuOH concentration below the toxic level (2 g L^{-1}), but also to control glucose concentration at <80 g L^{-1} and pH between 4.5 and 5.5. The amount of glucose consumed could approximatly be estimated as 4 times the volume of gas evolved, and BuOH was produced from glucose with an average yield of 0.173. It was thus possible to estimate the concentration of glucose and BuOH at any fermentation time using the volume of gas evolved as an indicator. As oleyl alcohol was an excellent extracting solvent for BuOH, a fed-batch culture system for the microorganism was developed, where withdrawing and feeding operations of the solvent were done automatically based on gas evolution [162]. Ohno combined the fermentation by C. beijerinckii ATCC 25752 which perfectly inhibited the process at the BuOH concentration of 12 kg m^{-3} with the extraction with oleyl alcohol and removing the butanol from its mixture with oleyl alcohol which was carried out by prevaporation with hollow fiber membrane. When the BuOH concentration in oleyl alcohol was 22 kg m^{-3}, the BuOH flux was 3.6 ´ 10^{-4} kg m^{-2} h^{-1} at 35 °C [163].

Extraction with the non-toxic immiscible solvent, oleyl alcohol was combined with fermentation performed with immobilized C. acetobutylicum to ferment glucose to ABE solvents in a fluidized-bed bioreactor. The extracting solvent had a distribution coefficient of near 3 for butanol. Nonfermenting system tests indicated that equilibrium between the phases could be reached in one pass through the column. Steady-state results are presented for the fermentation with and without extractive solvent addn. One run, with a continuous aqueous feedstream containing 40 g L^{-1} glucose, was operated for 23 d. Steady state was established with just the aqueous feedstream. About half of the glucose was consumed, and the pH fell from 6.5 to 4.5. Then, during multiple intervals, the flow of organic extractive solvent (oleyl alc.) began into the fermenting columnar reactor. A new apparent steady state was reached in about 4 h. The final aqueous butanol concentration was lowered by more than half. The total butanol production rate increased by 50-90% during the solvent extraction as the organic-to-aqueous ratio increased from 1 to 4, respectively. A maximal volumetric productivity of 1.8 g butanol h^{-1} L^{-1} was observed in this nonoptimized system. The butanol yield apparently improved because of the removal of the inhibition. More substrate is going to the desired product, butanol, and less to maintenance or acid production, resulting in a 10-20% increase in the ratio of butanol relative to all products [164].

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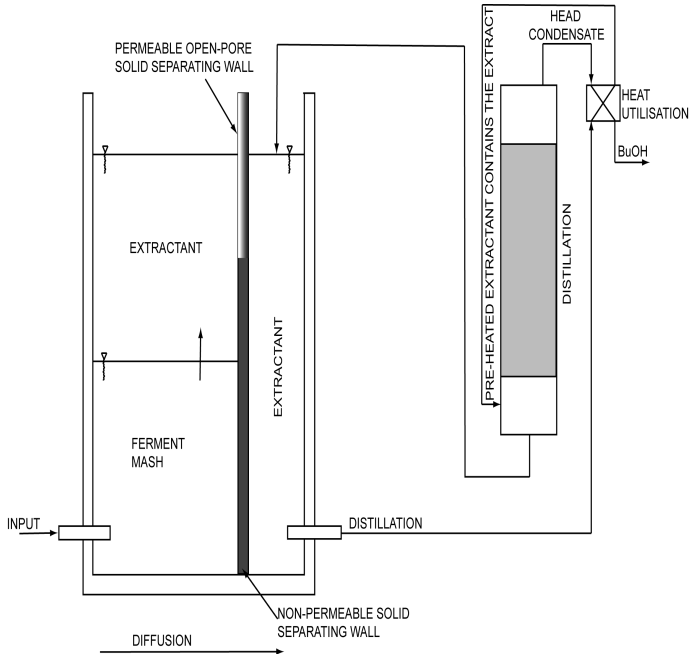

Figure 4. An in-situ extractive fermentor construction [166].

Whole broth containing viable cells of C. acetobutylicum was cycled to a Karr reciprocating plate extraction column in which acetone and butanol were extracted into oleyl alcohol flowing counter-currently through the column. A concentrated solution containing 300 g L^{-1} glucose was fermented at an overall butanol productivity of 1.0 g L^{-1} h^{-1}, 70% higher than productivity of normal batch fermentations. The continuous extraction process provides flexible operation and lends itself to process scale-up [165].

A new type of bioreactor containing a porous permeable wall to recover the biobutanol produced in anaerobic ABE fermentation processes was developed [166, 176]. The ferment liquor is contacted with a non-toxic organic solvent as oleyl alcohol and the butanol in the fermentation liquor distributes between the organic phase and the ferment liquor. The butanol containing solvent located at one side of the permeable wall is in diffusion equilibrium with a same kind of auxiliary solvent with lower butanol concentration located at the other side of the permeable wall. Due to concentration difference, butanol diffuses from one side of the wall to the other side. The concentration difference is kept to be constant by continuous removal of the butanol form the auxiliary solvent phase in which the butanol concentration is always lower than in the extractant phase but much higher than the butanol concentration in the ferment liquor phase. In this way, the primary extractant solvent contacting the ferment liquor is only a transmitting media between the ferment liquor and a small volume of the auxiliary solvent separated with the permeable wall. Energy demand of the distillation to remove the butanol from the auxiliary sol-

vent is less than energy demand of the direct butanol recovery from the ferment liquor or from the extractant phase [166]. The porous composite membranes used as permeable walls for ABE production can be prepared by the method of Tamics et al. [167].

Not only simple alcohols but polyols can also be used in extractive fermentation systems for ABE production. Mattiasson et al. [168] produced acetone and BuOH by C. acetobutylicum in an aqueous two-phase system using 25 % polyethylene glycol 8000. Bacteria remained in the lower phase, and the partition coefficients of acetone and BuOH favoring the upper phase were 2.0 and 1.9, resp. Mean productivity was estimated at 0.24 g BuOH L^{-1} h^{-1}, producing 13 g BuOH L^{-1} in 50 h. Poly(propylene)glycol 1200 is the highest partition coefficient reported to date for a biocompatible ABE extracting solvents. Extractive fermentations using concentrated feeds produced ~58.6 g L^{-1} acetone and BuOH in 202 h, the equivalent of 3 control fermentations in a single run. Product yields (based on total solvent products and glucose consumed) of 0.234-0.311 g g^{-1} and within-run solvent productivities of 0.174-0.290 g L^{-1} h^{-1} were consistent with conventional fermentation reported in the literature. The extended duration of fermentation resulted in an overall improvement in productivity by reducing the fraction of between-run down-time for fermentor cleaning and sterilization [141].

Two aqueous two-phased systems involving polyol-type extractants were investigated to determine their ability to reduce product inhibition in the acetone-BuOH-EtOH fermentation. An industrial-grade dextran (DEX) and a hydroxylpropyl starch polymer (Aquaphase PPT (APPT)) were tested as a copolymer with polyethylene glycol (PEG) to form a two-phased fermentation broth. Two-phase fermentation performances in the DEX-PEG and APPT-PEG 2-phase systems were compared to a single-phase conventional fermentation through a series of batch runs. Effects of the phase-forming polymers on C. acetobutylicum also were investigated. With a BuOH partition coefficient of 1.3, the BuOH yield with the two-phase system was increased by 27% over conventional fermentation [169].

Dibutyl phthalate is one of the ester-type extractants used in extractive fermentation of glucose, glucose-xylose mixtures and hydrolyzates of lignocellulosics to acetone-butanol solvents. Dibutyl phthalate has satisfactory physical properties, nontoxic and mildly stimulates the growth of the organism used, C. acetobutylicum P262. Sugar concentrations mainly in the range of 80-100 g L^{-1} resulted in solvent concentrations of 28-30 g L^{-1} in 24 h extractive fermentation compared to 18-20 g L^{-1} for nonextractive control fermentation. Conversion factors of 0.33-0.37 g solvents g^{-1} sugar consumed were obtained. Rapid fermentation was achieved by high cell concentrations and cell recycling from every 24 h fermentation to succeeding similar 24 h fermentation. Somewhat higher nutrients were also helpful. By this means, 255 L of acetone-butanol solvents were obtained per ton of aspen wood, 298 L per ton of pine, and 283 L per ton of corn stover. Such high product yields from inexpensive substrates offer the prospect of economic viability for the process [170].

Induction of flocculation of Clostridia led to a reduction of the specific solvent production rate. Cells adhering to sintered glass are better than flocculating cells for continuous BuOH-acetone fermentation. Due to low toxicity, in-situ application of paraffin, oleic alcohol or stearic acid butyl ester with the cells in the fermenter is possible. Solvent production by Clostridia can be considerably enhanced by the extractive process. Extraction may be directly integrated into a

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continuous fermentation. Separation of BuOH from oleic acid is easy due to the high boiling point of the extractant (260 °C) being far above the boiling point of BuOH (117 °C). Thus, BuOH can be obtained by normal distillation and the extractant can be recycled [171].

BuOH could be manufactured by cultivating BuOH-producing microorganisms such as C. acetobutylicum in a medium containing a fluorocarbon extractant. The generation time, the mean BuOH production rate, and the mean final BuOH concentration in the C. acetobutylicum culture medium containing Freon-11 (1 g L^{-1}) were increased by 29, 19, and 12%, respectively. Production of acetone and EtOH was not affected [172]. Continuous fermentation of a carbohydrate substrate with continuous extraction of the product by $CFCl_3$ took place in a cylindrical fermentor, with an inlet at the center and a filter membrane concentric with the outer wall, allowing the medium to diffuse outward and to retain microorganisms. The collected medium is pumped to an extractor, where it contacts $CFCl_3$ or another material with a high solvency for BuOH and a low solvency for H_2O and then separated into two phases. The extracted medium is recycled to a feed tank. The solvent is removed from BuOH in an evaporator, where BuOH is collected and the solvent pumped to a compressor and re-utilized [173].

Organic solvents having relatively high distribution coefficients for BuOH against water, often higher alcohols, esters, and organic acids, are very toxic to the microorganisms for BuOH fermentation. Most fermentation inhibition caused by solvent toxicity was eliminated by re-extracting the primary extractant solvent from the residual phase, to be recycled from the product extraction column to the fermentor by paraffin as an extractive fermentation process applied externally to product extraction. After selecting 2-octanol as the extractant from the standpoint of energy consumption in BuOH recovery, a two-stage-extraction BuOH extractive fermentation process having the possibility of reducing the production cost of BuOH was proposed [174]. Heptanal shows strong toxic effect towards C. Acetobutylicum R1 and T5 strains [175] but it has extremely high distribution coefficent (11.5) for butanol [175,176]. Ex-situ extraction with heptanal and recycling the residual broth into a new fermentation cycle proved to be unsuccesfull because the broth contained approx. two times higher heptanal concentration than the toxic limit. Diluting the recycled broth or extracting it with a secondary non-toxic apolar solvent such as hexane to remove the residual dissolved heptanal, inoculate the recycled broth with fresh bacteria in each cycle showed that 4-5 cycles of fermentation could be obtained without important decreasing in the ABE yields and productivity [175]. A multiple solvent extraction is described by Shi et al [177].

Mathematical formulation was made for the performance evaluation considering two types of solvent-supplying strategies. One is to add multiple solvents simultaneously and the product is removed at one time. Another is to add them one by one consecutively. Computer simulations were made for batch, fed-batch, and repeated fed-batch operation of acetone-BuOH fermentation to show the power of the approach. Significant improvement in terms of productivity and product concentration is expected when two extractants such as oleyl alcohol and benzyl benzoate are used, as compared to using only one solvent [177]. A two-stage-extraction butanol extractive fermentation process was developed and studied using a bench-scale extractive fermentation plant with a butanol production capacity of ~10 g h^{-1}. The production rate equation for extractive fermentation was simply expressed by a previously

reported equation multiplied by an equation for the extraction raffinate recycling effect. A butanol production-cost calculation program for the two-stage-extraction process determined the optimum operational conditions to be when butanol concentration, residual sugar concentration and recycling ratio were 6 kg m^{-3}, 15 kg m^{-3} and 3, respectively. These optimal conditions were achieved in the bench-scale plant when it was operated with total sugar concentration, dilution rate and recycling ratio of 113 kg m^{-3}, 0.158 h^{-1} and 3, respectively [178].

A special kind of in-situ extractive fermentation is the so-called perstraction, where a selective membrane is located between the broth and the extractant phase. Both sides of the membrane contact with each phase and ensures a medium betwen two immiscible phases to exchange butanol content. Due to lack of direct contact betwen two phases, toxicity or other problems can be eliminated and a dispersion-free extraction is possible, leading to an easy operation of the equipment, but the mass transfer in the membrane becomes important. This extraction processes were coupled to batch, fed-batch, and continuous BuOH fermentation to affirm the applicability of the recovery techniques in the actual process. In batch and fed batch fermentation a 3-fold increase in the substrate consumption could be achieved, while in the continuous fermentation it increases by~30% [142]. Jeon and Lee [179] described a fed-batch operation for enhanced separation with a semipermeable silicon membrane which showed high specific permeability to BuOH and acetone. Among various solvents examined, oleyl alcohol and polypropylene glycol were the most suitable as extractants. In fed-batch operation of the membrane-assisted extractive BuOH fermentation system, significant improvements were found in comparison to a straight batch fermn. The total glucose uptake per run was raised to 10 times of the value normally found in batch fermentation. The solvent productivity increased by a factor of 2. The total solvent yield increased by 23% due to reduction of acid production and reuse of cells in the fed-batch operation [179]. A continuously operated membrane bioreactor was connected to a 4-stage mixer-settler cascade and Clostridium acetobutylicum was cultivated in this reactor. BuOH was selectively extracted with butyric acid-saturated decanol from the cell-free cultivation medium, and the BuOH-free medium was refed into the reactor. Due to high boiling point of decanol, recovery of BuOH from the decanol solution is easy. Both partition coefficient and selectivity of BuOH in the cultivation medium-decanol system are sufficiently high for removing it from the medium. Direct contact of cells with the decanol phase causes cell damage. However, decanol is practically insoluble in the fermentation medium, thus the contact of the cell-free medium with the solvent phase does not influence cell growth neither product formation. At a dilution rate of D=0.1 h^{-1}, BuOH productivity was increased by a factor of 4 by removing BuOH from the medium [180].

6.6. Membrane techniques and other methods

Pervaporation is an energy-efficient alternative to distillation for removing volatile organic compounds from water, especially ABE solvents from their dilute solutions in a fermentation broth. Pervaporation is able to enrich acetone, BuOH, and EtOH with respect to water. The selectivity of this process is based mainly on superposition of the thermodinamical liquid-vapor selectivity, the chemical affinity selectivity, and the kinetic diffusional selectivity of the materials used. The liquids to be separated are not stressed in any chemical, thermal, or mechanical

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way. Gudernatsch et al. demonstrated the technical feasibility of the pervaporation process in continuous fermentation runs. Composite hollow fiber membranes with transmembrane fluxes in the range of 2 kg m^{-2} h^{-1} and sufficient selectivity were prepared and characterized [182]. El-Zanati et al. designed a special cell to separate the butanol from butanol/water solutions of different butanol concentrations between 6 and 50 g L^{-1}. The temperature of the mixture feed to the cell was 33 °C while the pressure of permeation side was about ~0 bar. Results revealed that butanol concentration changes non-linearly during the first 3 h, and then proceeds linearly. The percentage of butanol removal increases with increasing feed concentration [183]. A new type of pervaporation apparatus was designed and tested by Vrana et al. to develop an integrated fermentation and product recovery process for ABE fermentation. A cross-flow membrane module able to accommodate flat sheet hydrophobic membranes was used for the experiments. Permeate vapors were collected under vacuum and condensed in a dry ice/ethanol cold trap. The apparatus containing polytetrafluoroethylene membranes was tested using butanol-water and model solutions of ABE products. Parameters such as product concentration, component effect, temperature and permeate side pressure were examined [184].

Various kinds of polymeric, ceramic, and liquid membranes can be used for selective separation of solvent vapors at the temperature of fermentation. Polymeric and ceramic membranes have rather poor solvent selectivity compared to liquid membranes even though they achieve reasonable solvent mass fluxes. Liquid membranes have stability problems due to various losses. Groot et al. used silicon tubing membrane technology in the BuOH/iso-PrOH batch fermentation and the substrate conversion could be increased by simultaneous product recovery [185,186]. Geng and Park carried out fermentation by using a low acid producing C. acetobutylicum B18 and a pervaporation module with 0.17 m^2 of surface area was made of silicone membrane of 240 mm thickness. During batch and fed-batch fermentation, pervaporation at an air flow rate of 8 L min^{-1} removed butanol and acetone efficiently. Butanol concentration was maintained below 4.5 g L^{-1} even though C. acetobutylicum B18 produced butanol steadily. With pervaporation, glucose consumption rate increased as compared to that without pervaporation, and up to 160 g L^{-1} of glucose was consumed during 80 h [187]. Experiments using make-up solutions showed that BuOH and acetone fluxes increased linearly with their concentration in the aqueous phase. Fickian diffusion coefficients were constants for fixed air flow rates and increased at higher sweep air flow rates. During batch and fed-batch fermentation, pervaporation at an air flow rate of 8 L/min removed BuOH and acetone efficiently. BuOH concentration was maintained at <4.5 g/L even though C. acetobutylicum B18 produced BuOH steadily. Cell growth was not inhibited by possible salt accumulation or O_2 diffusion through the silicone tubing. The culture volume was maintained relatively constant during fed-batch operation because of offsetting effects of water and product removal by pervaporation and addition of nutrient supplements [188]. Fadeev et al evaluated poly[1-(trimethylsilyl)-1-propyne] (PTMSP) dense films for n-butanol recovery from ABE fermentation broth. Flux decline of a PTMSP film during pervaporation of 20 g L^{-1} BuOH/water mixture was linear. PTMSP films change their geometry when exposed to alcohol and alcohol/water mixtures and then dried. As a result of the relaxation process, polymer film becomes thicker and denser, effecting membrane performance. PTMSP films that were treated with 70% iso-propanol/water show linear flux decline vs. pervaporation time. Strong lipid

adsorption seems to occur on the membrane surface when fermentation broth is used as a feed causing flux decline within short period of time [189].

Oya and Matsumoto used a hydrophobic polypropylene porous hollow fiber membrane of surface area 0.3 m^2, porosity 45%, and bubble point 12.5 kg/cm^2, under reduced pressure [190]. By Knapp et al, a vinyl-type norbornene polymer with average molar weight ~5000 was found to be useful as pervaporation membranes with separation factor of ~10 for separation of n-butanol and isobutanol [191]. Various membranes like styrene Butadiene Rubber (SBR), ethylene propylene diene rubber (EPDM), plain poly di-Me Siloxane (PDMS) and silicalite filled PDMS were studied for the removal of ABE solvents from binary aqueous mixtures and from a quaternary mixture. It was found that the overall performance of PDMS filled with 15% wt./wt. of silicalite was the best for removal of butanol in binary mixture study. SBR performance was best for the quaternary mixtures studied [192].

Composite membranes containing adsorbents such as silicalite or liquid extractants such as oleyl alcohol or other solvents proved to be effective materials in ABE solvent removal from fermentation broth. Thin-film silicalite-filled silicone composite membranes were fabricated by incorporating ultrafine silicalite-1 particles, 0.1-0.2 mm. It was found that with the increase of silicalite content in the top active layer, selectivity for n-butanol and n-butanol flux increased, while the total flux decreased. When the silicalite-1 content was over 60%, the active layer appeared to have defects, aggregation of silicalite-1 particles, which influenced the separation factor. By controlling the membrane thickness and silicalite-1 content, membranes with total flux of 600-700 g m^{-2} h^{-1} (n-butanol flux of 300 g m^{-2} h^{-1}) and selectivity of 90-100 at 70 °C using 10 g L^{-1} of n-butanol as feed solution were obtained. The effects of operation temperature and feed solution concentration on membrane performances were studied [193]. A membrane with a silicalite-1 (its adsorption capacity for a mixture of acetone, butanol and ethanol were 8-12, 85-90 and <5 mg g^{-1}, respectively, there was no apparent difference in absorption rate of butanol at 36° C and 79°C and desorption of butanol occurred efficiently at 78 °C and 1-3 Torr) to polymer ratio of 1.5:1 (g:g) (306 mm thick) had butanol selectivities of 100-108 and a flux of 89 g m^{-2} h^{-1} at feed butanol concnentrations ranging from 5 to 9 g L^{-1} and a retentive temperature of 78°C. A 170 mm silicone membrane under identical conditions had selectivity and flux of 30 and 84 g m^{-2} h^{-1}, respectively. A thin silicalite membrane offered low selectivity and high flux, while a thick membrane offered high selectivity and low flux. The effect of butanol concentration (0.37-78 g L^{-1}) on flux and selectivity was also studied [194].

Thongsukmak and Sirkar developed a new liquid membrane-based pervaporation technique to achieve high selectivity and avoid contamination of the fermentation broth. Trioctylamine as a liquid membrane was immobilized in the pores of a hydrophobic hollow fiber substrate having a nanoporous coating on the broth side. The coated hollow fibers demonstrated high selectivity and reasonable mass fluxes of solvents in pervaporation. The selectivities of butanol, acetone, and ethanol achieved were 275, 220, and 80, respectively, with 11.0, 5.0, and 1.2 g m^{-2} h^{-1} for the mass fluxes of butanol, acetone and ethanol, respectively, at a temperature of 54 °C for a feed solution containing 1.5 wt.% butanol, 0.8 wt.% acetone, and 0.5 wt.% ethanol. Mass fluxes were increased by as much as five times with similar selectivity of solvents when an ultrathin liquid membrane was used [195]. Other long-chain trialkylamines such as tri-

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laurylamine or tri-decylamine could also be used as liquid membranes [196]. Acetic acid in the feed solution reduced selectivity of the solvents without reducing the solvent fluxes due to coextraction of water which increases the rate of water permeation to the vacuum side. The liquid membrane present throughout the pores of the coated substrate demonstrated excellent stability over many hours of experiment and essentially prevented the loss of liquid membrane to the feed solution and the latter's contamination by the liquid membrane [195].

In order to exclude toxic effect of the released liquid membrane ingredient, an oleyl alcohol based liquid membrane was developed. This liquid membrane was energy efficient and did not affect microorganism growth. Oleyl alcohol liquid membrane was proved to be useful for the separation of BuOH and isobutanol in a fermentation culture with immobilized Clostridium isopropylicum IAM 19239 [197].

An ionic liquid (IL)-polydimethylsiloxane (PDMS) ultrafiltration membrane (pore size 60 nm) guaranteed high stability and selectivity during ABE fermentation carried out at 37 °C. Overall solvent productivity of fermentation together with continuous product removal by pervaporation was 2.34 g L^{-1} h^{-1}. The supported ionic liquid membrane (SILM) was impregnated with 15 wt.% of a novel ionic liquid (tetrapropylammonium tetracyano-borate) and 85 wt. % of polydimethylsiloxane. Pervaporation, accomplished with the optimized SILM, led to stable and efficient removal of the solvents butan-1-ol and acetone out of a C. acetobutylicum culture [198].

Reverse osmosis for recovering water from broth can also be used to concentrate ABE fermentation products. Polyamide membranes exhibited BuOH rejection rates ≤85%. Optimum rejection of BuOH occurred at a pressure of 5.5-6.5 MPa and hydraulic recoveries of 50-70%. The flux range was 0.5-1.8 L m^{-1} [199]. Other membranes exhibited rejection rates as high as 98% and the optimal rejection of BuOH in the ferment liquor occurred at recoveries of 20-45% with flux ranging between 0.05-0.6 L m^{-2} min^{-1} [200].

Dialysis fermentation relieves BuOH toxicity with increased yield of product, and solvent extraction can be applied to the nongrowth side of the fermentor for concentration of the BuOH. C. acetobutylicum ATCC 824 and several other strains were studied for the fermentation of corn, potato, and glucose [201].

The ability of cyclodextrins to form crystalline insoluble complexes with organic components was explored as a selective separation of dilute ABE products from Clostridium fermentation systems. A product or a product mixture at a concentration of 0.150 mM each was treated with α-cyclodextrine or β-cyclodextrine in aqueous solutions or nutrient broth. In the acetone-butanol-ethanol system and in the butanol-isopropanol system, α-cyclodextrine selectively precipitated 48% and 46% butanol after 1 h agitation at 30°. However, β-CD was superior for the butyric acid-acetic acid system because it selectively precipitated 100% butyric acid under the same conditions. Cooling the three-product system with α-CD to 4° for 24 h significantly increased the precipitates but decreased the selectivity for either butanol or butyric acid [202].

Hypercrosslinked microporous ion-exchanger resins proved to be suitable agents to adsorb butanol into solid phase from fermentation broth. This ensures fermenting with a microorganism capable of producing butanol in a suitable fermentation medium and recovering butanol from the fermentation medium [203].

Integration of the abovementioned (Chapter 6) methods ensures new possibilities in the economic ABE solvent recovery. Some representative examples without demand of completeness are discussed here.

Mawasaki et al performed continuous extractive butanol fermentation with the microbe immobilized in gel beads and presented the recovery system of butanol from the solvent by pervaporation with hollow fiber membrane. This system was expected to be advantageous to prevent the fouling of membrane because butanol-oleyl alcohol mixtures obtained from extractive fermentation do not include solid particles [204]. Pervaporation method could also be used for *in situ* alcohol recovery in continuous iso-PrOH-BuOH-EtOH fermentation with immobilized cells. Fermentation was performed in a stirred tank and in a fluidized bed reactor as well. In the integrated process, the substrate consumption could be increased by a factor of 4 if compared to continuous fermentation without pervaporation product recovery. Experiments with a pilot plant plate-and-frame pervaporation module were described for the separation and dehydration of alcohols. This module was also coupled to continuous BuOH fermentation, however, sterilization of the module was troublesome, and it was frequently plugged by microbial cells [205]. ABE solvents were produced in an integrated fermentation-product recovery system using C. acetobutylicum and a silicalite-silicone composite membrane. Cells of C. acetobutylicum were removed from the cell culture using a 500,000 molecular weight cut-off ultrafiltration membrane and returned to the fed-batch fermentor. The ABE solvents were removed from the ultrafiltration permeate using a silicalite-silicone composite pervaporation membrane. The silicalite-silicone composite membrane (306 mm thick) flux was constant during pervaporation of fermentation broth at the same concentration of ABE solvents. Acetone butanol selectivity was also not affected by the fermentation broth, indicating that the membrane was not fouled by the ABE fermentation broth. The silicalite-silicone composite membrane was exposed to fermentation broth for 120 h. Acetic acid and ethanol did not diffuse through the silicalite-silicone composite membrane at low concentrations. The fed-batch reactor was operated for 870 h. Totally 154.97 g L^{-1} solvents was produced at solvent yield of 0.31-0.35 [206].

Application of membrane-assisted extraction to butanol fermentation was investigated as a means of product separation and also as a way of alleviating the problems concerning the end-product inhibition. The coupled reactor-separator system was stable enough to sustain continuous operation lasting several weeks. The data on continuous run reaffirmed most of the advantages found in a previous study on fed-batch system in that the reactor separator system rendered high productivity and yields due primarily to reduced product inhibition. Improvement in productivity was particularly notable, as a fourfold increase over straight batch operation was obtained. In normal continuous operation, spontaneous cell deactivation occurred after 200-400 h of operation despite the removal of inhibitory products. The presence of autolysin was one of the probable causes of cell deactivation. The cell viability, however, was prolonged significantly when the bioreactor was operated under glucose-limited conditions [207].

A calcium alginate-immobilized continuous culture was used in a novel gas-sparged reactor to strip the solvents from the aqueous phase and reduce their toxicity. A dilution rate of 0.07 h^{-1} was found to give maximal solvent productivity at 0.58 g dm^{-3} h^{-1}, although at 0.12 h^{-1} the productivity was slightly lower. In order to increase glucose uptake by the culture, feed glucose

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concentration was increased over time to attempt to acclimatize the culture. This resulted in productivity as high as 0.72 g dm^{-3} h^{-1} although this production rate was unstable [208].

An extractive acetone-BuOH fermentation process was developed by integrating bioproduction, ultrafiltration, and distillation, providing simultaneous retention of biomass, selective removal of inhibitors from permeate and separation and purification of acetone, BuOH, and EtOH. Successive batch fermentations were performed with normal pressure distillation (98°) which permitted prolonging and enhancing (by a factor of 3) solvent production with very few volume exchanges of medium (average dilution rate was 0.002 h^{-1}), and recovering the concentrated solvents online. Different operating conditions were also tested in order to study the presence of extracellular autolytic enzymes as inhibition factors. Extracellular autolytic activity was low most of the time, even without enzyme-inactivating heat treatment in the distillation boiler, and high-temperature distillation was deleterious to the culture medium. Improvements of the process were achieved, first, by managing continuous runs, providing a minimal renewal of the culture medium and, mainly, by decreasing the temperature and pressure of distillation. Solvent productivity reached 2.6 g L^{-1} h^{-1} for a 0.036 h^{-1} average dilution rate, corresponding to a feed concentration of 156 g L^{-1} glucose actually consumed [209].

Continuous extractive bioconversion processes were described for conversion of native starch granules to ABE solvent production using a selective adsorbent. In fermentation of carbohydrates with C. acetobutylicum selective synthetic zeolite or crosslinked divinylbenzene-styrene copolymer sorbents are integrated in the process to adsorb the products from the medium continuously [210]. The conversion of glucose to ABE solvents by C. acetobutylicum employing extractive fermentation by using a combination of membrane technology and solid adsorbents integrated into the fermentation process was studied. The adsorbent used was a nitrated divinylbenzene-styrene copolymer. Its ability to adsorb fermentation broth constituents was as follows: BuOH 82, EtOH 36, Me_2CO 51, butyric acid 99, and AcOH 21 mg/g sorbent. The polymer was then heat treated to release the bound solvents. In a long term experiment using an adsorption column, 400 g glucose was added successively to the column and fermentation allowed to proceed for 320 h. A total amount of 67 g of solvent was recovered by heating 930 g polymer [211]. It was found that the *in situ* adsorption process using polyvinylpyridine as the adsorbent enhanced the fermentation rates and the reactor productivity by C. acetobutylicum. In typical traditional acetone-butanol fermentation process only about 60 g/L of glucose could be used in a batch operation mode and thus, at maximum only 21 g L^{-1} of the total final products concentration could be achieved. In the adsorption-coupled system an initial glucose concentration of 94 g L^{-1} was fermented when a weight ratio of the adsorbent to the fermentation broth of 3/10 was used. An overall product concentration of 29.8 g L^{-1} and a productivity of 0.92 g L^{-1} h^{-1} were achieved in the adsorptive batch fermentation system. Compared with the controlled traditional batch acetone butanol fermentation, the integrated process increased the final product concentration by 54% and the productivity by 130% [212]. Integration of a repeated fed-batch fermentation (C. acetobutylicum) with continuous product removal (poly(vinylpyridine) adsorption) and cell recycling resulted in inhibitory product concentration reduction. Because of the reduced inhibition effect, a higher specific cell growth rate and thus a higher product formation rate were achieved. The cell recycle using membrane

separation increased the total cell mass density and, therefore, enhanced the reactor productivity. The repeated fed-batch operation overcame the drawbacks typically associated with a batch operation such as down times, long lag period, and the limitation on the maximum initial substrate concentration allowed due to the substrate inhibition. Unlike a continuous operation, the repeated fed-batch operation could be maintained for a long time at a relatively higher substrate concentration without sacrificing the substrate loss in the effluent. As a result, the integrated process reached 47.2 g L^{-1} in the equivalent solvent concentration (including acetone, BuOH, and EtOH) and 1.69 g L^{-1} h^{-1} in the fermentor productivity, on average, over a 239.5-h period. Compared with controlled traditional batch acetone-BuOH fermentation, the equivalent solvent concentration and the fermentor productivity were increased by 140% and 320%, respectively [213].

Cells of C. acetobutylicum were immobilized by adsorption onto bonechar and used in a packed bed or fluidized bed reactor for the continuous production of ABE solvents from whey permeate. At dilution rates in of 0.35-1.0 h^{-1}, ABE solvent productivities of 3.0 to 4.0 g L^{-1} h^{-1} were observed, but lactose utilization values were poor. When operated in an integrated system with product removal by liquid-liquid extraction, there was a decrease in productivity, but lactose utilization was increased markedly. Of the three extractants tested, oleyl alcohol proved to be superior to both benzyl benzoate and dibutyl phthalate [214].

Shah and Lee studied simultaneous saccharification and extractive fermentation (SSEF) to produce ABE solvents from aspen tree. In SSEF employing cellulase enzymes and C. acetobutylicum, both glucan and xylan fractions of pretreated aspen are concurrently converted into acetone and butanol. Continuous removal of fermentation products from the bioreactor by extraction allowed long-term fed-batch operation. The use of membrane extraction prevented the problems of phase separation and extractant loss. Increase in substrate feeding as well as reduction of nutrient supply was found to be beneficial in suppressing the acid production, thereby improving the solvent yield. Because of prolonged low growth conditions prevalent in the fed-batch operation, the butanol-to-acetone ratio in the product was significantly higher at 2.6-2.8 compared to the typical value of two [215]. Integrated bioreactor-extractor was also tested in SSEF and production of ABE solvents from pretreated hardwood by C. acetobutylicum and cellulase enzymes. The SSEF system was constructed so that products of fermentation were extracted from the broth through a semipermeable membrane. *In situ* removal of inhibitory products was found to be beneficial in sustaining cell viability, thus allowing fed-batch operation of the bioreactor over a period of several weeks. Hardwood chips were pretreated by monoethanolamine in such a way that hemicellulose and cellulose were retained in high yield. The feed material thus prepd. was readily converted by SSEF. The ability of C. acetobutylicum to ferment both glucose and xylose was a major factor in simplifying the overall process into a single-stage operation [216].

7. Perspectives of butanol as biofuel

Biobutanol has excellent fuel properties compared to ethanol, thus it can be used directly as fuel or blending component for both diesel and gasoline powered internal combustion engines

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[217-221]. Butanol has no corrosive properties and its miscibility with gasoline and water tolerance is higher than the appropriate properties of ethanol or methanol [222]. Butanol can also be used as hydrogen source for fuel cells [223] and proved to be useful as esterification alcohol in fatty acid ester type biodiesel production [229-233] or as raw material in the production of dibutyl ether [236] butoxylated butyl diesels [237] or can be converted into aromatic hydrocarbons on zeolite catalysts [238-241].

7.1. Butanol as fuel and blending component in fuel mixtures

Although ethanol as a gasoline extender has received a great deal of attention, this fluid has numerous problems, such as aggressive behaviour toward engine components and a relatively low energy content, the properties of butanol or butanol containing gasoline, diesel and biodiesel fuel compositions are more advantageous than the analogous properties of ethanol or ethanol containing fuels [222]. The performances of gasoline and diesel engines powered with gasoline contained 0-20% BuOH and diesel fuel contained 0-50% BuOH were evaluated. Tests showed that BuOH can be used as a gasoline or diesel fuel supplement in amounts of ≤20% and ≤40%, respectively, without significantly affecting unmodified engine performance. BuOH slightly decreased the octane rating of a blend of 20% BuOH in gasoline but in diesel fuel ≤40% BuOH had no detectable effect on the ignition of the fuel blend [217]. Diesel engines can be powered with 25-75% of a Bu-alcohol and 25-75% of vegetable oil mixtures which were normally liquids under operating conditions. A fuel mixture composed of 50% corn oil and 50% n-BuOH was used as the fuel for 2 tractors when the engine performance in both tractors and the behaviour of the fuel was entirely satisfactory, the engine running smoothly and evenly without significant smoke or odor, with quick acceleration and smooth idling. The above blend could be mixed in any proportion to no. 2 diesel oil without significant change in engine performance [218]. A diesel precombustion chamber engine powered with 70% BuOH-30% diesel fuel had, at an av. 5.9-bar pressure, an ignition delay of operation which was only 10% more than that when operated with diesel alone. The maximum pressure increase during the operation remained higher in both combustion chambers in operation with 70 vol.% BuOH than in operation with diesel alone. There is high potential of improvement of the exhaust gas quality with BuOH-diesel fuel mixtures, especially with regard to smoke value, particulate emissions, and nitrogen oxides. The engine performance under such conditions is similar to that with diesel fuel alone. The starting problem of the engine powered with diesel-BuOH mixture is avoided by using an electrically heated spark plug which maintains ~1000 ºC in the precombustion chamber. More than 200 h of satisfactory operation was attained in a BuOH-diesel mixture powered engine [219]. Substitute diesel fuel compositions consist of gas oil (b. 167-359 °C) 20-55, a 75:25 (wt.) mixture BuOH-Me_2CO 30-40, fatty acid esters 15-40 wt.%. Thus, substitute diesel fuel composition containing gas oil 20, BuOH-Me_2CO mixture 40, and gas oil and BuOH-Me2CO mixture 40 wt.% had cetane no. 40.6 and resulted in normal tractor operation for 50 h [220].

Coupled biodiesel and ABE production technology proceeds by extraction of the ABE containing broth with biodiesel oils forms a mixture which can directly be applied as fuel for diesel engines [224]. Using soybean-derived biodiesel as the extractant with an aqueous phase volume

ratio of 1:1, butanol recovery ranged from 45 to 51% at initial butanol concentration of 150 and 225 mM, respectively. Using biodiesel-derived glycerol as feedstock for butanol production, the production of a biodiesel/butanol fuel blend could be a fully integrated process within a biodiesel facility [225]. The presence of surfactants had important influence on the amount of extracted butanol with biodiesel oil prepared from waste cooking oil [226]. This extraction was integrated into the fermentation process, when large quantity of gas (H_2 and CO_2), was released and the produced butanol and acetone were brought into extractant phase. Surfactants decreased the tension of gas-liquid interface and made the large bubble break down, therefore, the releasing gas passed through the extractant phase in form of small bubbles. The mass transfer rate of products from the aqueous phase to the extractant phase was enhanced and the balance time was shortened accordingly by addition of surfactants, consequently, the fermentation productivity was improved. Using waste cooking oil derived biodiesel as extractant the butanol concentration in the extractant phase was increased by 21.2% as compared to the control, while the concentration of surfactant (Tween-80) in culture medium was 0.140% (w/v). Under these conditions, gross solvent productivity was increased by 16.5% [226]. When the biodiesel derived from crude palm oil was used as extractant, the fuel properties of the biodiesel-ABE mixture were comparable to that of No.2 diesel, but its cetane no. and the boiling point of the 90% fraction were higher [227]. Biodiesels prepared from some waste oils proved to be somewhat toxic toward C. Acetobutylicum. Under this condition, the butanol concentration in the biodiesel phase also reached a level of 6.44 g L^{-1} [228].

7.2. Butyl–ester type biodiesels

Biodiesel is typically synthesized from triacylglycerides derived from vegetable oils and an alcohol with base catalysis, yielding the fatty acid ester type biodiesel. Wahlen et al. determined conditions that allowed rapid and high yield conversion of oil feedstocks containing significant concentrations of free fatty acids into biodiesel using an acid-catalyzed reaction with longer chain alcohols such as n-butanol at a slight molar excess. Biodiesel yields >98% were achieved in <40 min. Key properties of the resulting butyl-diesel were determined, including cetane number, pour point, and viscosity [229]. The batch and continuous-flow preparation of biodiesel derived from vegetable oil and 1-butanol using a microwave apparatus has been reported. The methodology allows for the reaction to be run under atmospheric conditions and in continuous-flow mode. It can be utilized with new or used vegetable oil with 1-butanol and a 1:6 molar ratio of oil to alcohol. Sulfuric acid or potassium hydroxide can be used as catalyst [230]. High conversion could be reached when the transesterification of triglycerides with 1-butanol was performed under near-critical or supercritical conditions with microwave heating [231].

Biodiesel synthesis by butanolysis of vegetable oils (soybean, sunflower, and rice bran) catalyzed by Lipozyme RM-IM), and the optimization of the enzyme stability over repeated batches has been described. The enzyme showed the highest activity at a 9:1 BuOH:oil molar ratio and in the 30-35 °C temperature range [232]. Transesterification reaction using sunflower oil and butanol catalyzed by immobilized lipases can be carried out without auxiliary solvent. Immobilized porcine pancreatic lipase (PPL) and Candida rugosa lipase (CRL) showed

http://dx.doi.org/10.5772/52379

satisfactory activity in these reactions. Activities of immobilized lipases were highly increased in comparison with free lipases because its activity sites became more effective. Immobilized enzyme could be repeatedly used without difficult method of separation and the decrease in its activity was not largely observed [233].

7.3. Other types of butanol–based biofuels

Preparation of a fuel blending mixture characterized by viscosity breaking and clouding point decreasing abilities was carried out in the reaction of acetone (by-product of biobutanol production) and glycerol (by-product of biodiesel or butyldiesel production) in presence of acidic catalysts such as sulphuric acid, p-toluenesulfonic acid or strongly acidic cation exchangers. A mixture of 2,2-dialkoxy-propanes, 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane and 2,2-dimethyl-5-hydroxy-1,3-dioxane was formed [176]. Similar reaction of an oxidized ABE mixture consist of butyraldehyde, acetaldehyde and acetone was carried out with formation of a mixture contained 2,2-dialkoxy-propanes, 1,1-dialkoxyethanes, 1,1-dialkoxybutanes, and 2,2-dimethyl-, 2-methyl or 2-propyl derivatives of the appropriate 4-hydroxymethyl-1,3-dioxolane and 5-hydroxy-dioxane[176]. In this way, the by-products of the biodiesel or butyl-biodiesel production (glycerol) and the acetone from the biobutanol producing (or the oxidized ABE solvent mixtures) can completely be used as fuel components [176]. Fuel characteristics of a blended (15 %) biofuel prepared from oxidized ABE mixture and glycerol contains methanol can be seen in Table 4.

Parameter	Values measured by EU standardized methods [176, 243]		
	Commercial biodiesel	Experimental biodiesel	Commercial diesel No.2
Fatty acid methyl ester content, wt.%	100.00	85.00	4.00
Density at 15 °C, kg dm^{-3}	0.8879	0.8938	0.8495
Kinematic viscosity, 40 °C, $mm^2 s^{-1}$	2.98	4.23	5.65
Flash point, °C	179	56.5	76
Sulphur content, mg kg^{-1}	12	5.4	36
Conradson number, wt. %	0.08	0.02	0.16
Sulphate ash, wt. %	0.012	0.001	0.002
sClouding point, °C	-3	-15	-12

Table 4. Fuel parameters of a biodiesel oil contains 15 % acetal mixture prepared from mixture of glycerol, etanol, butanol and acetaldehyde, butyraldehyde and acetone [176].

Butanol and butyric acid prepared by optimized batch or fed-batch fermentation of wheat flour hydrolysate with selected strains of Clostridium strains, then butanol was recovered from the fermentation broth by distillation and butyric acid by solvent extraction. Esterification could be performed with a lipase in the solvent of extraction [234]. The butylbutyrate formed has a great value as novel biofuel [235]. D'amore at al. developed a catalytic process for making dibutyl ether as transportation fuel and diesel blending component from aq. butanol solutions

[236]. Butoxylation of the unsaturated fraction of biodiesel offers the potential benefit of reduced cloud point without compromising ignition quality or oxidation stability. Butyl biodiesel derived from canola oil was epoxidized via the *in situ* peroxyacetic acid method then the epoxy butyl biodiesel was butoxylated with n-butanol with sulfuric acid catalyst without use of solvents. Optimal conditions for the butoxylation of epoxy butyl biodiesel were 80 °C, 2% sulfuric acid, and a 40:1 molar ratio of n-butanol over a period of 1 h. Conversion of epoxy butyl biodiesel was 100%, and selectivity for butoxy biodiesel was 87.0%. Butoxy biodiesel is able to prevent an earlier onset of crystallization due to the decrease in unsaturated content, but only at lower concentrations [237]. One-step conversion reactions of the title products (6:3:1 volume BuOH-acetone-EtOH) with and without water to aromatic hydrocarbons over molecular shape-selective zeolite were carried out by Anunziata et al. The presence of water in the feed resulted in increased catalyst life. Deactivation reactions toward aromatic hydrocarbon synthesis with product-H_2O mixtures (50:50, 85:15, 99:1, vol.) shown the influence of secondary alkylation reactions leading to substituted aromatic hydrocarbons whose yields were related to the deactivation time of the catalyst [238]. Costa et al. studied the conversion of n-BuOH/Me_2CO mixtures to C_{1-10} hydrocarbons on ZSM-5-type zeolites with different Si-Al ratios. Best results were obtained with a HZSM-5 zeolite (Si/Al=36:1), using a 30 wt% Na montmorillonite binder. The formation of gaseous olefins and non-aromatic liquid hydrocarbons decreased with increasing reaction temperature or space velocity, whereas the amount of aromatic hydrocarbons and gaseous paraffins increased. The total yield of liquid hydrocarbons increased with pressure, although the aromatic content showed a smooth maximum at 1 atm. The yield of aromatic hydrocarbons decreased with increasing water content in the feed. A hydrocarbon distribution similar to that obtained from the anhydrous mixture can be obtained with water-containing feedstock, but lower space velocities were necessary [239]. Orio et al. described the conversion of low molecular-weight oxygenated compounds as ABE solvent mixture into gasoline components over HZSM-5 zeolites. Reagents were used in non-anhydrous form. Formation of C_{2-4} hydrocarbons decrease and aromatic hydrocarbons increase with increasing temperature, formation of C_{5-8} hydrocarbons increases to a maximum at ~300 °C and then decreases. The yields of aromatics from all reactants were ~60 to ~90%; the yields of C_{2-4} and C_{5-8} hydrocarbons were <30% and <10%, respectively. Highest production of aromatic hydrocarbons was attained with the fermentation products of starch (6:3:1 BuOH-acetone-EtOH) [240]. Butanol produced by the fermentation of starch can be presented as a key compd. to produce diesel and jet fuel. Butanol could be converted into Bu esters or into 1-butene which was catalytically oligomerized in a H_2 atmosphere into a hydrocarbon fuel [241].

8. Conclusion

Biobutanol proved to be a superior fuel substitute and blending component in gasolines or diesel fuels. It can be used as raw material in the preparation of so-called butyl-diesel (long-chain fatty acid butyl esters), in the butoxylation of unsaturated fatty acid esters and in the preparation of dibutoxy-acetals. Butanol can easily be transformed via butyraldehide into 2-butoxy-4-hydroxymethyl-1,3-dioxolane or 2-butoxy-5-hydroxy-1,3-dioxane fuel additives

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with using waste glycerol of biodiesel or butyldiesel production. The new fermentation techniques use renewable lignocellulosic raw materials, and integration with various recovering technologies, membrane techniques, together with new fermentor types and genetically engineered microorganisms make a solid base of a new generation of economic biobutanol production processes.

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