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REVIEW
Bioprocess Engineering

Biotechnological Approaches to Generate Biogenic Solvents and Energy Carriers from Renewable Resources

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Abstract

Background: Current threats connected to the ongoing depletion of fossil resources and elevated levels of greenhouse gases accelerating climate change and global warming provoke a renaissance of biotechnological production of various organic bulk chemicals, which, particularly during the second half of the 20th century, were almost exclusively produced from fossil resources via chemosynthetic processes.

Scope: Besides the manufacture of bioethanol, a product obtained by microbial fermentation, biogenic production of solvents and energy carriers like acetone, isopropanol, 2,3-butanediol, or 1-butanol, hence, processes known since the beginning of the last century, experiences now a substantial revival.

Summary of new synthesis and conclusions reached in the review: The review illustrates how to produce these products by resorting to fossil raw materials instead of petrochemical production processes, and how this can be accomplished by the cultivation of anaerobic organisms, namely facultatively anaerobic yeasts and bacteria (production of ethanol or 2,3-butanediol), and strictly anaerobic Clostridia (1-butanol, acetone, or isopropanol) on renewable resources. Moreover, novel methods for producing biodiesel-like methyl-esters of aerobically produced bacterial polyhydroxyalkanoate biopolyester building blocks combine the synthesis of microbial biopolyesters from wastewater with the progress of innovative renewable energy carriers. The biochemical background, the current state of research and development, and the status of industrialization of these processes are reviewed.

Conclusion: Challenges to make these bioprocesses, based on inexpensive renewable resources, competitive with or even superior to petrochemical production routes in terms of sustainability, scalability, and economic feasibility still exist: however, they can be overcome by the concerted action of various scientific disciplines.

Keywords: acetone; anaerobes, biofuels, 2,3-butandiol, Clostridia, energy carriers, ethanol, fermentation, hydroxyalkanoate methyl esters; inexpensive feedstocks, isopropanol, *Klebsiella*, polyhydroxyalkanoate (PHA), *Saccharomyces cerevisiae*, solvents, yeasts

Introduction

Using various biogenic solvents as biofuels currently is a topical issue, and it is globally considered an appealing approach to combat one of the most pressing problems of our days: rising greenhouse gas emissions caused by the combustion of fossil-fuel-based energy carriers sustaining the climate shift. However, running engines with biogenic fuels does not constitute a novel invention. Already in 1826, the American inventor Samuel Morey designed an internal combustion engine powered by ethanol and turpentine oil to run a boat at seven mph [1]. Both Nikolaus August Otto and Henry Ford showed that pure ethanol of biological origin is a suitable fuel to run the respective engines. In 1912, the engine pioneer Rudolf Diesel tested plant oils as a fuel in his newly developed "Diesel engine" [2]. Considerably later, the application of 1-butanol as a novel biofuel has been reported; it was only in 2005, when David Ramey drove a car across the USA, using 1-butanol instead of "biosprit" (ethanol) as a fuel [3].

Solvents such as 1-butanol, isopropanol, acetone, or 2,3-butanediol (2,3-BD) experienced heavy production from petrochemical resources based on well-established chemical processes, especially during the third quarter of the last century. Although of excellent productivity and yields, these petrochemical processes, similar to other industrial

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processes like plastic manufacturing, are characterized by depletion of fossil resources, harsh reaction conditions regarding temperature and pressure, and the need for expensive and toxic catalysts. Similar to the combustion of waste petroplastics in incineration plants, running motors on petrochemistry-derived fuels produces excess CO_2 [4].

Living organisms biosynthesize renewable resources, such as carbohydrates or lignocellulose, and convert them under survival conditions. Thus, these bioprocesses take place at low temperature and low pressure, corresponding to the optimum conditions of the organisms. Biosynthetic processes do not require exogenous catalysts, because biocatalysts are already present in the enzymatic toolbox of the production strains. Finally, the carbon present in bio-based solvents and fuels is embedded into the natural carbon cycle: Combustion of these compounds does not contribute to increased atmospheric CO_2 levels and does therefore not accelerate global warming. This life cycle of biogenic solvents is analogous to the generation, biodegradation, composting, or incineration of biopolymers used for plastic replacement: After use, the materials derived from renewable resources release exactly the same quantity of carbon into the atmosphere as required by green plants or microalgae to photosynthetically replenish biosynthesis of the raw materials needed for their production. This way, the carbon cycle is closed, and the entire life cycle of these materials is circular [5].

Figure 1 provides an illustration of these principal differences between, on the one hand, petrochemistry for production of fuels, solvents, or plastics from fossil resources, and, on the other hand, biotechnological production of biofuels, biogenic solvents, or biopolymers from renewable resources.

Going back in history, it becomes clear that interest in biosynthesis of organic solvents to be used, *inter alia*, as fuels, is directly proportional to current prices for fossil feedstocks used for their chemosynthesis. This exactly matches the trend observed in the decades following WWII, when petrochemical processes were already well established, and fossil feedstocks were available at a low and stable price, which contrasted the rather high costs for renewable feedstocks those days. At that time, biotechnological processes, e.g., for production of 2,3-BD, 1-butanol, acetone, or isopropanol, were more or less forgotten, or at best considered unproductive scientific shenanigans [6].
The situation changed *de facto* from one moment to another, when the Jom-Kippur-War in 1973, followed by the OPEC oil export embargo of 1973–1974, caused the first oil crisis, leading to a sudden increase of the crude oil price, which had remained stable before for many years, by about 70%. This first oil price crisis unmasked the industrialized nations' dependence on fossil energy carriers, especially fossil fuels. After the Islamic Revolution 1978-1979 in Persia/Iran and the following First Gulf War, the second oil crisis started, rocketing the crude oil price from 13 to 39 US-$ per barrel in 1979/80, again unbosoming the vulnerability of industrialized nations to a sudden shortage of crude oil [7]. All of a sudden, the scientific community and industry re-discovered processes independent of the availability of fossil fuels for producing fuels, polymers, and other heavily required products. Not only were old fermentation protocols for these procedures pulled out of the drawer, but intensified worldwide research and development activities contributed to making these processes more efficient, sustainable, and partly even superior to the established petrochemical processes. These activities are ongoing until today, or even are only nowadays picking up speed.

Especially in the last two decades, we witness a recent significant change in the bio-production of these compounds, in contrast to the first-generation biofuels like bioethanol or biodiesel, which were based on edible renewables as biogenic feedstocks (sugars, oils, etc.). The search for low-cost waste streams ("second-generation feedstocks") such as lignocellulose, whey, molasses, and other waste streams for producing second-generation biofuels authenticated to save money on raw materials, help the vast majority of people by reserving food resources, which, in turn, make industrial-scale bioprocesses economically feasible [8], and even matches the topical UN Sustainable Development Goals (SDGs) [9]. Genetic engineering approaches to tailor microbial production strains for enhanced productivity, broader substrate spectra, better production yields, and to close metabolic bottlenecks become more advanced and sophisticated. More recent developments have combined bioproduct formation with CO_2 mitigation, for example, by photoautotrophic accumulation of lipids or starch by microalgae, which can then be converted to biodiesel or bioethanol, respectively. These fuels are known as "third-generation biofuels", while "fourth-generation biofuels" (biohydrogen, biomethanol, and methane) resort to genetically engineered autotrophs with expedient CO_2 scavenging capacity.

Most importantly, such new processes for biotechnological production of marketable biofuels, solvents, biopolymers, etc., based on inexpensive, abundantly and locally available raw materials need to be integrated into "waste biorefinery" concepts, where as many material cycles as possible are closed, and the carbon footprint and greenhouse gas emissions are kept as low as possible. For illustration, the bio-production processes need to be integrated into existing industrial production lines, where the substrates directly accrue as waste streams. Such concepts of upgrading waste to valuable bioproducts match contemporary ideas of the "circular bioeconomy" as a game-changer on the way towards a sustainable, future-oriented global economy [10].

2) BIOETHANOL

2.1) Background

Because of the above-discussed oil price shocks during the 70ies and early 80ies of the last century, considerable global interest existed both in the scientific community and industry to re-explore the fermentative production of alcohols for production of biogenic fuels, stemming from amply available renewable resources. The bioconversion of diverse abundantly available raw materials to ethanol fuel ("biosprit") has been mixed success in different countries. Crude oil prices have rapidly

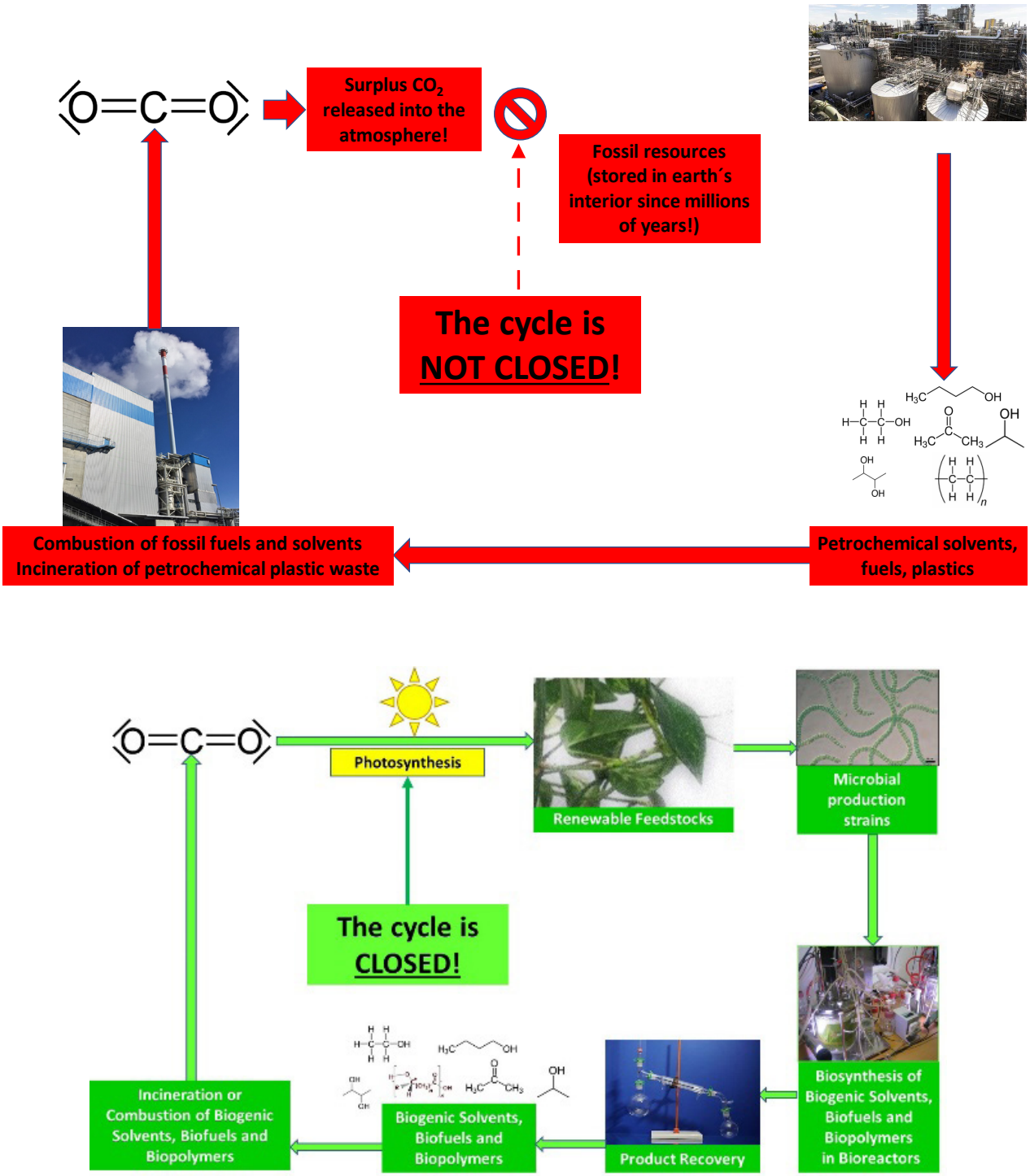


Figure 1. Fundamental differences between petrochemistry for production of fuels, solvents, or plastics from fossil resources (*top*; carbon cycle is not closed), and biotechnological production of biofuels, biogenic solvents, or biopolymers from renewable resources (*bottom*; carbon cycle is closed).

risen, leading to a global shift in fuel production, independent of fossil resources and market prices. As a frontrunner in this aspect, Brazil had introduced its bioethanol production program in 1975, followed by the USA only two years later. Many other countries followed this example during the subsequent years, while in 1982, this situation changed again because of significantly lower crude oil prices. For the umpteenth time, biofuel production became a widely neglected topic. However, in 1990, after Iraq's occupation of Kuwait and the following Second Gulf War in the Middle East, the dependence of global industry to fossil resource supply by changing political situations in involved crude oil-exporting countries was once more demonstrated [7].

2.2) Today´s situation

Bioethanol is currently the most widely used renewable fuel, produced in the United States in 2012 at around 4 x 10^9 gallons and around twice that in the world [11]. Brazil is the second-largest bioethanol producer globally, with half the production capacity than the USA. Brazilian bioethanol production is based on cane sugar [12], while corn starch is the fundamental renewable feedstock for fermentative bioethanol production in other countries, especially in the USA. Almost half of the US-American corn cultivation area is dedicated to corn used for biofuel production. Genetically modified corn harboring a thermostable bacterial α-amylase (EC 3.2.1.1), called Enogen™ corn ("amylase corn") was developed by the company Syngenta, and it gains importance in the USA and other countries [13]. From 2013 to 2016, the agricultural area for farming Enogen™ corn increased from 300 km² to 1,600 km² in the USA. In Canada and Japan, the genetically engineered plants are approved for cultivation, too, and products made of its starch are approved in 15 countries. The heat-stable microbial enzyme (α-amylase) facilitates starch degradation and thus promotes the fermentation process towards ethanol.

According to more recent data, global bioethanol output reached 11 x 10^{10} liters in 2018 and increased by around 30% by 2022 [14]. The current market price of bioethanol is about 2-3 US-$ per gallon (4.546 L). As a car engine fuel, ethanol is added to different fractions of petrol in many countries; while in the USA, an admixture of 10% vol-% is usual ("E10"), 5% bioethanol in petrol ("E5") is found in most EU countries according to the European norm EN 228. Because of its higher resistance to engine knock, ethanol-containing fuels even improve the performance of combustion engines.

For bioethanol production, too, alternatives to above-described first-generation feedstocks of nutritional importance (cane sugar and cornstarch) are researched. The US Department of Agriculture and the Department of Energy have reported that about 1 Gt of biomass of different origin, predominately second-generation feedstocks of agricultural derivation, would be available in the USA per year; this would replace about 30% of the suggested fuel demand of about 8 x 10^{10} gallons. According to current estimates, only about 3% of global bioethanol production comes from second-generation feedstocks because of a variety of challenges related to pre-treatment of these raw materials (mainly hydrolysis to convertible carbon sources), fermentation (selection and engineering of yeast with a broad substrate spectrum), and product recovery. Especially an optimal yeast to be used for efficient bioethanol production from inexpensive second-generation feedstocks with high specific ethanol productivity and inhibitor tolerance is needed. Azhar *et al.* summarized the ability to convert a broad range of sugars stemming from hydrolysis of lignocellulosic raw materials (hexoses and pentoses) [15]. The feasibility of bioethanol production from abundantly available lignocelluloses requires integrated utilization systems [16]. These authors presented a demonstration-scale continuous process for pre-treatment and liquefaction of lignocellulose (wheat straw), using only steam and enzymes. Generated cellulose was saccharified to sugars and simultaneously fermentatively converted to bioethanol, while remaining lignin was used as energy carrier to provide the process energy, and sugars stemming from hemicelluloses, remaining in dried distillers' grains and solubles (DDGS) after the distillation of ethanol, are utilized as fodder. The authors claim that by such integrated processes, the price of second-generation bioethanol approaches the price of established first-generation bioethanol from edible corn starch.

Beyond the use of inexpensive second-generation feedstocks, there is also a lot of research ongoing today to use algal strains that accumulate different carbohydrates like starch, glycogen, or cellulose, which can be used as feedstocks for bioethanol production. Biosynthesis of these carbohydrates occurs via photosynthetic fixation of CO_2 by the microalgae. This production of third-generation bioethanol combines mitigation of CO_2, which can stem, e.g., from industrial flue gas, with the generation of a renewable energy carrier. *Chlamydomonas reinhardtii*, *Porphyra* sp., or *Chlorella vulgaris* are microalgae species with a high capacity for carbohydrate storage [17]. Theoretically, the yield of bioethanol from microalgae is much higher (15,000 gallons per acre) than when using corn starch (430 gallons per acre) [12]. However, currently, industrial-scale production of third-generation bioethanol is not realized yet due to high investment costs, low volumetric productivity of microalgal biomass due to typically low cell densities in the fermentation broth, excessive energy input needed for harvesting, risks of contamination in open microalgal cultivation systems, and challenging downstream processing (reviewed by [14]). Only in the stage of academic research is the use of genetically engineered microalgae with enhanced CO_2-fixation capacity; as mentioned above, bioethanol produced from carbohydrates accumulated by such engineered photosynthetic cell factories is termed fourth-generation biofuel in the scientific literature [18].

2.3) Biosynthesis of ethanol

2.3.1) Bioethanol by eukaryotic facultatively anaerobic yeasts

Biosynthetic production of ethanol from carbohydrates by the most important production strain, namely the facultatively aerobic yeast *Saccharomyces cerevisiae*, starts with glucose (e.g., from hydrolyzed corn starch) breakdown by the glycolysis (Emden-Meyrhof-Parnas, EMP) pathway. Via the intermediate compounds glucose-6-phosphate (reaction catalyzed by hexokinase, EC 2.7.1.1), fructose-6-phosphate (phosphoglucose isomerase; a.k.a. glucose-6-phosphate isomerase or phosphohexose isomerase, EC 5.3.1.9), fructose-1,6-bisphosphate (phosphofructokinase, a.k.a fructose-6-phosphate kinase, EC 2.7.1.11), glycerolaldehyde-3-phosphate (GAP; catalyzed by the lyase fructose bisphosphate aldolase, EC 4.1.2.13), 1,3-bisphosphogluconate (glyceraldehyde phosphate dehydrogenase, EC 1.2.1.12), 3-phosphogluconate (phosphoglycerate kinase, EC 2.7.2.3), 2-phosphogluconate (phosphoglycerate mutase, EC 5.4.2.1), and the energy-rich compound phosphoenolpy-

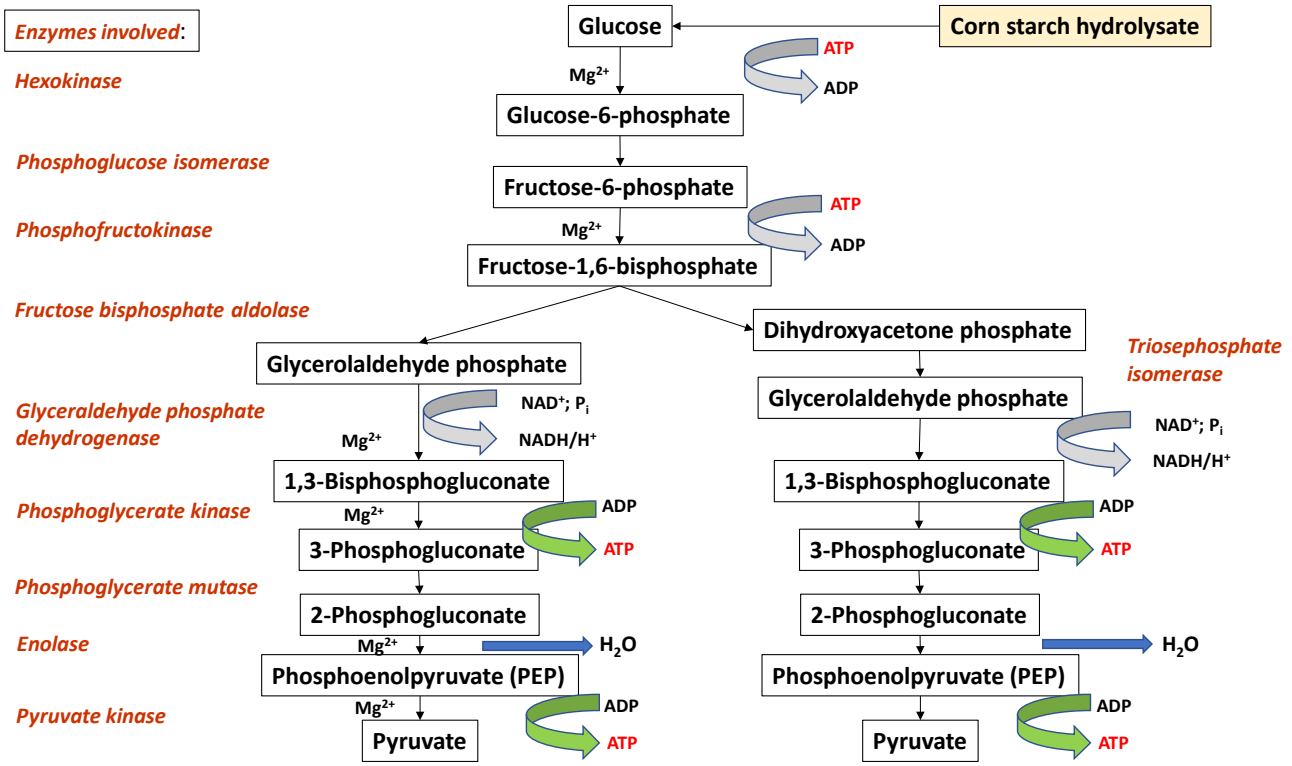


Figure 2. Emden-Meyerhof-Parnas (EMP, glycolysis) pathway from glucose to pyruvate, the starting point for various fermentation products described in this chapter.

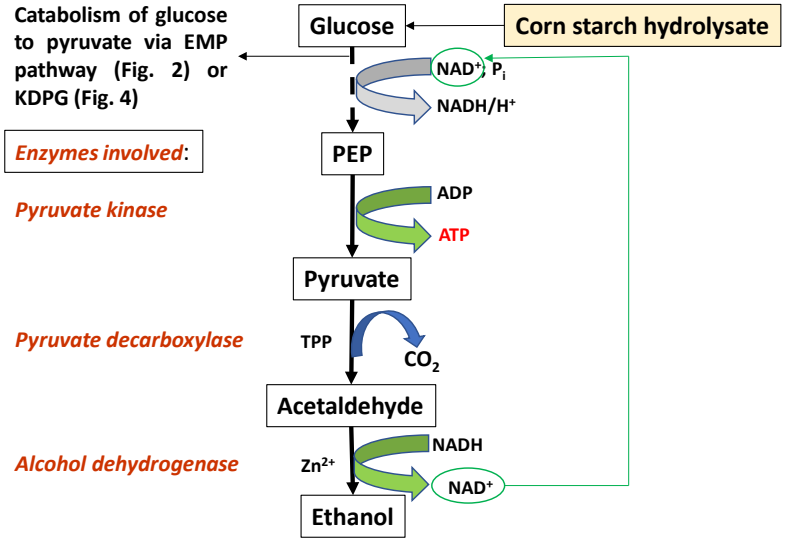


Figure 3. Fermentation pathway from pyruvate to ethanol under anaerobic conditions in *S. cerevisiae*.

catalyzed by the well-known reversible enzyme alcohol dehydrogenase (EC 1.1.1.1); this reaction depends on the presence of bivalent zinc ions polarizing the carbonyl group of acetaldehyde, thus enabling the said transfer of two electrons and one proton from NADH to the carbonyl group; ethanol is finally generated and excreted as an extracellular product, and NAD^+ is regenerated.

This ethanol biosynthesis shows the fundamentals of anaerobic fermentation processes: Under anaerobic conditions, NAD^+ regeneration is needed, too, in order to maintain substrate chain phosphorylation (substrate catabolism), which is needed to generate energy in form of ATP; hence, fermentation basically can be understood as an oxygen-independent strategy to indirectly generate ATP by keeping the substrate chain phosphorylation active, while not directly leading to ATP formation. Hereafter, under anaerobic conditions, the generation of reduced extracellular products like alcohols is a strategy of cells to recycle NAD^+ by oxidizing NADH, which happens via hydrogen transfer to higher oxidized organic intermediates (acetaldehyde in case of ethanol formation). Figure 3 shows a scheme of this fermentative generation of ethanol from pyruvate.

Comparing aerobic yeast growth (biomass formation) with anaerobic fermentation to ethanol, it is well known that the aerobic pathway (via acetyl-CoA, tricarboxylic acid cycle (TCC), and oxidative phosphorylation) by far generates more ATP per mol consumed glucose (38 mol ATP per mol substrate) than anaerobic fermentation (only 2 mol ATP per mol substrate). This, in turn, means that per mol ATP to be yielded, much more substrate needs to be consumed under anaerobic conditions. This phenomenon was already recognized by Luis Pasteur, which caused the common terminus "Pasteur effect". In addition, also under aerobic conditions, yeasts can switch to ethanol fermentation when too high glucose concentrations prevail in the cultivation medium. Such high glucose levels cause substrate inhibition by repressing the expression of genes responsible for the respiration chain [19]; this so called "Crabtree effect" causes ethanol formation even under optimum oxygen supply, and can already be observed at glucose levels as low as 0.15 g/L [20]. Switching to ethanol formation, a process depleting excessive amounts of glucose according to Pasteur, is a strategy of yeast cells to quickly get rid of surplus glucose, which in turn restores the full expression of the respiration genes. Once glucose is completely depleted, such yeasts can to a certain extend re-consume ethanol they excreted before into the cultivation medium for ATP formation; however, this process requires ATP for acetyl-CoA formation, which makes it energetically little favorable [19]. This makes it clear that when yeast biomass propagation under aerobic conditions is intended, substrate feeding needs to be done in a well-dosed way in accordance to the substrate consumption kinetics of the strain in order to avoid unwanted substrate loss towards ethanol formation. In contrast, when ethanol is the desired main product, higher substrate concentrations can be applied under anaerobic conditions.

At that point, it should also be stressed that the terminus "fermentation" *sensu stricto* exclusively refers to anaerobic bioprocesses, as originally postulated at the end of the 19th century by Luis Pasteur ("*la fermentation, c´est la vie sans air*" (fermentation, that´s the life without air)) [21]. Aerobic bioprocesses, e.g., industrial bioproduction of acetic acid, citric acid, penicillin, propagation of yeast, maturation of tea, etc., should rather be termed "cultivations". That time, this was a paradigm shift in the fundamental understanding of life, which was believed before to be not existing under anaerobic conditions. Scientifically, this exploration was the starting shot for detection of other important anaerobic fermentation processes, such as the different lactic acid fermentations, propionic acid fermentation, mixed acid fermentation, biogas production via fermentative methanogenesis, or the production of the solvents and/or biofuels 2,3-BD, acetone, isopropanol, and 1-butanol, the topics of the subsequent chapters.

2.3.2) Bioethanol by facultatively anaerobic bacteria

Besides eukaryotic yeasts like *S. cerevisiae*, also prokaryotic microbes are described as proficient ethanol producers. In this context, the Gram-negative, rod-shaped facultative anaerobic bacterium *Zymomonas mobilis* deserves special attention. This organism is applied for production of pulque by fermentation of hydrolyzable sugars from the stems of *Agave tequilana* Weber var. Azul; distillation of the fermentation product pulque generates the original Mexican tequila [22]. This bacterium is of interest for enhanced bioethanol production due to its convenient accessibility towards genetic manipulation (reviewed by [23]). In contrast to yeasts, *Z. mobilis* does not convert glucose and fructose via the glycolysis (EMP) pathway (Figure 2), but via the 2-keto-3-desoxy-6-phosphoguconate (KDPG) pathway, also termed the Entner-Doudoroff (ED) pathway. Different to the EMP, the ED, a typical prokaryotic pathway, yields only one instead of two mol ATP per mol converted hexose. The initial step, conversion of glucose to glucose-6-phoshate catalyzed by hexokinase, is identical for both pathways. In the KDPG pathway, glucose-6-phosphate is oxidized to 6-phosphogluconolactone (catalyzed by glucose-6-phosphate dehydrogenase, EC 1.1.1.49) under reduction of $NADP^+$, which later on needs to be regenerated. Subsequently, 6-phosphogluconolactone is hydrolyzed by the hydrolase 6-phosphoglucolactonase (EC 3.1.1.31) to 6-phosphogluconate, which finally gets converted to KDPG, the central compound of this pathway, by the catalytic action of phosphogluconate dehydratase (EC 4.2.1.12). The C6-compound KDPG, in turn, gets split by the key enzyme KDPG aldolase (EC 4.1.2.14) into the two C3-compounds pyruvate plus glycerolaldehyde-3-phosphate, which, analogously to the EMP pathway, gets also converted to pyruvate. This metabolic path is illustrated in Figure 4.

Again, two mol NAD^+ are depleted per mol glucose/fructose to be catabolized to pyruvate (see Figure 4), which, under anaerobic conditions, need to be regenerated via a fermentation reaction. This, in analogy to ethanol generation by yeasts, occurs via decarboxylation of pyruvate to acetaldehyde, which

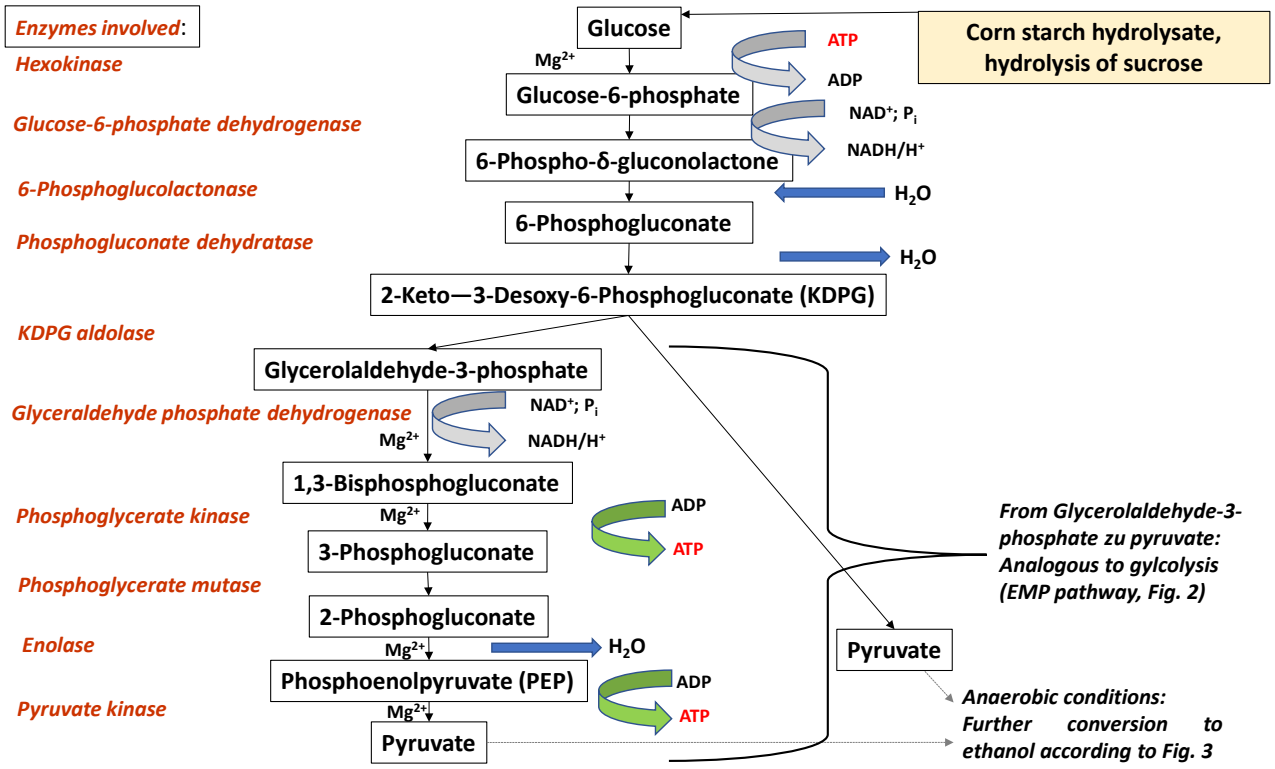


Figure 4. 2-Keto-3-desoxy-6-phosphogluconate (KDPG, a.k.a. Entner-Doudoroff (ED)) pathway for glucose catabolism to pyruvate by the bacterium *Zymomonas mobilis*.

gets reduced by acceptance of two electrons and one proton from NADH by the aldehyde group. Finally, 2 mol ethanol plus two mol CO_2 are generated per mol catabolized glucose, which results in the EMP (yeasts) and ED (*Z. mobilis*) pathway having the same theoretical sugar-to-ethanol yield. *In praxi*, in both cases, about 96% of sugar undergo this conversion to ethanol plus CO_2, with similar final ethanol concentrations of about 12%. Advantages of using *Z. mobilis* for ethanol biosynthesis are a significantly higher specific productivity (0.67 g/(g·h) for *S. cerevisiae vs.* 5.67 g/(g·h) for *Z. mobilis*) and volumetric productivity (29 g/(L·h) for *S. cerevisiae vs.* 200 g/(L·h) for *Z. mobilis*). Moreover, sucrose, especially when present in inexpensive molasses, constitutes an excellent substrate for *Z. mobilis*-based bioethanol production.

Current efforts to make the *Z. mobilis* process for biosprit production competitive on industrial scale focus on closing metabolic bottle necks associated to sensitivity against osmotic pressure, product concentration, and elevated temperature [23]. Moreover, efforts are dedicated to open the substrate spectrum of *Z. mobilis* to other inexpensive carbohydrates like dairy whey lactose [24], sugar cane molasses [25], soybean molasses [26], cellulose [27], starch [28], or pentoses [29]; these processes often resort to tools of genetic engineering.

2.3.3) Bioethanol by strict anaerobic Clostridia

An emerging topic for bioethanol production is the use of acetogenic Clostridia. These autotrophic organisms are able to utilize syngas, a mixture consisting of CO_2, CO and H_2, for generation of the fermentation products ethanol, acetic acid, and 2,3-BD. Syngas can be produced by pyrolysis of almost all organic waste materials, such as inexpensive and abundant lignocellulose. The metabolic branch enabling this process is the so-called Wood-Ljungdahl pathway (a.k.a. reductive acetyl-CoA pathway), which serves microbes for energy generation and autotrophic carbon assimilation. In this branch, CO_2 is reduced to formic acid, which, under consumption of ATP, generates a methyl molecule bound on the corrinoid-iron-sulfur protein. In this process, H_2 serves as hydrogen donor, CO_2 as electron acceptor and biomass building block. The reaction between CO_2 (converted to a methyl group) and CO (generated in the carbonyl branch or consumed directly from syngas), catalyzed by the acetyl-CoA synthase complex, generates acetyl-CoA, which is further metabolized to 2,3-BD (regeneration of NAD^+!) and acetic acid, while a part of acetyl-CoA is further reduced to ethanol for additional NAD^+ regeneration [30]. Figure 5 provides a simplified illustration of this process.

The currently most important representative of these acetogenic Clostridia is *Clostridium autoethanogenum*, an organism already implemented in PR China on industrial scale. There, a commercial plant exists for production of about 46 kt ethanol per year from flue gases. Importantly, this Clostridium (in contrast to many precarious members of this group, like, e.g., *Clostridum botulium*, *Clostridium tetanii,* or *Clostridium perfringens* (*welchii*), which are highly hazardous to health) is

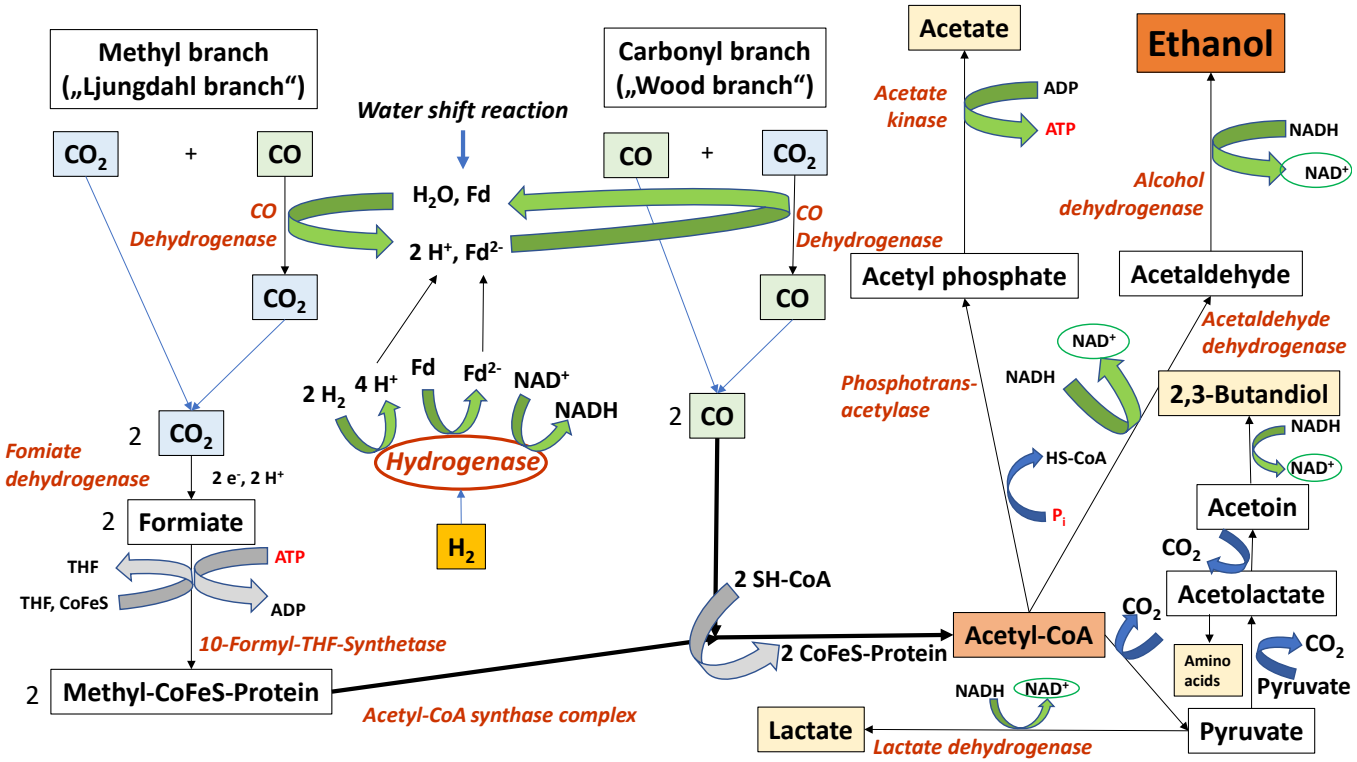


Figure 5. Simplified illustration of the Wood-Ljungdahl pathway (reductive acetyl-CoA pathway) for biosynthesis of ethanol and by-products (2,3-BD, acetate, lactate) by acetogenic Clostridia cultivated on syngas. *Fd:* Ferredoxin; *THF:* Tetrahydrofolate; *HS-CoA:* Coenzyme A; *CoFeS protein*: Corrinoid-iron-sulfur protein

not pathogenic, which allows the utilization of the generated Clostridia biomass as protein-rich fodder (reviewed by [31]). In this process, acetic acid is a rather undesired by-product; therefore, cultivation conditions are currently explored to produce maximum amounts of the energy carrier ethanol and only low amounts of acetic acid. In this context, Abubackar *et al.* reported that after addition of tungsten and at a low pH-value of 4.75, ethanol concentration in batch set ups with *C. autoethanogenum* reached its maximum with 867 mg/L with only 46 mg/L 2,3-BD and no acetic acid generated at all [32]. From the substrate side, it needs to be emphasized that almost all inexpensive and abundantly available organic waste streams can be converted to the feedstock syngas; this is a fundamental advantage of such Clostridia compared to obligate heterotrophic yeasts contemporarily implemented in ethanol production, which have a substrate spectrum predominantly restricted to sugars. Other currently researched acetogenic Clostridia following this metabolic pathway are *C. ragsdalei* [33] or *C. ljungdahlii* [34].

3) 2,3-Butandiol

3.1) Chemical production of 2,3-Butandiol

2,3-butanediol (2,3-BD) is a widely used, odorless, hygroscopic C4 platform chemical, which acts as a basic compound for the production of different follow-up solvents, such as the fuel additive and resin- and lacquer-solvent methyl ethyl ketone (MEK; IUPAC: butanone; via dehydration of 2,3-BD [35]), or 1,3-butadiene (a significant feedstock for synthetic rubber production; produced via further dehydration of MEK, or via direct dehydration of 2,3-butandiol with alumina as catalyst [36]). 2,3-BD has an estimated market volume for 2018 of about 74 megatons (Mt) and a price of about 1.6 US-$ per kg [37]. In a pure form, it acts as anti-freezing agent due to its low freezing point of -60°C (reviewed by Li [38]). In addition, dehydrogenation of 2,3-BD generates diacetyl, which is applied as flavoring compound and food preservative [39]. Moreover, its high heating value and capability to increase the octane number of fuels make 2,3-BD a promising drop-in biofuel and fuel additive [40]. It exists in three stereoisomeric forms, namely (*2R,3R*)-2,3-butandiol (*dextro* form), (*2S,3S*)-2,3-butandiol (*levo* form), and its *meso*-form.

Petrochemically, 2,3-BD is produced from crack gas. After removal of butadiene and isobutene from crack gas, the C4 hydrocarbon fraction ("C4 raffinate II") remains, consisting of about 77% butenes (1-butene, *cis*-2-butene, *trans*-2-butene) and 23% of butanes. This C4 fraction is chlorohydrinated by an aqueous chlorine solution, and thus generated halohydrins (chlorohydrins) get cycled with sodium hydroxide; generated mixture contains 55% *trans*-2,3-butene oxide, 30% *cis*-2,3-butene oxide, and 15% 1,2-butene oxide. Hydrolysis of this

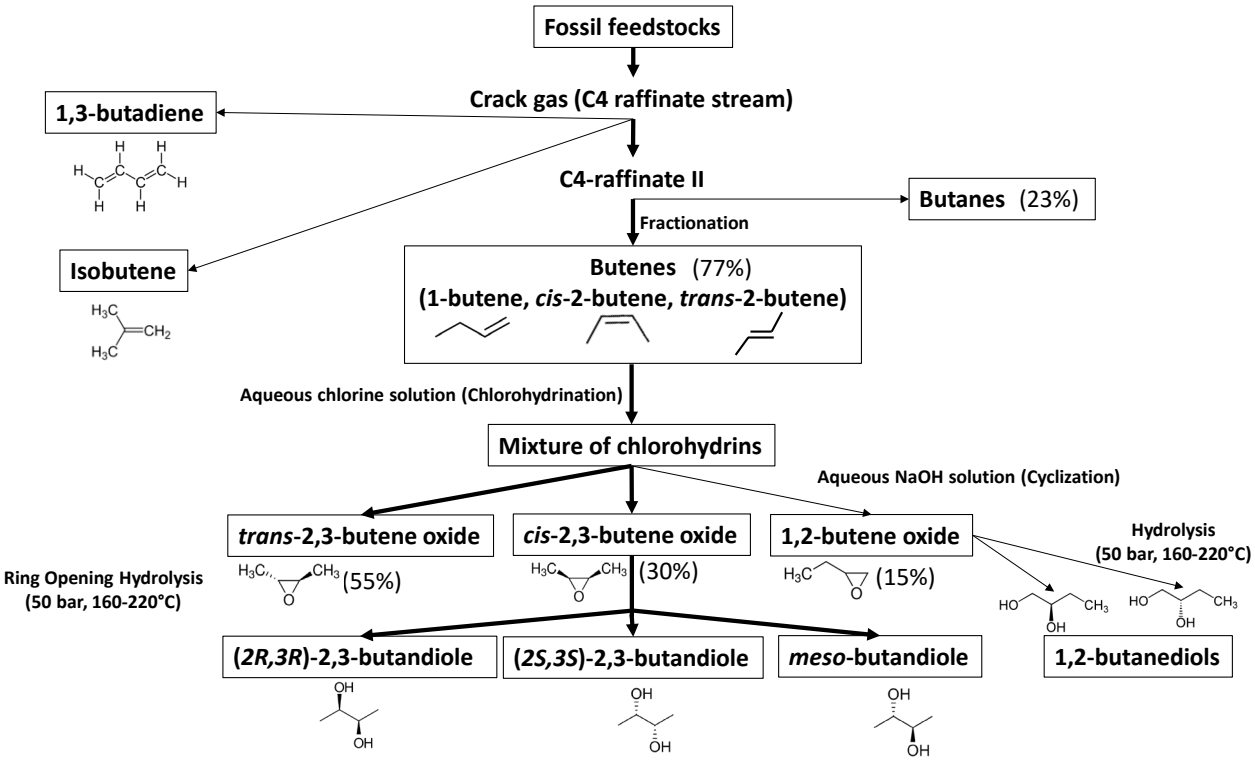


Figure 6. Chemosynthesis of 2,3-butandiol (2,3-BD) starting from fossil resources

mixture at extreme conditions (50 bar and 160-220°C) yields a mixture of 1,2- and 2,3-butanediols, which can be vacuum-fractionated [41]. Figure 6 illustrates this chemosynthetic route starting from fossil feedstocks.

3.2) Biotechnological production of 2,3-Butandiol (2,3-BD)

Principle knowledge about the biotechnological production of 2,3-BD dates back to the early 20th century, when *Klebsiella pneumoniae* (formerly *Klebsiella aerogenes* or *Aerobacter aerogenes*) and other facultative anaerobes, especially enterobacteria and bacilli, were studied by Harden and Walpole [42] and Harden and Norris [43] already in 1906 and 1912, respectively. That time, biosynthetic production of 2,3-BD still outperformed the petrochemical route, since petrochemistry was still in its infancy. Product formation by these organisms occurs via the 2,3-butanediol pathway (Figure 7). Side products are ethanol, CO_2, and formic acid (disintegrates to CO_2 and H_2 in many cases). In 1933, Fulmer first proposed the industrial scale production of 2,3-BD, especially as precursor for 1,3-butadiene, which, as an intermediate in synthetic rubber production, was a strategically important compound in WWII; therefore, biotechnological production of 2,3-BD on pilot scale started that time [44].

However, in the post-war time, this process got widely forgotten due to the triumphant advance of the petrochemical industry those days, which allowed convenient and cost-efficient 1,3-butadiene production from fossil feedstocks. Similar as for other bioproducts, such as microbial polyhydroxyalkanoate (PHA) biopolyesters, which were discovered in the 1920ies, but ignored until the oil crisis and shortage of fossil raw materials [5], or other solvents discussed in the next sections, the 1970ies brought a revival of the biosynthetic 2,3-BD production, first in the USA, and, since the 2010ies, especially in PR China. Already in 2013, Savakis estimated annual growth rates for biotechnological 2,3-BD production of about 3-4% [45]. Currently, a restricted number of companies such as LanzaTech and GS Caltex Corporation already produce bio-based 2,3-BD on an industrial scale ([46], and reviewed by [47]). Different microorganisms employed produce different 2,3-BD stereoisomers to varying degrees, while a mixture of two stereoisomers is typically obtained [48].

Classical biotechnological 2,3-BD production starts, similar to bioethanol production, with acidic hydrolysis of corn starch. Generated glucose is catabolized via glycolysis (EMP pathway, see Figure 2) or, to a lower extend, the KDPG pathway (ED pathway, see Figure 4) to the energy-rich compound phosphoenolpyruvate (PEP), which depletes 2 mol NAD^+ per mole converted glucose. PEP, in turn, is converted to pyruvate, which is the energy-generating step, yielding 1 mol ATP per mol pyruvate as the microbes´ energetic gain of the substrate chain phosphorylation. The further fate of the C3 compound

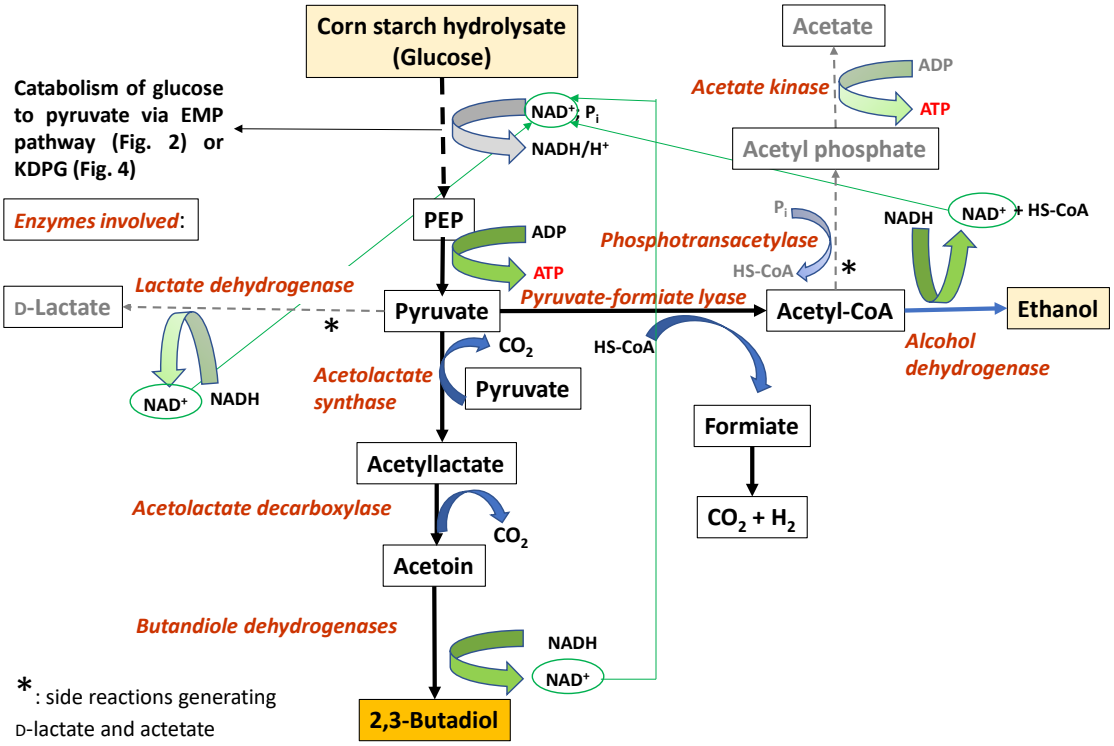


Figure 7. Biosynthetic pathway for 2,3-butandiol (2,3-BD) generation by facultative anaerobes like *Klebsiella* sp.

pyruvate in the 2,3-butanediol pathway is diverted into two directions: A part of pyruvate undergoes condensation towards formation of acetyl lactate (C5 compound; loss of one molecule CO_2 per molecule of acetyl lactate formed). This reaction is catalyzed by a thiamin pyrophosphate (TPP)-dependent acetolactate synthase (EC 4.1.3.18). Acetyl lactate in turn gets decarboxylated by acetyl lactate decarboxylase (EC 4.1.1.5) to the C4 compound acetoin (3-hydroxy-2-butanone), the compound undergoing reduction to the main product 2,3-BD for NAD^+ regeneration (catalyzed by acetoin reductase, EC 1.1.1.4). Another fraction of pyruvate reacts with free acetyl-CoA in a reaction catalyzed by pyruvate formate lyase (formate C-acetyltransferase, EC 2.3.1.54), generating formic acid (partially further yielding CO_2 and the potential renewable energy carrier H_2) and acetyl-CoA, the hydrogen acceptor from NADH, which results in formation of ethanol. To a lower extent, D-lactate (by direct reduction of pyruvate by the oxidoreductase lactate dehydrogenase (EC 1.1.1.27), a step which also regenerates NAD^+) and acetate (via conversion of acetyl-CoA by inorganic phosphate to acetylphosphate – catalyzed by phosphate acetyltransferase (phosphotransacetylase, EC 2.3.1.8) - and subsequent conversion with ADP to generate additional ATP and acetate – catalyzed by acetate kinase (EC 2.7.2.1)) can be found as side products. In contrast to related pathways like the mixed acid fermentation, less acid, but more neutral solvents (2,3-BD and ethanol) are generated. Figure 7 illustrates described biosynthetic pathway for 2,3-BD production.

Roughly, 1 mol of glucose yields 1.72 mol CO_2, 0.7 mol ethanol, 0.66 mol 2,3-BD, 0.36 mol H_2, 0.18 mol formic acid, 0.03 mol D-lactic acid, and 0.01 mol acetate. This means that the theoretical conversion yield for glucose to 2,3-BD and ethanol is about 0.33 and 0.18 g/g, respectively. *In praxi*, 2,3-BD yields from glucose of even more than 0.4 g/g were reported by several authorships for fed-batch feeding processes using different production strains (reviewed by [48]), with product concentrations up to 150 g/L for 2,3-BD, corresponding to a volumetric productivity of 4.2 g/(L·h) for the strain *K. pneumoniae* SDM [49].

As mentioned above, the ratio of stereoisomers of 2,3-BD obtained biosynthetically can vary drastically in dependence on the microbial production strain. While *K. pneumoniae*, *Klebsiella oxytoca*, and *Enterobacter aerogenes* are described as producers of the *dextro-* plus the *meso*-form [40], *Paenibacillus polymyxa* predominantly produces 2,3-BD in its *levo*-form at 98% purity [50]. *Bacillus subtilis*, in turn, yields a mixture of *levo-* and *meso*-2,3-BD. More recently, Li and colleagues achieved production of enantiomerically pure *meso*-2,3-BD from glucose when cultivating a recombinant *Escherichia coli*

(harboring a high-copy plasmid with 2,3-BD biosynthesis genes, but subjected towards deletion of mixed acid fermentation genes in order to avoid formation of major side product (e.g., succinic acid)) under low oxygen concentration in a 6 L bioreactor [38].

In order to make 2,3-BD production economically more competitive, inexpensive heterotrophic feedstocks were tested instead of pure sugars, such as wood residues, glycerol, food waste or diverse lignocellulosic agricultural feedstocks (reviewed by [51]). In this context, it was shown already in 1997 by Cao *et al.* that corn cobs can be pre-treated to remove the lignin and hemicellulose fraction, while remaining cellulose can be simultaneously saccharified by cellulase addition and converted to 2,3-BD by *K. oxytoca*. In this lab-scale process, 80 g corn cob cellulose yielded 25 g 2,3-BD plus 7 g ethanol [52]. More recently, Pasaye-Anaya *et al.* used solid lignocellulosic agave bagasse waste, which, after acid pre-treatment, was enzymatically hydrolyzed to a cocktail containing glucose, xylose, and arabinose as fermentable sugars. About 52.5% sugar release from waste bagasse was obtained. In a batch cultivation setup, this sugar cocktail was converted by a *K. oxytoca* strain to 10.3 g/L of 2,3-BD plus 0.28 g/L ethanol, which corresponds to a 2,3-BD yield of 0.40 g/g. Noteworthy, not only the hexose glucose was readily consumed by the strain, but also the pentose xylose was completely depleted until the end of the process [53]. This procedure is analogous to production of other value-added bioproducts from agave bagasse, as shown, e.g., in 2019 by González-García *et al.* for PHA biopolyester production [54]. In addition, sweet sorghum juice, an inexpensive, sugar-rich raw material, was applied for 2,3-BD biosynthesis by Yuan *et al.*, who used a *Serratia marcescens* strain, another facultative anaerobe. In fed-batch studies carried out in 5 L bioreactors, about 110 g/L 2,3-BD were obtained at a volumetric productivity of 1.40 g/(L·h) and a conversion yield of 0.83 g/g [55].

As a downside, most common and proficient heterotrophic microbes used for 2,3-BD production based on sugar conversion, such as *Klebsiella* sp., are described as pathogens (risk group 2 microorganisms). This gave reason to screen alternative innocuous, non-pathogenic 2,3-BD production strains, such as, e.g., *Bacillus licheniformis*. In repeated batch/fed-batch experiments, this organism showed 2,3-BD productivity and yields from glucose competitive with data reported for established risk group 2 strains from the *Klebsiella* genus [56]. Moreover, attempts were made to remove pathogenicity by genetic engineering tools. Such genetic engineering approaches for enhanced 2,3-BD production involve genetic engineering of diverse microbes, which are no natural 2,3-BD production strains, to make them proficient 2,3-BD producers from inexpensive feedstocks, as shown in 2013 by Savakis *et al.* for recombinant cyanobacteria (*Synechocystis* sp.). These recombinant strains heterologously expressed the catabolic pathway from enterobacteria and lactic acid bacteria, consisting of above-mentioned enzymes acetolactate synthase, an acetolactate decarboxylase and acetoin reductase. Fixation of CO_2 and formation of the needed intermediate product pyruvate occurred via the Calvin Benson cycle constitutively present in these cyanobacteria. This way, it was possible to produce 2,3-BD from CO_2 as sole carbon source with maximum product concentration of 0.4 g/L, besides about 0.3 g/L of remaining acetoin (3-hydroxy-2-butanone; flavor additive in the food and tobacco industry) [45]. In the context of genetic engineering, the bacterium *Vibrio natriegens* heterologously expressed the enzymes needed for pyruvate conversion to 2,3-BD and was cultivated in a microaerophilic fed-batch process supplied with glucose, sucrose and sugar beet molasses. 50 g/L 2,3-BD plus acetoin were obtained at an extraordinary high volumetric productivity of almost 4 g/(L·h). When running the process in continuous chemostat mode, a volumetric productivity of 2 g/(L·h) was obtained [57]. Currently, efforts are dedicated to modify innocuous *E. coli* strains for 2,3-BD production from inexpensive substrates. In this context, Fernández-Gutierrez *et al.* tested 2,3-BD production by rec. *E. coli* on whey lactose as inexpensive substrate, with 2,3-BD yields competitive to natural 2,3-BD producers from lactose. The authors observed high sugar-to-2,3-BD conversion yields (0.38 g/g, which is about 80% of the theoretical values) when testing lactose and glucose, but lower conversion yields (0.19 g/g) from galactose as the second monomer of lactose. These experiments were the first report on 2,3-BD biosynthesis from the whey-derived substrates lactose and galactose [58]. Moreover, metabolic engineering of *E. coli* for 2,3-BD biosynthesis from cellulosic biomass (hydrolyzed empty palm fruit bunches) was described by Sathesh-Prabu *et al.*, who used a glucose-inducible gene expression system. In this study, *E. coli* was genetically modified by deleting pathways leading to side products others than the 2,3-BD precursor pyruvate (i.e., acetate, formate, ethanol, and lactate formation was prevented). Moreover, the recombinant *E. coli* was equipped with a glucose-inducible expression system from *Pseudomonas putida* to convert substrates via the KDPG- instead of the EMP pathway, and the *Klebsiella aerogenes* 2,3-BD biosynthesis genes *budA*, *budB*, and *budC*, encoding acetolactate decarboxylase, acetolactate synthase, and acetoin reductase, respectively. This change in carbon source catabolism (KDPG instead of EMP) was needed because the compound KDPG activates the expression of the 2,3-BD biosynthesis genes, and enables 2,3-BD formation from pyruvate. The thus constructed recombinant strain produced 11 g/L 2,3-BD at a yield of 0.48 g/g from the empty palm fruit bunches hydrolysate, which predominantly contained glucose and xylose as convertible sugars [59].

As an alternative to heterotrophic 2,3-BD biosynthesis, Köpke *et al.* demonstrated 2,3-BD production by strictly anaerobic nonpathogenic acetogenic Clostridia, such as *C. autoethanogenum*, *C. ljungdahlii*, and *C. ragsdalei*, as reported above as by-product of ethanol production by such organisms (Figure 5) [60]. As mentioned, these organisms are able to use CO-containing industrial effluent gases or syngas as the sole carbon and energy source based on the Wood-Ljungdahl pathway (described before in the bioethanol section of this chapter), which enables these microbes to utilize this typically toxic

gas. As described in the ethanol section of present review, this pathway consists of a methyl-branch and a carbonyl-branch, which synergistically interact to form acetyl-CoA. In the methyl-branch, CO or CO_2 is converted in a cascade of tetrahydrofolate (THF)- and cobalamin-dependent reactions into a methyl group (intermediates: CO_2 and formic acid), which in turn combines with CO, forming acetyl-CoA by the carbon monoxide dehydrogenase/acetyl-CoA synthase enzyme complex. This reaction requires reduction equivalents, either provided via molecular hydrogen, or via the so-called water-shift reaction, which describes the conversion of CO plus H_2O to CO_2, H^+ and electrons. Generated acetyl-CoA can undergo carboxylation to pyruvate, which, in analogy to above described 2,3-BD production from sugars, again is further metabolized to acetolactate, the precursor of acetoin and finally 2,3-BD. Again, part of pyruvate is reduced to lactate, while part of acetolactate does not undergo conversion to acetoin, but acts as precursor for synthesis of amino acids (valine, leucine).

4) Organic solvents as clostridial fermentation products - ABE and IBE fermentation

Solventogenic clostridia, a group of strictly anaerobic, rod-shaped, Gram-positive and endospore-forming microbes, can convert numerous carbohydrates like hexoses, pentoses, or their oligo- and polysaccharides to value-added organic solvents and biofuels in rather simple cultivation setups. Most important examples for such solvent producers are *Clostridium acetobutylicum*, *Clostridium beijerinckii* (*C. butylicum*), and *Clostridium saccharoperbutylacetonicum*.

Cultivation patterns (time courses of concentrations of substrates, products, and biomass during the fermentation) of these organisms are highly characteristic: During the exponential growth phase, high quantities of organic acids (butyric acid and acetic acid) are produced as products of the primary metabolism for energy (ATP) generation, resulting in drastic acidification of the cultivation medium ("acidogenic phase" of cultivation). During this acidogenic phase, molecular hydrogen and CO_2 are generated as by-products, thus resulting in characteristic heavy gas formation. As soon as a too low pH-value is reached (typically pH ~4.2-4.5, about 50-70 mM undissoci-

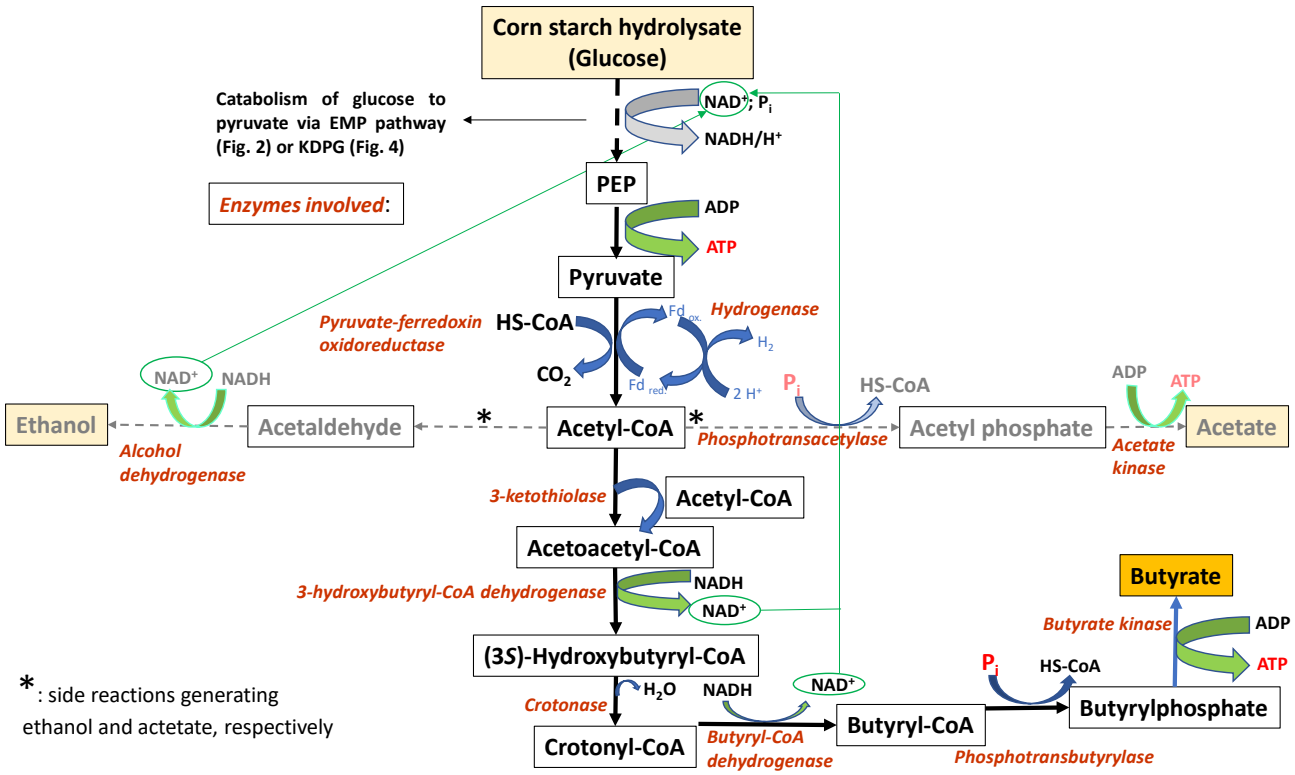


Figure 8. Acidogenic phase of butyric acid fermentation as basis for ABE solvent fermentation

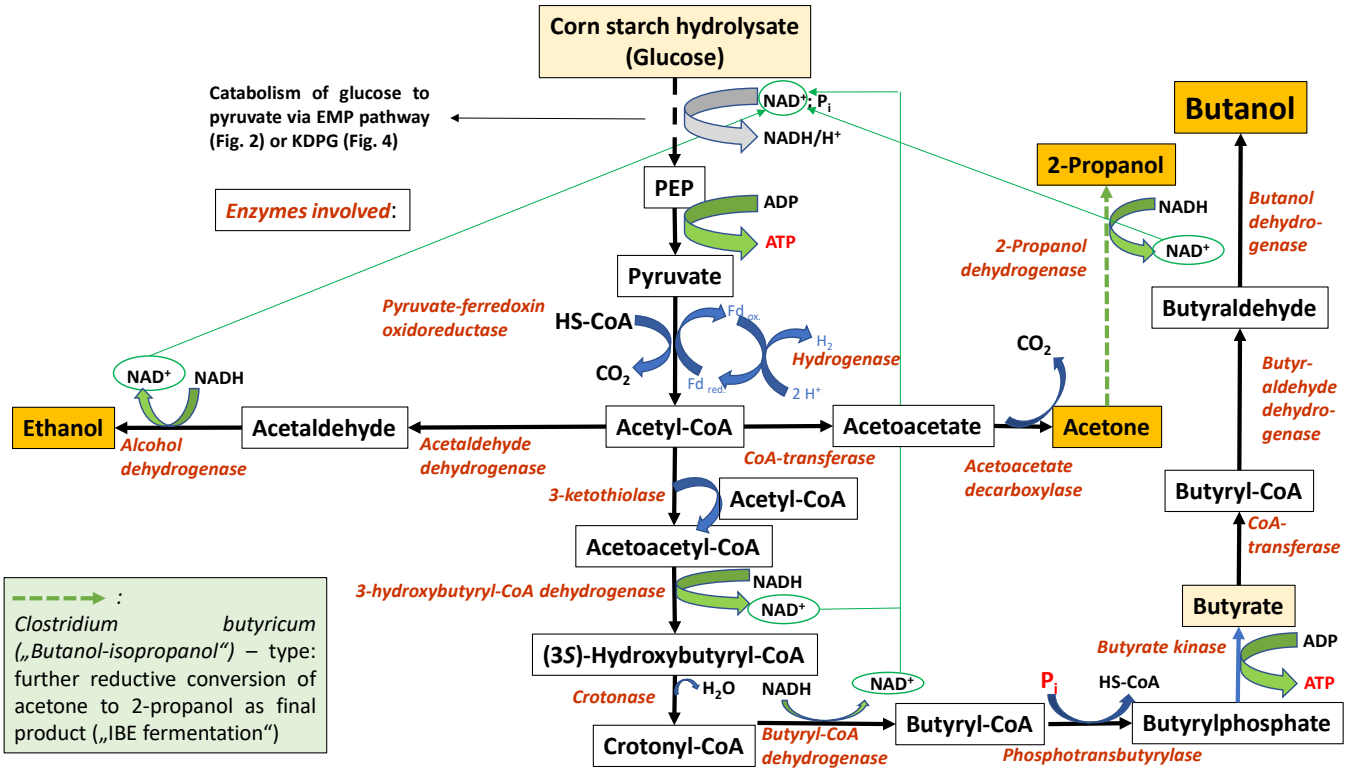


Figure 9. ABE and IBE solventogenesis by Clostridia after onset of the "acid crash".

ated acids formed), the gene expression pattern for solventogenic bacteria like *C. acetobutylicum* suddenly changes; now, excreted acids are re-assimilated by the cells in the so-called "solventogenic phase", yielding the solvents acetone, butanol, and ethanol ("ABE fermentation"). In some *C. beijerinckii* (*C. butylicum*) strains, all generated acetone gets further reduced to isopropanol, an important disinfectant, solvent, and fuel additive ("IBE fermentation") [61].

The time point of switch from acidogenesis to solventogenesis is also known as the "acidic crash"; it typically also marks the onset of sporulation. Hence, the switch from acidogenesis to solventogenesis can be regarded a detoxification reaction, giving solventogenic organisms a survival advantage over pure acidogenic fermentation organisms (e.g., *C. saccharobutylicum, C. pasteurianum, Clostridium tyrobutyricum*). Mechanistically, at lower pH-value (4.5 and less), extracellular acids (acetic and butyric acid) get protonated, which allows them diffusing into the cells; inside the cells, they would deprotonate again, which would severely drop the intracellular pH-value, thus disrupting the fine-tuned proton gradient between the intra- and extracellular space, which in turn causes cell death (reviewed by [3]).
During the phase of biomass growth (acidogenesis), carbohydrates like glucose are catabolized to pyruvate by well-described pathways (Fig. 1), in analogy to the above-described process for bioethanol or 2,3-BD formation. Pyruvate, in turn, is converted to acetyl-CoA by the catalytic action of pyruvate-ferredoxin oxidoreductase; major part of generated acetyl-CoA, in turn, undergoes a condensation reaction to acetoacetyl-CoA (catalyzed by the thiolase acetyl-CoA-acetyl-transferase (EC 2.3.1.9)), while part of acetyl-CoA is phosphorylated to acetyl-phosphate (catalyzed by phosphotransacetylase (EC 2.3.1.8)), which reacts with ADP, finally yielding ATP and acetate (catalyzed by acetate kinase (EC 2.7.2.1)); an additional share of acetyl-CoA gets reduced to ethanol via acetaldehyde. Acetoacetyl-CoA gets reduced by 3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157) to 3-hydroxybutyryl-CoA, which undergoes dehydration to crotonyl-CoA, catalyzed by crotonase enoyl-CoA-hydratase (EC 4.2.1.17). Both reactions regenerate NAD^+, depleted before during glucose catabolism via the KDPG pathway (see Figure 4). Butyryl-CoA in turn gets phosphorylated by phosphotransbutyrylase (EC 2.3.1.19) to butyryl-phosphate, which, catalyzed by butyrate kinase (EC 2.7.2.7), subsequently generates butyrate and ATP. This pathway from the substrate glucose to the product butyric acid is illustrated in Figure 8.

After the onset of the acidic crash, both acetate and butyrate get activated by CoA-transferase, generating acetyl-CoA and butyryl-CoA by coupling with CoA. CoA needed for these reaction stems from acetoacetyl-CoA, which, under solventogenic conditions, undergoes conversion to acetoacetate

(CoA-transferase, EC 2.8.3.8) and finally to the solvent acetone (acetoacetate decarboxylase, EC 4.1.1.4). Hence, acetoacetyl-CoA gets depleted, and can no longer be catabolized towards butyric acid. On the one hand, acetyl-CoA gets reduced to acetaldehyde (catalyzed by acetaldehyde dehydrogenase; EC 1.2.1.10) and finally to the first solvent, ethanol (catalyzed by ethanol dehydrogenase, EC 1.1.1.1). Both steps regenerate one mol NAD^+ per mol reduced substrate. On the other hand, butyryl-CoA gets reduced to butyraldehyde by butyraldehyde dehydrogenase (EC 1.2.1.57), which in turn undergoes reduction to the solvent 1-butanol. Based on the three generated solvents, these Clostridia-driven fermentations are also known as "acetone-butanol-ethanol fermentation" ("ABE fermentation"). Typically, the A:B:E ratio amounts to 3:6:1 [62]. Figure 9 illustrates this pathway.

4.1) 1-Butanol

1-butanol (butan-1-ol according to IUPAC) is a colorless compound of distinct odor and a high boiling point of 118°C. This alcohol, one of the narcotic fusel alcohols produced during schnapps distillation, is a frequently used solvent in chemical and textile industry. Especially for latex surface coating, enamels, and lacquers, it is used in form of the heavily applied solvents butyl acetate, butyl acrylate and methacrylate esters. To a certain extent, it is also utilized as artificial flavor compound in the US, where it is a food additive approved for use in butter, creams, fruits, spirits, candies, ice cream, or bakery products. Moreover, grace to its properties similar to gasoline, it is a potential biofuel and fuel additive (ROZ number 96, hence, in the same range as gasoline with ROZ 91-99); its chemical composition (four carbon atoms to be oxidized during combustion to CO_2 and only one oxygen atom per molecule), low freezing point, higher calorific value (plus 25% compared to bioethanol; 29.2 MJ L^{-1} vs. 19.6 MJ L^{-1}), lower volatility, lower hygroscopicity, and lower corrosivity make it superior to ethanol in this regard. Indeed, 1-butanol is nowadays considered a "top-tier biofuel" [63]. Due to its lower oxygen content, it can be blended with gasoline at a rate of up to 100%, compared to only 85% for bioethanol [64]. In 2007, the annual production of 1-butanol was estimated with about 2.8 Mt, accounting for a market price of about 5 x 10^9 US-$ [65], while its production capacity for 2010 already amounted to 3.6 Mt (reviewed by [66]).

4.1.1) Chemical production of 1-butanol

Chemical production of 1-butanol starts from the fossil product propene (formerly "propylene"), which, homogenously catalyzed by metal (Co, Ru, or Rh) hydride complexes, gets hydroformylated with CO and H_2 by the "oxo-process" to a mixture of n-butanal and iso-butanal (2-methylpropanal). These compounds can further be catalytically hydrogenated to yield the respective butanols (mixture of 1-butanol and 2-butanol). This petrochemical standard process was operated until the 1970ies

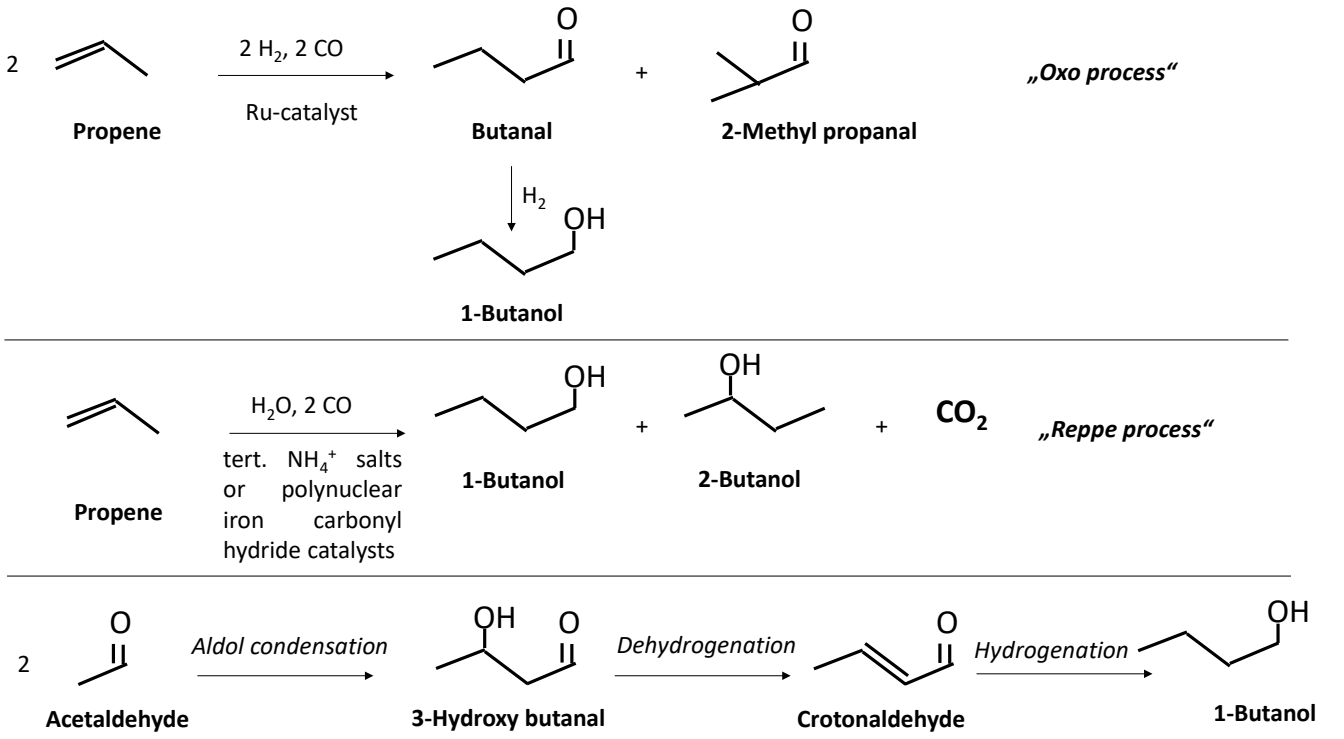


Figure 10. Scheme of different routes for chemical production of 1-butanol (top: "oxo process"; center: "Reppe process"; bottom: route via acetaldehyde and crotonaldehyde)

at high pressure of up to 3×10^7 Pa for the CO/H_2 mixture at 100–180 °C. Nowadays, it is catalyzed by a Rh-catalyst, and operates at a reduced pressure ($1–5 \times 10^6$ Pa) with a 1-butanol yield of about 95% [66]. For a schematic of this process, please refer to the top of Figure 10.

Alternatively, propene can undergo reaction with water and CO via the so called "Reppe process", yielding 2-butanol (ratio 1- to 2-butanol: 86 to 14) and CO_2 as side products (see center of Fig. 10). This process is operated at a reduced pressure of $0.5–2 \times 10^6$ Pa and a temperature of 100 °C, using tertiary ammonium salts or polynuclear iron carbonyl hydrides as catalysts [67].

In former times, 1-butanol was also produced via acetaldehyde (C2 compound), which undergoes aldol condensation towards 3-hydroxybutanal. 3-hydroxybutanal in turn gets dehydrated to the unsaturated compound crotonaldehyde, which finally can get hydrogenated to 1-butanol. This synthesis route from acetaldehyde, which is illustrated at the bottom of Fig. 10, was popular some decades ago, but was later outperformed by the above-described propene-based routes [66]. It might be possible that this route will regain importance due to the fact that crotonaldehyde is obtainable from renewable resources, such as via pyrolysis of microbial poly(3-hydroxybutyrate) biopolyester [68], and also acetaldehyde can easily be produced starting from biogenic materials via oxidation of ethanol. Figure 10 summarizes the discussed chemical production routes towards 1-butanol.

4.1.2) Biotechnological production of 1-butanol

Fermentative 1-butanol production via above-described "ABE-fermentation", in combination with generation of acetone and ethanol in the same fermentation setup, is a biotechnological process that was well-established already in the early 20th century. Indeed, the first commercial plant for biotechnological 1-butanol production started its operation in UK already in 1911 [69]. Biotechnologically, the organism today called *Clostridium acetobutylicum* was used as the first described 1-butanol production strain, which converted carbohydrates mainly to 1-butanol and acetone. However, the first detection of 1-butanol bioproduction even dates back to 1862, when Luis Pasteur described this process when studying "*Vibrion butyrique*", which presumably was a mixed culture containing *Clostridium butyricum* or *C. acetobutylicum* [70]. In 1915, Chaim Weizmann, Israel´s first president from 1949-1952, patented a process for biotechnological production of acetone and alcohols (1-butanol, ethanol) from starch-based materials by a mixed microbial culture (Weizmann´s "strain BY"), which mainly contained the species later known as *C. acetobutylicum*. Importantly, at that time (WWI), it was acetone, not 1-butanol, which was the product of major interest due to its military importance: Acetone is an essential compound to produce the explosive cordite. Cordite, an invention of Sir Frederick Abel, was the most important smokeless propellant applied in ballistic weaponry at the beginning of the 20th century; in cordite production, acetone serves as gelatinizing agent to support incorporation of the ingredients (cellulose trinitrate, nitroglycerol, and a petroleum jelly) (reviewed by [71]). Indeed, during WWI, acetone was the desired product of ABE fermentation, and 1-butanol was considered merely as an undesired by-product reducing the acetone yield in the process. After WWI, the need for explosives and thus for acetone suddenly decreased. However, the interest in this fermentation process increased again when automotive production emerged in the USA, and amyl acetate was needed as component of car lacquers. Yet, amyl acetate production was dependent on the availability of the fusel alcohol amyl alcohol (1-pentanol), a by-product of bioethanol and spirit production. During the prohibition, official ethanol fermentation processes became scarce, which in turn resulted in severe shortage of amyl alcohol. As an alternative, butyryl acetate soon turned out as viable substitute, where the amyl alcohol moiety is replaced by 1-butanol. This alcohol was that time produced biotechnologically by described ABE fermentation [72]. Indeed, in the early 1920ies, biotechnological 1-butanol production became the second most important industrial fermentation process after bioethanol production, and now, acetone was the product of minor interest of the ABE fermentation process. Similar to many other processes (*vide supra*), interest in the biotechnological production mode lost attractiveness after WWII, and almost completely stopped in the 1960ies, when prices for biogenic raw materials increased, and petrochemical production processes became more and more efficient and less expensive [64]. Nowadays, it is clear that 1-butanol by far outperforms acetone as desired main product of ABE fermentation due to its significance as energy carrier, and current efforts are often dedicated to shift the product composition towards high butanol and low acetone shares [73]. Numerous efforts were made to make the 1-butanol fermentation process economically more competitive. When using a traditional production plant and corn starch as raw material, about 79% of 1-butanol production costs goes to substrates, and 14% to energy provision. Based on a techno-economic assessment, Qureshi and Blaschek estimated that, when assuming a corn price of 71 US-$ per ton, 1-butanol production costs amount to 0.55 US-% per kg, 56% thereof being caused by the substrate costs. These considerations were made based on a total ABE yield of 0.42 g/g, an ABE productivity of 0.38 g/(L·h) in batch processes and an annual capacity of 150,000 metric tons, based on three batch-reactors operated sequentially (each day, another reactor is inoculated). As production strain, the butanol-hyperproducer *C. beijerinckii* BA101, an α-amylase-active strain able to directly use corn starch without the need for starch saccharification, was used. Authors calculated that further improvements, such as increased ABE yields of 0.45 g/g and savings in electricity and energy could reduce the production price to 0.44 US-$ per kg 1-butanol [74].

1-butanol production plants already in operation primary resort to sugar cane, sugar beet and sugar molasses as substrates when operated in Brazil (8,000 annual tons plant based on sugar cane juice [62]) or the UK, or to corn starch, when the plant is located in the US (reviewed by [3]). In 2008, Qureshi

and Ezeji reviewed the use of different inexpensive lignocellulosic materials like wheat straw, corn stover, barley straw, and switchgrass as second-generation feedstocks for 1-butanol biosynthesis [75]. In this context, as an inexpensive, abundantly available substrate, lignocellulose-rich DDGS, the remnants of bioethanol distillation, were hydrolyzed to a mixture of hexoses and pentoses and used as feedstock for ABE fermentation by different solventogenic bacteria (*Clostridium beijerinckii* BA101, *C. acetobutylicum* 260, *C. acetobutylicum* 824, *C. saccharobutylicum* 262, and *C. butylicum* 592) by Ezeji and Blaschek (2008). Authors of the study noticed that all sugars generated by hydrolysis were utilized by all tested strains, with glucose being the preferred carbohydrate, and fermentable sugars are needed in excess for the onset and maintenance of solventogenesis. Interestingly, it was shown that presence of inhibitors stemming from lignocellulose hydrolysis, namely furfural and 5-hydroxymethylfurfural, even stimulated growth and solvent formation, while other compounds like syringaldehyde, ferulic and p-coumaric acid severely inhibited ABE production, which makes their removal from the hydrolysis cocktail, e.g., via overliming, necessary. Increasing such tolerance against inhibitors from upstreaming inexpensive raw materials, together with a high product tolerance, are considered the major research directions to be pursued in order to make 1-butanol production more cost efficient in the future [76]. In addition, *in situ* product recovery during the bioprocess to keep the actual solvent concentration low might be a technologically more advanced solution to overcome the problem of product inhibition [62]. In the meantime, different other agro-industrial waste streams were successfully tested for 1-butanol production, such as empty palm fruit bunches by *C. acetobutylicum* [77], cassava bagasse hydrolysate by the butanol-hyperproducing strain *C. acetobutylicum* JB200 [78], corncob hydrolyzate using *C. acetobutylicum* SE-1 [79], organosolv-pretreated rice straw using *C. acetobutylicum* [80], rice straw delignified by alkali treatment using a co-culture of cellulose-degrading *C. thermocellum* and solventogenic *C. saccharoperbutylacetonicum* [81], steam-exploded corn straw using *C. acetobutylicum* [73], or pineapple waste using *C. acetobutylicum* NRRL B 527 [82].

Besides substrate costs, the typical end-product inhibition of best-established ABE producers *C. acetobutylicum* or *C. beijerinckii* at already rather low ABE concentrations of about 20 g/L makes the product recovery via distillation cost-demanding. In most cases, clostridial cell mass is separated from the solvent-rich supernatant by centrifugation, stored at 4°C, and can be commercialized as fodder at a market price similar to yeast biomass from ethanol production. From the supernatant, the solvents are stripped in a stripping distillation column. After solvent stripping, water is removed from the solvent-free waste supernatant by a membrane technique, and recycled for use in subsequent fermentation processes. The mixture of solvents and water undergoes distillation into a mixture of ethanol and acetone plus a mixture of 1-butanol and water; both fractions are separately distilled to yield the pure solvents [74]. Already in 1983, Phillips and Humphrey estimated that a doubling of the ABE concentration could reduce energy requirements for solvent recovery by about 62% [83]. Therefore, efforts were dedicated to make production strains more tolerable towards their fermentative end-products; especially a higher concentration of 1-butanol in the cultivation broth is highly desirable. In this context, Chen *et al.* achieved an increase of total solvent and 1-butanol concentration by 69% when using the mutant strain *C. beijerinckii* BA101, a butanol-hyperproducer, when compared to its wild type strain NCIMB 8052. Increased and stabilized solvent production was further favored by addition of small amounts of sodium acetate. In a batch process, 32.6 g/L total solvents and 20.9 g/L 1-butanol were obtained [84].

Similar to enhancement of other bioprocesses, metabolic engineering is also a tool currently studied to boost 1-butanol formation. Either, the genes encoding enzymes required for 1-butanol biosynthesis can be transferred into a desired host production strain displaying other beneficial properties, such as faster growth or a broader substrate scope than established ABE producing Clostridia. Here, the transformed strain needs to reveal a certain solvent tolerance in order to avoid product inhibition already at quite low solvent levels. Moreover, the high oxygen sensitivity of ABE synthesis enzymes needs to be considered when using aerobic host strains like, e.g., *E. coli*. Alternatively, ABE producers like *C. acetobutylicum* can undergo transformation in such a way that they form 1-butanol as sole solvent, with only CO_2 and H_2 generated as by-products. It was successfully demonstrated that knocking out the acetoacetate decarboxylase gene in *C. acetobutylicum*, which is needed for acetone formation, results in a 1-butanol share of total solvents of 0.8, compared to 0.6 as shown for the wild type [85]. A different strategy involves transformation of Clostridia by inserting monooxygenase genes, which makes them able to produce 1-butanol from the hydrocarbon n-butane (reviewed by [3]).

Indeed, nowadays biotechnological 1-biobutanol production is considered economically at least competitive with routes of petrochemistry. A technoeconomic assessment by Pfromm *et al.* compared the efficiency of bioethanol production from yeasts with 1-butanol fermentation by *C. acetobutylicum* when starting from the same raw materials, namely corn and switchgrass. In the case of ethanol production, enzymes were needed to hydrolyze the electrically milled corn starch, while *C. acetobutylicum* directly utilized starch. The fermentation with yeasts did not require sterility precautions, while sterilization was needed for the strictly anaerobic Clostridia cultivation. For both processes, batch cultivation was considered. After the cultivations, 15 wt.-% ethanol (process with yeasts), but only 2 wt.-% 1-butanol (process with Clostridia) were obtained in fermentation broth. In the latter case, the by-product acetone was considered to be removed by distillation, with a mixture of 1-butanol and ethanol being the final product. Considering a quantity of 1.373 kt of corn starch (yellow dent corn) in both cases to be converted per day and 360 days per year of plant operation, ethanol generated by yeast fermentation was calculated to amount to 18.1 Megamol (Mmol; 10^6 mol) carbon daily, while the energy carrier mixture of 1-butanol and ethanol ob-

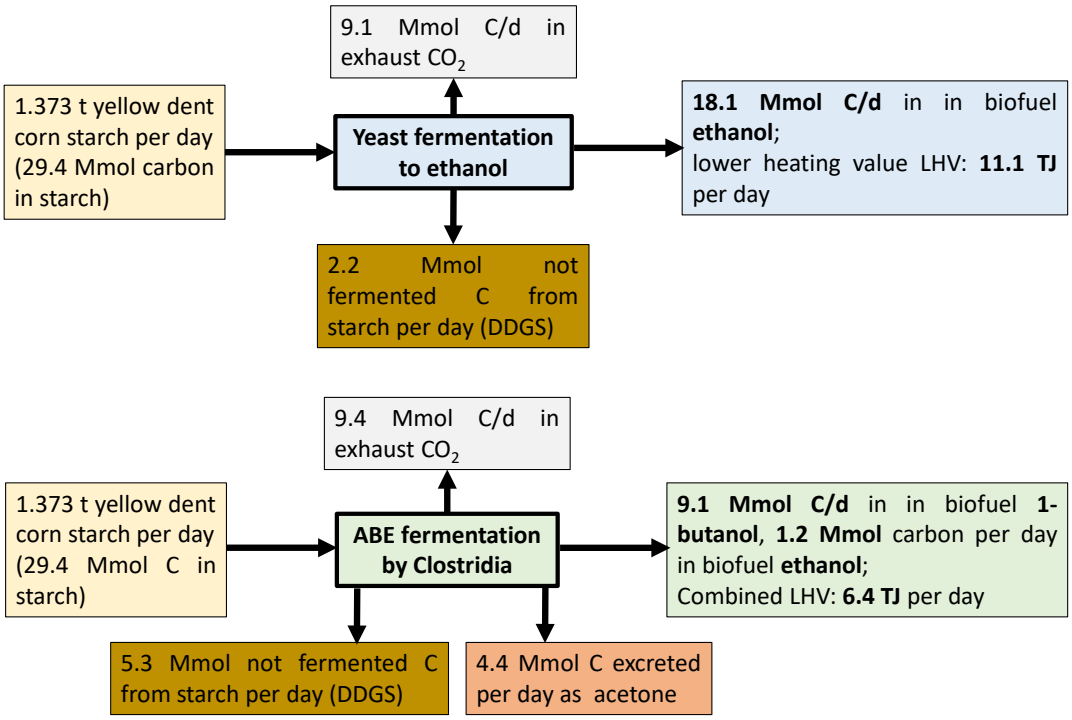


Figure 11. Production of biofuels ethanol and 1-butanol, and corresponding lower heating values (LHV) and by-products (CO_2, acetone). Comparison of yeast fermentation (top) and ABE fermentation (bottom) from the same amount of corn starch. Values from Pfromm *et al.*, 2010 [86]. Note: Mmol: 10^6 mol

tained by the Clostridia cultivation achieved 10.3 Mmol carbon per day (9.1 mmol 1-butanol, 1.2 Mmol ethanol) plus 4.4 Mmol carbon in acetone to be used, e.g., as solvent. 9.1 Mmol carbon get lost per day as CO_2 during yeast fermentation, which is almost equal to the ABE process. After downstream processing (100% solvent recovery assumed for the calculations), DDGS remain for every cultivation (2.2 Mmol carbon in DDGS for yeast fermentation, 5.3 Mmol for ABE fermentation), which can be sold as fodder or fertilizer, or burned for energy generation. Regarding the heating value of the products, the ethanol process was calculated to deliver 11.1 TJ per day, which is almost double the value as calculated for ethanol plus 1-butanol for the ABE process (6.3 TJ per day). Hydrogen generated as additional by-product of the ABE process might be another marketable side product, provided an economically feasible process for its separation from CO_2 will be available [86]. Figure 11 shows the balance for these processes for corn starch conversion to solvents to be used as energy carriers by yeasts and ABE fermentation by Clostridia, respectively.

4.2) Acetone

Acetone (propanone, dimethyl ketone; preferred IUPAC name: propane-2-one), is a colorless, highly flammable and volatile liquid, and characterized by its typical pungent smell. It is miscible with water in any ratio. It´s predominant applications are use as solvent (e.g., paint thinner, nail polish remover, etc.), as organo-chemical building blocks, and for manufacturing of methacrylate (the monomer of the polymer poly(methyl methacrylate) (PMMA)) and bisphenol A (precursor to epoxy resins, polycarbonates and other plastics. Caution: Bisphenol A has estrogen-mimicking, hormone-like properties!). Acetone peroxide, which is formed by the reaction between acetone and hydrogen peroxide, is a highly explosive compound with the impact sensitivity of an initial explosive, but, due to its extreme sensitivity, has no technical use. In the human body, acetone is formed in the liver as one of the ketone bodies, disposed of via normal metabolic processes, and is found in blood and urine. Elevated acetone levels in urine are a marker for diabetic ketoacidosis. The correct chemical structure of acetone was published, independently of each other, by Josef Johann Loschmidt (1861) and August Kekulé (1865). For 2010, a global productivity for acetone of about 6.7 Mt per year were estimated, with about a quarter thereof being produced in the US.

4.2.1) Chemical production of acetone

Well before WWI, acetone was predominantly produced as a product of wood distillation. Later, it was obtained by distillation of acetates, such as calcium or lead acetate at 380°C in a retort [71]. "Ketonic decarboxylation" of these acetates results in formation of metal oxides, acetone and CO_2. During WWI, the above-described famous Weizmann process for industrial, biotechnological acetone production based on ABE fermentation with *C. acetobutylicum* was developed for production of the explosive cordite (*vide supra*). However, during the last

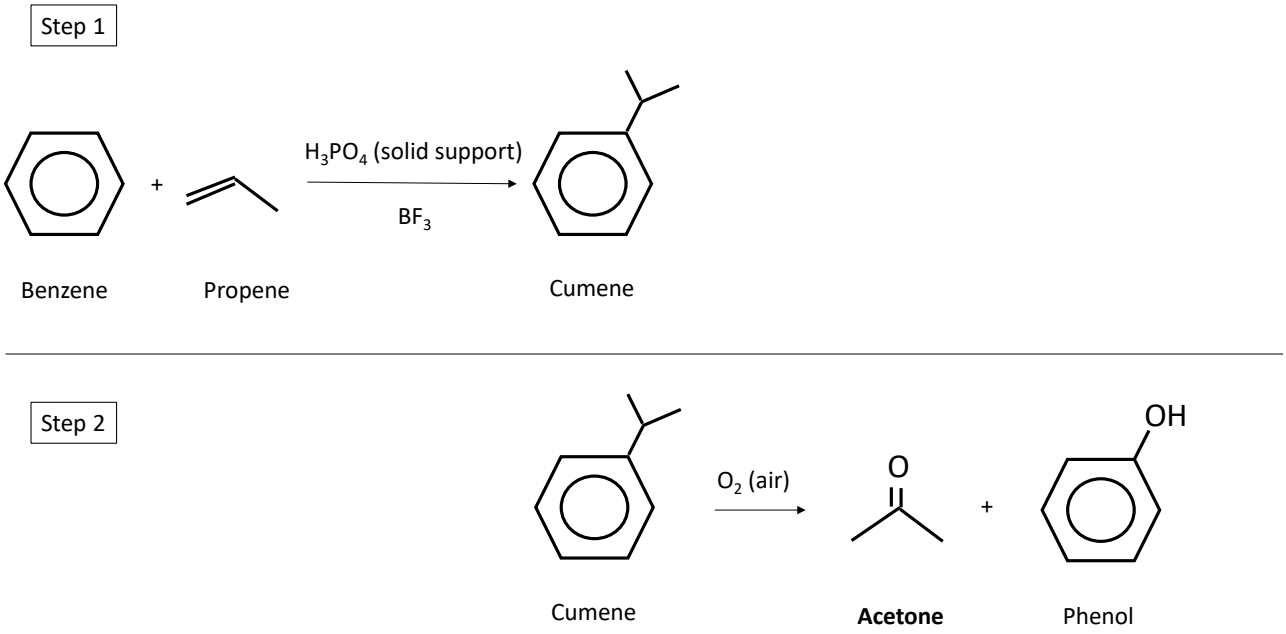


Figure 12. Chemical synthesis of acetone via the cumene process

decades, acetone was predominately produced directly or indirectly from the petrochemical product propene. As most important technique, propene is used to alkylate benzene via a Friedel-Crafts alkylation to generate cumene; this reaction is nowadays catalyzed by phosphoric acid on a solid support. Oxidation of cumene with air oxygen generates phenol and acetone (Figure 12).

4.2.2) Biotechnological production of acetone

The starting point of bio-based acetone manufacturing from fermentatively gained ethanol was connected to the fact that the above-described conversion of calcium acetate requires high quantities of acetic acid, which in turn is produced, via the intermediate acetaldehyde, by oxidation of ethanol. During WWI, also ethanol was a highly required chemical; therefore, novel microorganisms were explored to generate ethanol at high productivity for final production of acetone. Among these organisms, a few also produced acetone. Indeed, direct biotechnological acetone production via the Weizmann process was finally implemented for this purpose. Based on Weizmann´s patent from 1915, high quantities of acetone were produced for the British army during WWI in North America due to the lack of sufficient feedstocks (corn) in Great Britain, where the invention was made. As described above, acetone became rather the by-product of ABE fermentation after the end of WWI, when 1-butanol became an important raw material for automobile lacquers, a process considerably fueled by the rapid industrialization of automobile production by Henri Ford, and the need for acetone declined. This situation changed again, when WWII again increased the demand for acetone, which now took again the role as major product of ABE fermentation. In 1945, 10% of the global acetone (and 66% of 1-butanol) production was accomplished by Clostridia. Yet, soon after 1945, interest in ABE solvent fermentation suddenly decreased again due to the high competition by petrochemical processes. In PR China, however, these processes were never completely displaced by petrochemistry; even in the 1990ies, biotechnological processes accounted for about 50% of the entire acetone production in this country (reviewed by Dürre [72]).

4.3) Isopropanol

Similar to 1-butanol, isopropanol (IUPAC: propane-2-ol) is an important compound for various industrial applications such as solvent for lipids, lacquers, resins, or inks, as antifreeze, chemical intermediates, disinfectants, and for biofuels. In the context of biofuels, isopropanol can substitute methanol in biodiesel production, which generates biodiesel with reduced crystallization temperature, hence, the biofuel remains in liquid state at lower temperature [87]. When partially replacing gasoline by isopropanol, a fuel blend with increased octane number can be generated [88]. A recent study by Chen *et al.* (2020) demonstrated that blending isopropanol with gasoline has beneficial effects in combustion improvement and emission control [89]. This is in contrast to its biochemical precursor acetone, which plays no role as fuel or fuel additive because of its lower heating value (30.6 kJ/g for isopropanol vs. only 8.22 kJ/g for acetone) and high corrosiveness to engines and various rubber parts getting in contact with the fuel. Hence, the end-products of IBE

fermentations (isopropanol, butanol, ethanol) can be directly applied as biofuel in spark-ignition (SI) engines, and typically have lower emissions of pollutants and better engine performance than reported for products of ABE fermentations [90].

4.3.1 Chemical production of isopropanol

In analogy to acetone and 1-butanol production, industrial production of isopropanol also starts from the fossil feedstock

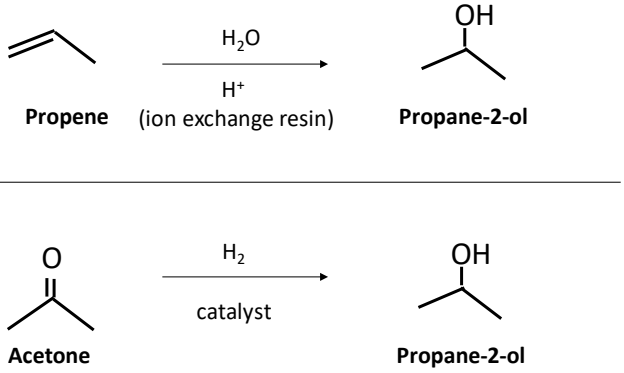


Figure 13. Chemical synthesis of isopropanol via acid-catalyzed hydration (top) or hydrogenation (bottom)

propene, which, for isopropanol synthesis, gets hydrated, typically by the catalytic aid of acidic ion exchange resins. Alternatively, acetone can be catalytically hydrogenated to isopropanol. Figure 13 illustrates these chemical reactions.

4.3.2) Biotechnological production of isopropanol

As mentioned above, the energy carrier 1-butanol nowadays is considered the industrial main product of solventogenic Clostridia, and acetone formation should be kept as low as possible. This makes such Clostridia of interest, which are able to further metabolize acetone towards isopropanol as final fermentation product ("IBE" fermentation; see Figure 9, dashed green line). The majority of isopropanol-producing bacteria belong to the species *C. beijerinckii* (synonym *C. butylicum*). Already in 1937, Osburn *et al.* published a paper on Clostridial butanol-isopropanol production [91]. In this context, Zhang *et al.* described high isopropanol production by a new *Clostridium beijerinckii* strain BGS1. This organism excreted 10.2 g/L butanol, 3.4 g/L isopropanol, and no acetone; this isopropanol concentration was considerably higher than values in previous reports for isopropanol production by wild type Clostridia. The authors explained this by a previously unknown secondary alcohol dehydrogenase found in this strain, which facilitates reductive acetone conversion to isopropanol. Moreover, this new isolate showed a broad spectrum of inexpensive carbohydrate substrates, which can be converted to 1-butanol plus isopropanol, such as sucrose, arabinose, xylose, galactose, or cellobiose, which considerably facilitates the use of this strain for fermentative conversion of second-generation feedstocks [90].

This natural process was mimicked by Wang *et al.*, who turned the ABE producer *Clostridium acetobutylicum* XY16 into an IBE producer by insertion of the *sadh* gene encoding for a secondary alcohol dehydrogenase. This way, acetone was completely converted to isopropanol by the recombinant strain. Importantly, these authors also underlined the role of NAD(P)H as reducing agent in solventogenic Clostridia; high levels of NAD(P)H are required for IBE fermentations to enable the catalytic conversion of the ketone acetone to the alcohol isopropanol by the secondary dehydrogenase. Authors found out that keeping the intracellular NADH and NADPH levels in an optimal range can be accomplished by efficient pH control [92].

5) 3-Hydroxyalkanoate Methylesters

A completely different group of potential liquid biofuels of the future, also deriving from biological processes, are methyl esters of hydroxyalkanoates. Hydroxyalkanoates, in turn, are the building blocks of microbial polyhydroxyalkanoate (PHA) biopolyesters. Such PHA biopolyesters are produced by numerous prokaryotic microbial species (mainly bacteria, but also by a steadily growing number of haloarchaea) and aerobic conditions (different to the anaerobic products described before in this chapter!). PHA serve the cells producing them as storage materials and stress protectants, and are biosynthesized from a variety of carbon sources, both heterotrophic (this is the case for most important industrially implemented production strains like *Cupriavidus necator*, *Azohydromonas lata*, *Paraburkholderia sacchari*, *Halomonas* sp., *Aeromonas* sp., or recombinant *E. coli*) and autotrophic (several cyanobacteria convert CO_2 to PHA, while some *Rhodospirilli* produce PHA from syngas) (reviewed by [93]).

PHA are stored in microbial cells as granules ("carbonosomes") surrounded by the enzymes needed for their formation and degradation, and are well visible in light microscopes as highly light-refractive intracellular inclusion bodies. From the technological point of view, PHA are considered to be among the top "green" materials of choice to sooner or later replace at least parts of the currently produced recalcitrant petrochemical plastics. This is due to PHA´s bio-based nature, biodegradability, industrial- and home-compostability, and biocompatibility, and the tuneability of PHA properties during biosynthesis by appropriate selection of feedstocks, production strains, and substrate feeding regimes (reviewed by [5, 94]). In dependence on the thus obtained monomeric composition, the properties of PHA can resemble petrochemical thermoplastics (short-chain-length PHA [*scl*-PHA] like poly(3-hydroxybutyrate) [P(3HB)]; such *scl*-PHA have building blocks with no more than five carbon atoms) or petrochemical elastomers (medium-chain-length PHA [*mcl*-PHA]; such *mcl*-PHA have building blocks with six or more carbon atoms) (reviewed by [93, 94]).

Typically, PHA of consistently high quality is produced by pure microbial cultures in monoseptic cultivation setups in

sterilized aerated bioreactors and aseptic nutrient broth. However, we currently witness an emerging trend in using mixed microbial cultures, e.g., from sewage sludge, to convert diverse carbon-rich waste streams under non-sterile conditions into PHA. Although being more cost-efficient in production, such PHA from mixed culture production setups are often of insufficient uniformity; typically, they constitute polymer blends of PHA produced by the individual species present in the mixed culture; this impedes their convenient processing to bioplastic materials [93]. That´s the moment when, finally, PHA´s role as raw material for biofuel production comes at play. A novel class of follow-up products of such low-quality PHA were produced by Zhang *et al.* (2009): Via acid methanolysis transesterification, these authors converted the polyesters P(3HB) and *mcl*-PHA to methyl esters of the individual hydroxyalkanoic acids [95]. This process is illustrated in Figure 14.

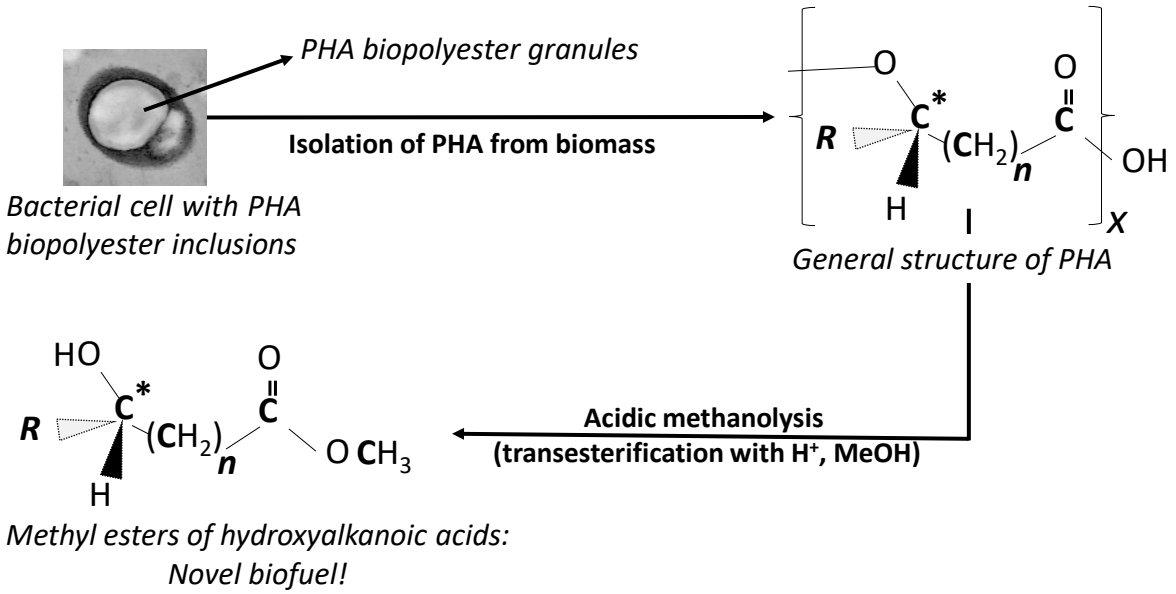


Figure 14. Generation of methyl esters of hydroxyalkanoic acids by acidic methanolysis of PHA biopolyesters isolated from microbial cells. *R*: side group in PHA monomers; *x*: degree of polymerization (number of monomers in PHA polymer chain); *n*: number of methylene groups in backbone of PHA monomers

These methyl esters display, apart from having one additional hydroxyl group, high structural similarity to well-established fatty acid methyl esters (FAME), which are widely used as "biodiesel". Indeed, these new materials were studied as possible innovative "biofuels"; their combustion heat was compared with values for currently used fuels and fuel additives such as gasoline or the biofuels ethanol and 1-butanol discussed before in this chapter. As main result, combustion heat values of 3-hydroxyalkanoate (3HA) methyl esters were in the range of 20 – 30 kJ/g, which is similar to values reported for ethanol (25.5 kJ/g), 1- butanol (33.1 kJ/g), or FAME (35.6 kJ/g) [96]. Moreover, the same team of scientists demonstrated that adding the methyl ester of 3-hydroxybutyrate (3HB) to gasoline even results in better quality regarding a lower oxygen content, higher boiling point, flash point, and dynamic viscosity than when adding ethanol. In addition, blending of gasoline with about 10% 3HB-methyl ester only insignificantly reduced the octane number [97]. It is pivotal to underline that PHA used for production of these methyl esters does not need to be of high purity and quality; on larger scale, it should definitely be produced in inexpensive, simple, non-sterile cultivation setups by mixed microbial cultures using, e.g., sewage water or other carbon-rich waste water as substrate, thus combining waste upcycling with generation of value-added bioproducts [96].

Very recently, an additional method to generate FAME connected to PHA biopolyester production was introduced by Siow and colleagues. These authors used PHA-rich bacterial biomass as fodder for meal worms (*Tenebrio molitor*). The larvae digested the non-PHA part of biomass (proteins, etc.), while the PHA biopolyester granules were excreted by the animals in pristine quality and astonishing purity as white feces. The physical and chemical properties of the oil present in thus nourished meal worms were investigated. The oil showed a high fraction of unsaturated fatty acids and an undesired high amount of free fatty acids, and was subjected towards acid-catalyzed methanolysis. A mixture of methyl esters of fatty acids (FAME) was generated, which was studied to evaluate its quality as biofuel. Indeed, the properties of generated biodiesel were found to be in accor-

dance with the US-biodiesel standard ASTM D6751 [97]. However, it appears doubtful if this technology is ethically clear; in a previous paper published by the same team of researchers some years ago, they postulate that the meal worm biomass obtained should be used as protein source for food and feed purposes [98]. Hence, the new concept to convert its lipid content to a biofuel might fuel the *food-vs.-fuel* controversy.

To sum up, the subsequent Table 1 gives an overview for the bioproducts discussed in this chapter (ethanol, 2,3-BD, 1-butanol, acetone, isopropanol, hydroxyalkanoate methyl esters), summarizing the production conditions, fields of application, heating values, production strains and essential raw materials for their production.

Table 1. Comparative summary of fermentation products discussed in the chapter

Product	Production conditions	Fields of application	Lower Heating Value (LHV) [kJ/g]	Production strain(s)	Most important feedstocks
Ethanol	Anaerobic	Engine fuel, solvent, alcoholic drinks	25.5	*Saccharomyces cerevisiae* (eukaryotic yeast), *Zymomonas mobilis* (Gram-negative bacterium; tequila production)	Corn starch (USA), cane sugar (Brazil), beet sugar (Europe), other carbohydrate-rich raw materials (molasses, whey, etc.); glycerol; lignocellulose (bagasse, etc.)
	Strict anaerobic	,,	,,	*Clostridium autoethanogenum* and other acetogenic Clostridia	Syngas (mixture CO_2, CO, and H_2; from conversion of organic waste streams)
2,3-Butandiol (2,3-BD)	Anaerobic	Basis for follow-up solvents; anti-freeze; 1,3-butadiene production (rubber)	33.1	*Klebsiella pneumoniae*; *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Bacillus polymyxa*, *Serratia marcescens*; rec. *E. coli*	Carbohydrates (starch, sucrose, lactose, galactose, etc.); glycerol; sweet sorghum juice; food waste; lignocellulose
	Strict anaerobic	,,	,,	*Clostridium autoethanogenum* and other acetogenic Clostridia	Syngas (mixture CO_2, CO, and H_2; from conversion of organic waste streams)
1-Butanol	Strict anaerobic	Biofuel; solvent, lacquer production, food additive, textile industry	33.1	*Clostridium acetobutylicum* and other ABE solventogens (e.g., *C. saccharobutylicum*, *C. aurantibutyricum*, *C. saccharoperbutylacetonicum*); rec. *E. coli*	Carbohydrates (starch, sucrose, etc.); lignocellulose
Acetone	Strict anaerobic	Solvent, explosives (cordite)	8.22	*Clostridium acetobutylicum* and other ABE solventogens (e.g., *C. saccharobutylicum*)	Carbohydrates (starch, sucrose, etc.); lignocellulose
Isopropanol (2-Propanol)	Strict anaerobic	Fuel additive, solvent, advanced biodiesel production; chemical intermediate, disinfectant	30.6	*Clostridium beijerinckii* (former *Clostridium acetobutylicum*); IBE solventogens	Carbohydrates (starch, sucrose, etc.); lignocellulose
Methyl esters of 3-hydroxyalkanoic acids	Aerobic (production of PHA as staring material)	Engine fuel	20-30*	Various prokaryotic microbes as PHA producers	Heterotrophic carbon sources from waste streams (e.g., carbon-rich urban or industrial effluent water)

* depends on type of hydroxyalkanoic acid; for comparison: "classical" biodiesel (FAME): 35.6 kJ/g

Conclusion

Given the increasing scarcity of fossil resources and elevating atmospheric CO_2 levels, the development and exploitation of biological alternatives to established crude oil-based industrial production processes for manufacturing solvents, fuels, polymers, and other bulk chemicals is the order of the day.

However, the switch from industrial technologies established since many decades to novel biocatalyst- and bioresource-based processes, under the ideas of a circular bioeconomy will require fundamental changes in the way industry operates nowadays, and changes in the mindset of scientists, engineers, and the public.

The first fundamental change addresses the feedstocks to be used: Petrochemical raw materials will be exchanged by renewables. Wherever possible, these renewables should constitute amply available carbon-rich waste- and surplus materials from other local industrial processes. Moreover, wherever possible, the new production facilities for biofuels, solvents, etc., should be integrated into existing industrial production lines, where the waste to be upcycled to raw materials for bioproduction directly accrues; in such biorefinery concepts, transport costs can be minimized.

The second fundamental difference between biotechnological manufacturing and petrochemical industry are the catalysts and production conditions to be applied. In the case of petrochemistry, expensive, often toxic metal-based catalysts drive the processes under typically extreme conditions of temperature and pressure, while biotechnological processes are operated under conditions optimal for given production strains, which harbor the enzymatic toolbox to carry out the biocatalysis from raw materials to marketable products. These optimum conditions are typically in the mesophilic range for temperature, pH-value, pressure, or redox potential.

Third, it is clear that biotechnological processes intrinsically cannot compete chemical processes in terms of yields, volumetric productivities or achievable product concentrations; biological systems are simply subject to their natural limitations. This poses new challenges in development of advanced downstream processing technologies for more efficient recovery of bioproducts, such as *in-situ* product recovery. Moreover, this aspect also covers the fact that, by means of evolutionary engineering, systems biology, genetic engineering tools, and multi-omics approaches, more efficient microbial production strains need to be screened and developed to close metabolic bottlenecks, increase productivity, stereoselectivity, or the range of inexpensive substrates converted by the organisms. In addition, such new microbial cell factories need to be less sensitive, on the one hand, to inhibitors stemming from upstream processing and pretreatment of inexpensive raw materials and, on the other hand, to high product titers. Finally, these organisms shall show reduced by-product formation, which can also be achieved by smart manipulation of intracellular carbon fluxes via metabolic engineering.

All this, in turn, needs the further intensified cooperation of specialists from different scientific disciplines like chemistry, biochemistry, biotechnology, microbiology, genetics, systems biology, bioengineering, chemical engineering, logistics, economics, etc., and from both the academic and industrial sector. By generation of transdisciplinary and trans-sectorial synergistic effects between such differently expertized specialists, considerable progress is expected to be accomplished in terms of switching into a bio-based and biology-inspired industry and transport sector in a not-too-distant future.

Acknowledgement

None declared

Conflict of Interest

No conflict of interest exists

References

1. Songstad D., Lakshmanan P, Chen J, Gibbons W, Hughes S, Nelson R. Historical perspective of biofuels: learning from the past to rediscover the future. In Vitro Cell Dev Biol Plant 2009; 45(3): 189-192.
2. Guo M, Song W, Buhain J. Bioenergy and biofuels: History, status, and perspective. Renew Sust Energ Rev 2015; 42: 712-725.
3. Dürre P. Biobutanol: an attractive biofuel. Biotechnology Journal: Healthcare Nutrition Technology 2007; 2(12): 1525-1534.
4. Mukherjee A, Koller M. Polyhydroxyalkanoate (PHA) biopolyesters - emerging and major products of Industrial Biotechnology. EuroBiotech J 2022; 6(2): 49-60.
5. Koller M, Mukherjee A. Polyhydroxyalkanoates – linking properties, applications, and end-of-life options. Chem Biochem Eng Q 2020; 34(3): 115-129.
6. Demirbas A. Biofuels securing the planet's future energy needs. Energy Convers Manag 2009; 50: 2239-2249.
7. Kilian L. Oil price shocks: Causes and consequences. Annu Rev Resour Economics 2014; 6: 133-154.
8. Aditiya HB, Mahlia TMI, Chong WT, Nur H,Sebayang, AH. Second generation bioethanol production: A critical review. Renew Sust Energ Rev 2016; 66: 631-653.
9. Online resource https://sdgs.un.org/goals (access February 10th, 2023)
10. Leong HY, Chang CK, Khoo KS, Chew KW, Chi SR, Lim JW, Chang JS, Show PL. Waste biorefinery towards a sustainable circular bioeconomy: a solution to global issues. Biotechnol Biofuels 2021; 14: 87.
11. Gray KA, Zhao L, Emptage M. Bioethanol. Curr Opin Chem Biol 2006; 10(2): 141-146.
12. Hossain N, Razali AN, Mahlia TMI, Chowdhury T, Chowdhury H, Ong HC, Shamsuddin AH, Silitonga AS. Experimental investigation, techno-economic analysis and environmental impact of bioethanol production from banana stem. Energies 2019; 12(20): 3947.
13. Waltz E. Amylase corn sparks worries. Nat Biotechnol 2011; 29: 294.
14. Sharma B, Larroche C, Dussap CG. Comprehensive as-

sessment of 2G bioethanol production. Bioresour Technol 2020; 313: 123630.
15. Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Faik AAM, Rodrigues K. F. Yeasts in sustainable bioethanol production: A review. Biochem Biophys Rep 2017; 10: 52-61.
16. Larsen J, Østergaard Petersen M, Thirup L, Wen Li H, Krogh Iversen F. The IBUS process–lignocellulosic bioethanol close to a commercial reality. Chem Eng Technol 2008; 31(5): 765-772.
17. Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Factories 2018; 17(1): 1-21.
18. Abdullah B, Syed Muhammad SAF, Shokravi Z, Ismai S., Kassim KA, Mahmood AN, Aziz MMA. Fourth generation biofuel: a review on risks and mitigation strategies. Renew Sust Energ Rev 2019; 107: 37-50.
19. Pfeiffer T, Morley A. An evolutionary perspective on the Crabtree effect. Front Mol Biosci 2014; 1: 17.
20. Verduyn C, Zomerdijk TPL, Dijken JP, Scheffers WA. Continuous measurement of ethanol production by aerobic yeast suspensions with an enzyme electrode. Appl Microbiol Biotechnol 1984; 19: 181–185.
21. Meyerhof O. Über den Einfluß des Sauerstoffs auf die alkoholische Gärung der Hefe. Naturwissenschaften 1925; 13: 980–984.
22. Rocha-Arriaga C, Espinal-Centeno A, Martinez-Sanchez S, Caballero-Pérez J, Alcaraz LD, Cruz-Ramirez A. Deep microbial community profiling along the fermentation process of pulque, a major biocultural resource of Mexico. Microbiol Res 2020; 241: 126593.
23. Panesar PS, Marwaha SS, Kennedy JF. *Zymomonas mobilis*: an alternative ethanol producer. J Chem Technol Biotechnol 2006; 81(4): 623-635.
24. Sadik MW, Halema AA. Production of ethanol from molasses and whey permeate using yeasts and bacterial strains. Int J Curr Microbiol Appl Sci 2014; 3(3): 804-818.
25. Maiti B, Rathore A, Srivastava S, Shekhawat M, Srivastava P. Optimization of process parameters for ethanol production from sugar cane molasses by *Zymomonas mobilis* using response surface methodology and genetic algorithm. Appl Microbiol Biotechnol 2011; 90(1): 385-395.
26. Letti LAJ, Karp SG, Woiciechowski AL, Soccol CR. Ethanol production from soybean molasses by *Zymomonas mobilis*. Biomass Bioenergy 2012; 44: 80-86.
27. Xia J, Yang Y, Li C.G, Yang S, Bai FW. Engineering *Zymomonas mobilis* for robust cellulosic ethanol production. Trends Biotechnol 2019; 37(9): 960-972.
28. Mazaheri D, Pirouzi A. Valorization of *Zymomonas mobilis* for bioethanol production from potato peel: fermentation process optimization. Biomass Conv Bioref 2022; 12: 3389-3398.
29. Zhang M, Eddy C, Deanda K, Finkelstein M, Picataggio S. Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*. Science 1995; 267(5195): 240-243.
30. Bengelsdorf FR, Beck MH, Erz C, Hoffmeister S, Karl MM, Riegler P, Wirth S, Poehlein A, Weuster-Botz D, Dürre P. Bacterial anaerobic synthesis gas (syngas) and CO2+H2 fermentation. Adv Appl Microbiol 2008; 103: 143–221.
31. Flüchter S, Follonier S, Schiel-Bengelsdorf B, Bengelsdor FR, Zinn M, Dürre P. Anaerobic production of poly(3-hydroxybutyrate) and its precursor 3-hydroxybutyrate from synthesis gas by autotrophic clostridia. Biomacromolecules 2019; 20(9): 3271-3282.
32. Abubackar HN, Veiga MC, Kennes C. Carbon monoxide fermentation to ethanol by Clostridium autoethanogenum in a bioreactor with no accumulation of acetic acid. Bioresour Technol 2015; 186: 122-127.
33. Sun X, Atiyeh HK, Zhang H, Tanner RS, Huhnke RL. Enhanced ethanol production from syngas by *Clostridium ragsdalei* in continuous stirred tank reactor using medium with poultry litter biochar. Appl Energy 2019; 236: 1269-1279.
34. Infantes A, Kugel M, Raffelt K, Neumann A. Side-by-side comparison of clean and biomass-derived, impurity-containing syngas as substrate for acetogenic fermentation with *Clostridium ljungdahlii*. Fermentation 2020; 6(3): 84.
35. Song D, Yoon YG, Lee CJ. Techno-economic evaluation of the 2,3-butanediol dehydration process using a hydroxyapatite-alumina catalyst. Korean Journal of Chemical Engineering 2018; 35(12): 2348-2354.
36. Liu X, Fabos V, Taylor SH, Knight DW, Whiston K, Hutchings GJ. One-step production of 1,3-butadiene from 2,3-butanediol dehydration. Chem Eur J 2016; 22(35): 12290-12294.
37. Radoš D, Carvalho AL, Wieschalka S, Neves AR, Eikmanns PBJ, Blombach B, Santos H. Engineering *Corynebacterium glutamicum* for the production of 2,3-butanediol. Microb Cell Factories 2015; 14: 171. doi:10.1186/s12934-015-0362-x.
38. Li ZJ, Jian J, Wei XX, Shen XW, Chen GQ. Microbial production of meso-2,3-butanediol by metabolically engineered *Escherichia coli* under low oxygen condition. Appl Microbiol Biotechnol 2010; 87(6): 2001-2009.
39. Bartowsky EJ, Henschke PA. The 'buttery' attribute of wine-diacetyl-desirability, spoilage and beyond. Int J Food Microbiol 2004; 96: 235–252.
40. Celińska E, Grajek W. Biotechnological production of 2,3-butanediol - current state and prospects. Biotechnol Adv 2009; 27: 715-725.
41. Gräfje H, Körnig W, Weitz HM, Reiß W, Steffan G, Diehl H, Bosche H, Schneider K, Kieczka H, Pinkos R. Butanediols, butenediol, and butynediol. In: Ullmann's Encyclopedia of Industrial Chemistry, 2000; 1-12: https://doi.org/10.1002/14356007.a04_455.pub2
42. Harden A, Walpole GS. Chemical action of Bacillus lactis aerogenes (Escherich) on glucose and mannitol: production of 2: 3-butyleneglycol and acetylmethylcarbinol. Proc

R Soc 1906; 77(519): 399-405.
43. Harden A, Norris D. The bacterial production of acetylmethylcarbinol and 2.3-butylene glycol from various substances. Proc R Soc 1912; 84(574): 492-499.
44. Fulmer EI, Christensen LM, Kendali AR. Production of 2, 3-butylene glycol by fermentation. Ind Eng Chem 1933; 25(7): 798-800.
45. Savakis PE, Angermayr SA, Hellingwerf KJ. Synthesis of 2,3-butanediol by *Synechocystis* sp. PCC6803 via heterologous expression of a catabolic pathway from lactic acid- and enterobacteria. Metab Eng 2013; 20: 121–130.
46. Online resource: LanzaTech. World's first commercial waste gas to ethanol plant starts up http://www.lanzatech.com/worlds-first-commercial-wastegas-ethanol-plant-starts/, 2018 (accessed April 15, 2021).
47. Tinôco D, Borschiver S, Coutinho PL, Freire DM. Technological development of the bio-based 2,3-butanediol process. Biofuel Bioprod Biorefin 2021; 15: 357–376.
48. Ji XJ, Huang H, Ouyang PK. Microbial 2, 3-butanediol production: a state-of-the-art review. Biotechnol Adv 2011; 29(3): 351-364.
49. Ma C, Wang A, Qin J, Li L, Ai X, Jiang T, Tang H, Xu P. Enhanced 2, 3-butanediol production by Klebsiella pneumoniae SDM. Appl Microbiol Biotechnol 2009; 82(1): 49-57.
50. Nakashimada Y, Kanai K, Nishio N. Optimization of dilution rate, pH and oxygen supply on optical purity of 2,3-butanediol produced by *Paenibacillus polymyxa* in chemostat culture. Biotechnol Lett 1998; 20(12): 1133-1138.
51. Białkowska AM. Strategies for efficient and economical 2,3-butanediol production: new trends in this field. World J Microbiol Biotechnol 2016; 32(12): 1-14.
52. Cao N, Xia Y, Gong CS, Tsao GT. Production of 2, 3-butanediol from pretreated corn cob by *Klebsiella oxytoca* in the presence of fungal cellulase. In: Davison, B.H., Wyman, C.E., Finkelstein, M. (eds.). Biotechnology for Fuels and Chemicals. Applied Biochemistry and Biotechnology, vol 63-65. 1997; Humana Press. pp. 129-139. https://doi.org/10.1007/978-1-4612-2312-2_13
53. Pasaye-Anaya L, Vargas-Tah A, Martínez-Cámara C, Castro-Montoya AJ, Campos-García J. Production of 2,3-butanediol by fermentation of enzymatic hydrolysed bagasse from agave mezcal-waste using the native *Klebsiella oxytoca* UM2-17 strain. J Chem Technol Biotechnol 2019; 94(12): 3915-3923.
54. González-García Y, Grieve J, Meza-Contreras JC, Clifton-García B, Silva-Guzman JA. Tequila agave bagasse hydrolysate for the production of polyhydroxybutyrate by *Burkholderia sacchari*. Bioengineering 2019; 6(4): 115.
55. Yuan J, He YZ, Guo ZW, Gao HF, Chen FB, Li LZ, Li Y-Y, Zhang LY. Utilization of sweet sorghum juice for efficient 2,3-butanediol production by Serratia marcescens H30. BioResources 2017; 12(3): 4926-4942.
56. Jurchescu IM, Hamann J, Zhou X, Ortmann T, Kuenz A, Prüße U, Lang S. Enhanced 2,3-butanediol production in fed-batch cultures of free and immobilized Bacillus licheniformis DSM 8785. Appl Microbiol Biotechnol 2013; 97(15): 6715-6723.
57. Erian AM, Freitag P, Gibisch M, Pflügl S. High rate 2, 3-butanediol production with Vibrio natriegens. Bioresource Technology Reports 2020; 10: 100408.
58. Fernández-Gutierrez D, Veillette M, Ramirez AA, Giroir-Fendler A, Faucheux N, Heitz, M. Production of 2,3-butanediol from diverse saccharides via fermentation. Can J Chem Eng 2020; 98(1): 54-61.
59. Sathesh-Prabu C, Kim D, Lee SK. Metabolic engineering of *Escherichia coli* for 2,3-butanediol production from cellulosic biomass by using glucose-inducible gene expression system. Bioresour Technol 2020; 309: 123361.
60. Köpke M, Mihalcea C, Liew F, Tizard JH, Ali MS, Conolly JJ, Al-Sinawi B, Simpson SD. 2,3-Butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. Appl Environ Microbiol 2011; 77(15): 5467-5475.
61. Chen J-S, Hiu SF. Acetone–butanol–isopropanol production by *Clostridium beijerinckii* (synonym, *Clostridium butylicum*). Biotechnol Lett 1986; 8: 371–376.
62. Green EM. Fermentative production of butanol—the industrial perspective. Curr Opin Biotechnol 2011; 22(3): 337-343.
63. Huzir NM, Aziz MMA, Ismail SB, Abdullah B, Mahmood NAN, Umor NA, Muhammad SAFAS. Agro-industrial waste to biobutanol production: Eco-friendly biofuels for next generation. Renew Sust Energ Rev 2018; 94: 476-485.
64. Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS. Fermentative butanol production by Clostridia. Biotechnol Bioeng 2008; 101(2): 209-228.
65. Anandharaj SJ, Gunasekaran J, Udayakumar GP, Meganathan Y, Sivarajasekar N. Biobutanol: Insight, Production and Challenges. In: Sivasubramanian V., Pugazhendhi A., Moorthy I. (eds.). Sustainable Development in Energy and Environment. Springer Proceedings in Energy. Springer, Singapore, 2020: pp. 25-37. https://doi.org/10.1007/978-981-15-4638-9_3
66. Uyttebroek M, Van Hecke W, Vanbroekhoven K. Sustainability metrics of 1-butanol. Catal 2015; 239: 7-10.
67. von Kutepow N, Kindler H. Die Alkoholsynthese nach W. Reppe. Angew Chem 1960; 72(22): 802-805.
68. Akgül A, Palmeiro-Sanchez T, Lange H, Magalhaes D, Moore S, Paiva A, Kazanç F, Trubetskaya, A. Characterization of tars from recycling of PHA bioplastic and synthetic plastics using fast pyrolysis. J Hazard Mater, 2022; 439, 129696.
69. Dürre P. Fermentative butanol production: bulk chemical and biofuel. Ann N Y Acad Sci 2008; 1125(1): 353-362.
70. Pasteur L. Quelques résultats nouveaux relatifs aux fermentations acétique et butyrique. Bull Soc Chim Fr 1862 : 52–53.
71. Bunch AW. How biotechnology helped maintain the supply of acetone for the manufacture of cordite during World

War I. Int J Eng Technol 2014; 84(2): 211-226.
72. Dürre P. New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. Appl Microbiol Biotechnol 1998; 49(6): 639-648.
73. Zhang X, Feng X, Zhang H, Wei Y. Utilization of steam-exploded corn straw to produce biofuel butanol via fermentation with a newly selected strain of *Clostridium acetobutylicum*. BioResources 2018; 13(3): 5805-5817.
74. Qureshi N, Blaschek HP. Economics of butanol fermentation using hyper-butanol producing *Clostridium beijerinckii* BA101. Food Bioprod Process 2000; 78(3), 139-144.
75. Qureshi N, Ezeji TC. Butanol, 'a superior biofuel' production from agricultural residues (renewable biomass): recent progress in technology. Biofuel Bioprod Biorefin 2008; 2(4): 319-330.
76. Ezeji T, Blaschek HP. Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia. Bioresour Technol 2008; 99(12): 5232-5242.
77. Noomtim P, Cheirsilp B. Production of butanol from palm empty fruit bunches hydrolyzate by *Clostridium acetobutylicum*. Energy Procedia 2011; 9: 140-146.
78. Lu C, Zhao J, Yang ST, Wei D. Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresour Technol 2012; 104: 380-387.
79. Zhang J, Wang M, Gao M, Fang X, Yano S, Qin S, Xia R. Efficient acetone–butanol–ethanol production from corncob with a new pretreatment technology - wet disk milling. BioEnergy Res 2013; 6(1): 35-43.
80. Amiri H, Karimi K, Zilouei H. Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production. Bioresour Technol 2014; 152: 450-456.
81. Kiyoshi K, Furukawa M, Seyama T, Kadokura T, Nakazato A, Nakayama S. Butanol production from alkali-pretreated rice straw by co-culture of *Clostridium thermocellum* and *Clostridium saccharoperbutylacetonicum*. Bioresour Technol 2015; 186: 325-328.
82. Khedkar MA, Nimbalkar PR, Kamble SP, Gaikwad SG, Chavan PV, Bankar S.B. Process intensification strategies for enhanced holocellulose solubilization: Beneficiation of pineapple peel waste for cleaner butanol production. J Clean Prod 2018; 199: 937-947.
83. Phillips JA, Humphrey AE. An overview of process technology for the production of liquid fuels and chemical feedstocks via fermentation. In: Wise DL (ed) Organic chemicals from biomass. Benjamins/Cummings Publishing, Menlo Park, Calif., 1983: pp 249–304
84. Chen CK, Blaschek HP. Acetate enhances solvent production and prevents degeneration in *Clostridium beijerinckii* BA101. Appl Microbiol Biotechnol 1999; 52(2): 170-173.
85. Jiang Y, Xu C, Dong F, Yang Y, Jiang W, Yang S. Disruption of the acetoacetate decarboxylase gene in solvent-producing *Clostridium acetobutylicum* increases the butanol ratio. Metab Eng 2009; 11(4-5): 284-291.
86. Pfromm PH, Amanor-Boadu V, Nelson R, Vadlani P, Madl R. Bio-butanol vs. bio-ethanol: a technical and economic assessment for corn and switchgrass fermented by yeast or *Clostridium acetobutylicum*. Biomass Bioenergy 2010; 34(4): 515-524.
87. Lee I, Johnson LA, Hammond EG. Use of branched-chain esters to reduce the crystallization temperature of biodiesel. J Am Oil Chem Soc 1995; 72: 1155–1160.
88. Rassadin V, Shlygin OY, Likhterova N, Slavin V, Zharov A. Problems in production of high-octane, unleaded automotive gasolines. Chem Tech Fuels Oil 2006; 42: 235–242.
89. Chen H, Zhou Z, He J, Zhang P, Zhao X. Effect of isopropanol and n-pentanol addition in diesel on the combustion and emission of a common rail diesel engine under pilot plus main injection strategy. Energy Reports 2020; 6: 1734-1747.
90. Zhang C, Li T, He J. Characterization and genome analysis of a butanol–isopropanol-producing *Clostridium beijerinckii* strain BGS1. Biotechnol Biofuels 2018; 11: 280.
91. Osburn O, Brown R, Werkman C. The butyl alcohol–isopropyl alcohol fermentation. J Biol Chem 1937; 121: 685–695.
92. Wang C, Xin F, Kong X, Zhao J, Dong W, Zhang W, Ma J, Wu H, Jiang M. Enhanced isopropanol–butanol–ethanol mixture production through manipulation of intracellular NAD (P) H level in the recombinant *Clostridium acetobutylicum* XY16. Biotechnol Biofuels 2018; 11(1): 1-10.
93. Koller M, Maršálek L, Miranda de Sousa Dias M, Braunegg G. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. N Biotechnol 2017; 37(A): 24-38.
94. Koller M. Chemical and biochemical engineering approaches in manufacturing polyhydroxyalkanoate (PHA) biopolyesters of tailored structure with focus on the diversity of building blocks. Chem Biochem Eng Q 2018; 32(4): 413-438.
95. Zhang X, Luo R, Wang Z, Deng Y, Chen, G-Q. Application of (R)-3-hydroxyalkanoate methyl esters derived from microbial polyhydroxyalkanoates as novel biofuels. Biomacromolecules 2009; 10(4): 707-711.
96. Wang SY, Wang Z, Liu MM, Xu Y, Zhang XJ, Chen G-Q. Properties of a new gasoline oxygenate blend component: 3-hydroxybutyrate methyl ester produced from bacterial poly-3-hydroxybutyrate. Biomass Bioenergy 2010; 34(8): 1216-1222.
97. Siow HS, Sudesh K, Murugan P, Ganesan S. Mealworm (*Tenebrio molitor*) oil characterization and optimization of the free fatty acid pretreatment via acid-catalyzed esterification. Fuel 2021; 299: 120905.
98. Chee JY, Lakshmanan M, Jeepery IF, Hairudin NHM, Sudesh K. The potential application of *Cupriavidus necator* as polyhydroxyalkanoates producer and single cell protein: A review on scientific, cultural and religious perspectives. Appl Food Biotechnol 2019; 6(1): 19-34.