Biotechnology for the production of commodity chemicals from biomass

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Current developments, especially in fermentation technologies, membrane technologies and genetic manipulation open new possibilities for the biotechnological production of market relevant chemicals from renewable resources. This article reviews possible fermentation strategies, actual research activities and future research demands to enhance the use of renewable resources as a source of chemicals. Special focus is put on commodity chemicals, which have a considerable market volume or which are believed to be key chemicals of the future.

1 The starting point

Up to the 19th century mankind used renewable resources not only for food but also for functional applications. At the turn of the century the situation changed completely, as coal became the key raw material for the production of coke, ammonia, tar, and gas. Finally, the developments and improvements in the area of fossil oil processing resulted in today's chemical industry mainly based on oil and natural gas. Nowadays, more than 2500 different oil-based products are on the market. Crude oil represents the major source for plastics, fibers and colours. At present, 10% of world-wide natural gas consumption, 21% of the natural gas liquids, and 4% of crude oil are used for chemicals. On a combined basis, the chemical industry requires about 7–8% of the total consumed liquid and gaseous hydrocarbons. Although this value seems to be quite low, it still represents a total market volume of more than 215 billion US dollars.[1]

Fig. 1 shows the most important low carbon organic chemicals by volume manufactured worldwide. Fossil resources, mainly oil and natural gas, are converted into key chemicals like ethylene, propylene or the C_4-fraction, which are intermediates for the synthesis of the respective chemicals. Ethylene represents the organic chemical consumed in largest quantities worldwide. The total volume of ethylene based chemicals and ethylene-based polymers amounted to about 79 Mio tons in 1995 and this is expected to increase by 5.4% annually. Propylene serves as a raw material for the production of C_3-chemicals with considerable market volumes as indicated

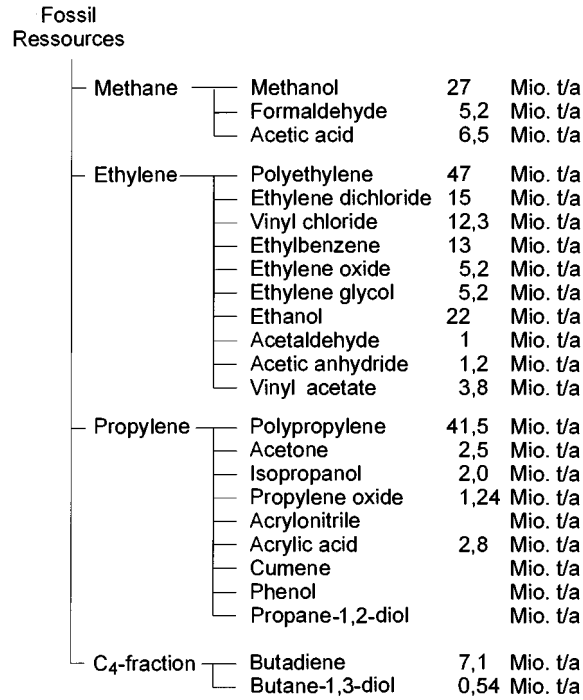

Fig. 1 Sources and production routes of the most important organic C_1–C_4-chemicals (Mio = 10^6).

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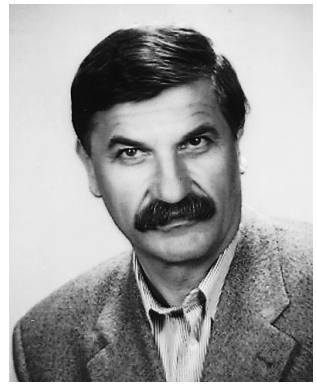

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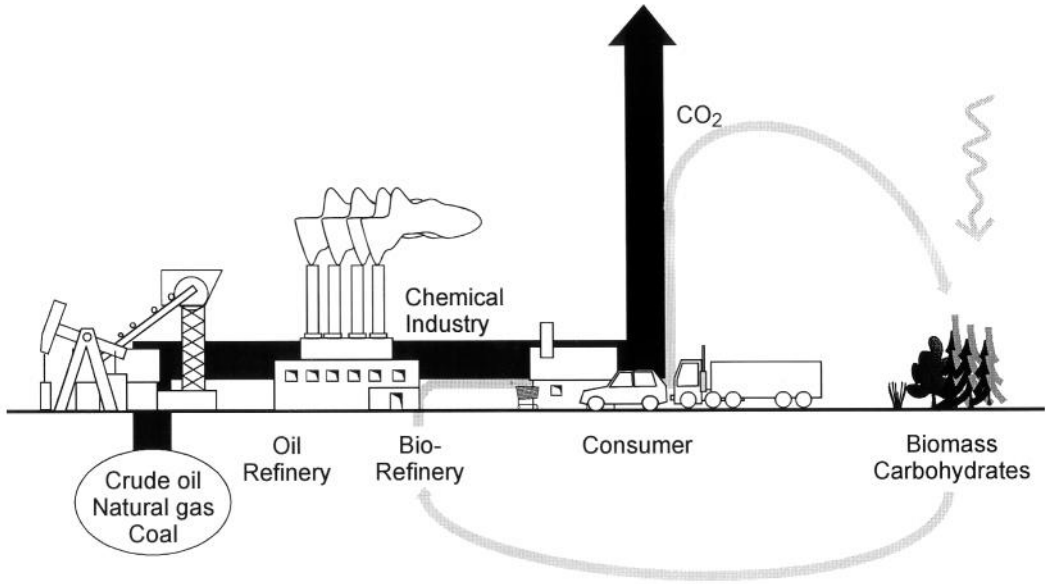

Fig. 2 Biorefinery industry based on renewable resources *versus* petroleum based industry.

in Fig. 1. The third major raw material, methanol, is derived from natural gas or from synthetic gas. Methanol is used as fuel blends (as methyl *tert*-butyl ether MTBE, or in various mixing ratios with conventional petroleum products) or for the chemical synthesis of various C_1, C_2 and C_3 compounds like formaldeyhde, acetaldehyde, acetic acid or glycol.

Some of these C_1–C_4-chemicals can be synthesized from renewable resources like starch, cellulose or other carbohydrates such as sugar from sugar cane or sugar beet. The petroleum crisis of the seventies resulted in a shift from total reliance on fossil resources and simultaneously triggered research into biomass based technologies. Renewable resources became a popular phrase and as a result of the oil crisis, countries like Brasil and the United States initiated national ethanol programmes to partly substitute fossil fuels (Proalcool and Gasohol programmes, respectively). Due to the decrease in oil prices in the eighties, most of the efforts for the production of substitutes for petrochemicals were given up. Nowadays, agricultural surpluses in Europe and worldwide efforts to reduce atmospheric CO_2 emmissions are the major driving forces for the implementation of renewable resource based technologies. The use of biomass as a source of energy and chemicals enables closed cycle material fluxes as indicated in Fig. 2

The objective of this article is to summarize the actual ongoing activities in the field of biotechnology for the production of bulk chemicals. Special focus is put on commercial processes and on genetical strain improvement to broaden substrate and product ranges of microorganisms.

2 State of the art

Today, only a small number of chemicals are produced from renewable resources *via* fermentation. In Europe, the biotechnological production of lactic acid, acetic acid and ethanol are the only processes which are currently applied in technical scales and which can compete with petrochemical routes. While the fermentation routes are limited by pathways of the microorganisms, together with chemical synthesis, a wide range of chemicals can be produced as indicated in Fig. 3.

2.1 Present industrial fermentations

2.1.1 Ethanol from carbohydrates. Ethanol represents the highest volume organic chemical produced predominantly *via*

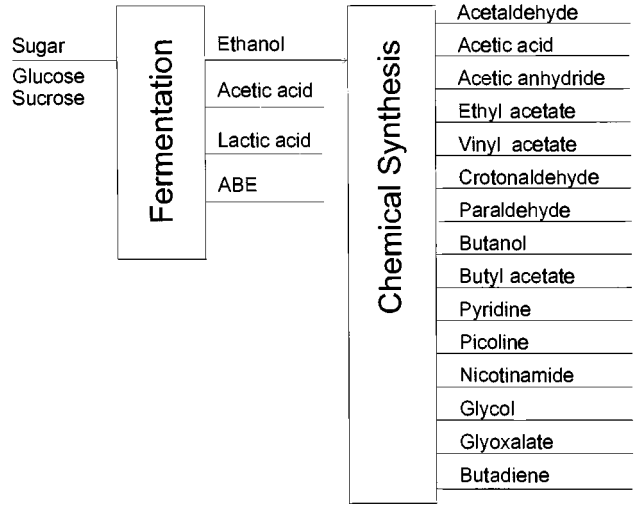

Fig. 3 Chemicals currently produced by fermentation from carbohydrates.

fermentation. While Brasil is producing all its ethanol (15.4 billion liters per annum) from sugar cane, the extent of fermentation ethanol in the United States amounts to 94% (5.3 billion liters per annum) based on starch hydrolysates and sucrose from sugar beet. In Europe, synthetic ethanol still has a considerable market and fermentation ethanol accounts for 60% (1.5 billion liters per annum) of total ethanol produced.

In Brasil, approximately 40% of the automobiles run on pure ethanol fuel. The remainder utilize a blend of 22% ethanol/78% gasoline. In the United States most of the ethanol is applied as fuel blend. However, the use of ethanol in high percentages, such as E85 (85% ethanol, 15% gasoline), remains limited. In european countries like France, the production of ETBE (ethyl *tert*-butyl ether) as a substitute of MTBE as a fuel additive is more favourable than direct blending with gasoline. Spain, Sweden and the Netherlands envisage national programmes for the production of bio-ethanol-ETBE.

Traditionally, ethanol fermentation is based on the conversion of sucrose from sugar cane and sugar beet or of glucose from corn or cereal starch hydrolysates by yeasts, mainly *Saccharomyces cerevisiae*. One glucose molecule is degraded to two molecules of carbon dioxide and two molecules of ethanol. This results in a weight loss of approximately 50% as carbon dioxide which represents less than 5% of the energy content. Product recovery is done by distillation. Steam requirement has been reduced significantly during the past years

resulting in steam consumption of less than 0.1 kg per kg of ethanol.

2.1.2 Acetic acid from carbohydrates. At present, most of the demand for technical acetic acid is met synthetically, which seemed to be the most economic way in the last 100 years. The fermentation by a species of *Acetobacter*, which converts ethanol to acetic acid with final concentrations of a small percentage (4–6%), has been used almost exclusively for vinegar production. Because of the loss of one carbon in the form of CO_2 in the glucose–ethanol fermentation, the theoretical maximum yield of the whole reaction is 2 moles of acetic acid from one mole of glucose or 0.67 g acetic acid per gram of glucose. In commercial practice, the actual yield is 0.50–0.55 g acetic acid per gram of glucose or roughly 75–80% of the theoretical yield.

2.1.3 Lactic acid from carbohydrates. Lactic acid represents a chemical with a small world market volume of 54500–59000 tonnes per annum. While the market for traditional applications of lactic acid is estimated to be growing at about 3–5% annually, new products based on lactic acid may increase the world market share significantly. New applications for lactic acid include the use of derivatives such as ethyl esters to replace hazardous solvents like chlorinated hydrocarbon solvents in certain industrial applications. Furthermore, lactic acid may be polymerized to biodegradable plastics as demonstrated by Danone Inc. in the form of yoghurt cups.

Industrial production of lactic acid is based on the conversion of pure sugar like glucose or sucrose with bacteria of the genus *Lactobacillus* at temperatures around 40–45 °C under anoxic conditions. One mole of glucose results in almost two moles of lactic acid. Unlike in ethanol fermentations, the recovery process for lactic acid is much more sophisticated involving various precipitation, chromatographic and/or distillation steps.

2.1.4 Acetone–butanol–ethanol fermentation. An acetone–butanol–ethanol (ABE) blend (ratio 3:6:1) may serve as an excellent car fuel, which can be easily mixed not only with 'super' fuels but also with diesel. ABE as a fuel additive has the advantage of a similar heat of combustion to hydrocarbons, and perfect miscibility with hydrocarbons, even when water is present. The fermentative production of ABE used to be the second largest industrial fermentation after ethanol production. Especially during the World Wars, acetone production increased while butanol was considered to be an undesirable byproduct. In 1945, 60% of butanol demand of the United States was produced *via* fermentation. Due to the shortage of raw materials, namely corn and molasses, and to decreasing prices of oil, all plants were closed in the years that followed. In 1981, the last plant in the world closed down in South Africa.

The traditional fermentative production of acetone–butanol–ethanol is a batch fermentation with *Clostridia*, a strictly anaerobic bacteria. The substrate consists of molasses, and phosphate and nitrogen sources. The substrate fomulation is sterilized prior to fermentation. The fermentation itself takes place under strictly anaerobic conditions and lasts 40–45 h followed by product separation by distillation. Solvent yields based on the fermentable sugars were usually around 29 to 33%. Instead of molasses other sugar sources like maize mash or sugar from polysaccharide type plant material may serve as a raw material for fermentation.[2]

2.2 Products derived from fermentation ethanol

In the seventies, ethanol was used to act as a basic building block in the organic chemical industry, when chemicals like ethylene and acetaldehyde were synthesized from fermentation ethanol. Currently, no ethylene plant based on this technology is in operation, but, in a few developing countries, some other ethanol based technologies are still applied, mainly because of relatively cheap ethanol and high-cost petroleum. In India for instance, more than 20 companies are producing not only ethanol from sugar cane or molasses but also chemicals like acetic acid, acetic anhydride or ethyl acetate from fermentation ethanol. Fig. 4 gives an example of the two most versatile organic companies: Somaiya Organo Chemicals Ltd and VAM Organic Chemicals Ltd. Other enterprises are mainly focusing on the production of acetic acid and ethyl acetate. These

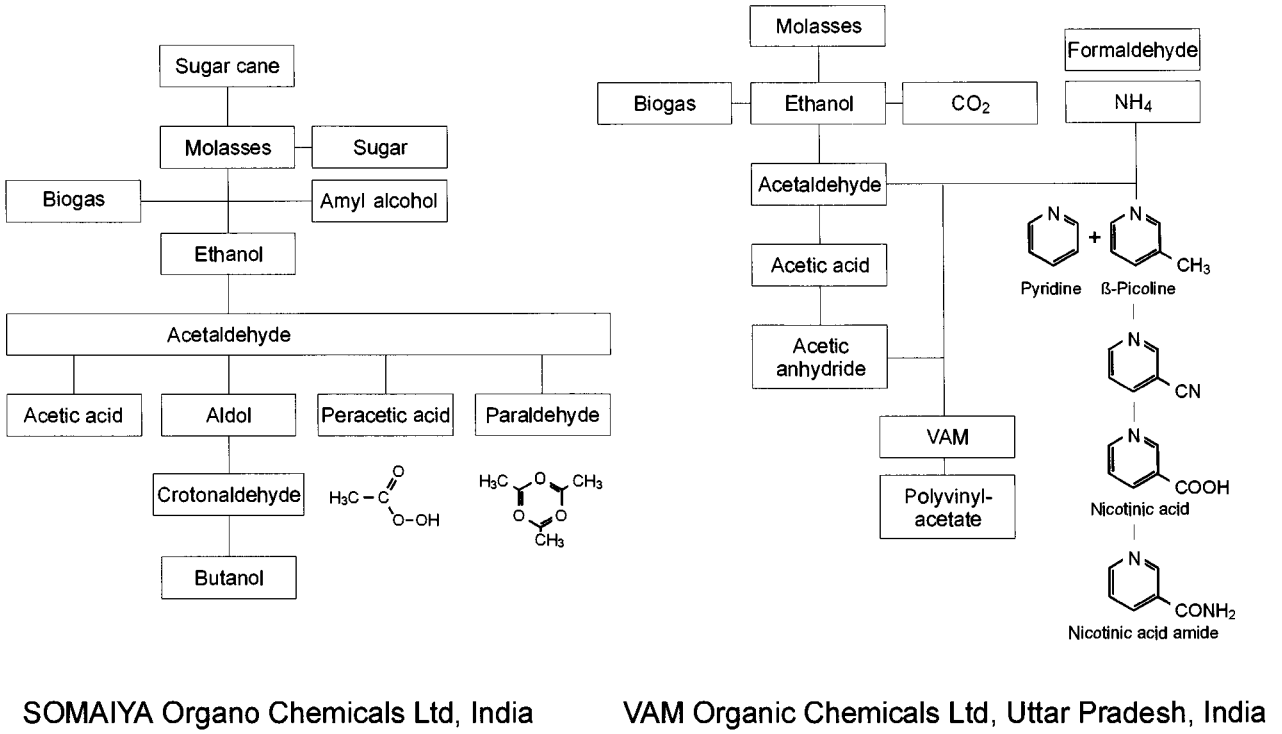

Fig. 4 Production of chemicals from fermentation ethanol in India.

activities meet 100% of the Indian demand for acetic anhydride and ethyl acetate and almost 80% of the acetic acid demand.[3] Production in Brasil has been decreasing during the last years leaving Cloroetil Solventes Acéticos S.A. as the only bio-ethanol based chemical company focusing on acetaldehyde, acetic acid and ethyl acetate.

2.2.1 Acetaldehyde and derivatives. Acetaldehyde is obtained from ethanol by catalytic dehydration or catalytic oxidation. The dehydration process involves Cr/Cu/Si-catalysts at temperatures of 350 °C resulting in acetaldehyde and hydrogen, which may be used as hydrating agents.[4] The oxidative process, which is still applied in India, involves silver catalysts at 550 °C. Yields of acetaldehyde from ethanol for both processes vary from 87 to 92% (w/w).

Aldol condensation can lead to butanediol, which is an important intermediate for polyesters. However, no plant could be found applying this process on a large scale today, with the exception of crotonaldehyde (but-2-enal) production by aldol condensation. Somaiya Organo Chemicals Ltd., India, is converting acetaldehyde to aldol which is then distilled with acetic acid as catalyst to produce crotonaldehyde. Further conversion to *n*-butanol with hydrogen from ethanol dehydration was performed until a few months ago when rising substrate costs (molasses) altered the economic feasibility of the process and resulted in a shut down of the production. To produce 100 kg of butanol, 145 kg acetaldehyde were required (79.8% of the theoretical yield).[5]

The cyclic trimer of acetaldehyde, paraldehyde, is still produced with mineral acids as catalysts at temperatures of 5–6 °C.[5]

Butadiene, which is almost entirely used in rubbers like styrene–butadiene rubber for the auto tyre industry may be manufactured from ethanol and acetaldehyde at 310 °C with tantalum oxide on silica gel. Union Carbide Corporation was operating plants using this process during World War II. Until the late seventies, India, Brasil and Russia were using this process, but today no plant could be found which is still in operation.

2.2.2 Acetic acid and derivatives. Acetic acid is obtained by oxidation of acetaldehyde. This oxidation is performed at moderate temperatures (50–60 °C) in the presence of catalysts like Mn/Co or mixtures of $KMnO_4$ and $Mn(CH_3COO)_2$. Yields are around 97% of the theoretical yield. The overall yield of acetic acid based on consumed sugar is 49–52 kg of acetic acid per 100 kg of glucose, which represents 74–78% of the theoretical yield (2 moles acetic acid per mole of glucose).

Acetic anhydride can be obtained by the reaction of acetic acid at 700 °C under vacuum or by synthesis of acetic acid and acetic anhydride from acetaldehyde at 5 bar with cobalt(II) acetate at moderate temperature (55 °C). Today, both processes are still applied in India by VAM Organic Chemicals Ltd. and The Dhampur Sugar Mills Ltd, respectively (Fig. 5). One major application of acetic anhydride is the production of cellulose acetate for textile yarns or cigarette filters.

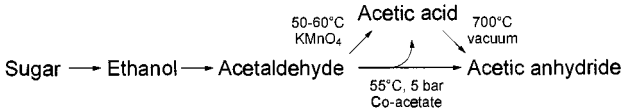

Fig. 5 Synthesis of acetic anhydride.

To produce 1000 kg of acetic anhydride 1260 kg of acetic acid are required. The direct synthesis from 100 kg acetaldehyde gives practical yields of 50 kg acetic acid and 50 kg acetic anhydride at theoretical yields of 58 and 68.2 kg.

Vinyl acetate monomer (VAM) is an intermediate in poly(vinyl acetate) production and therefore has a considerable market in the polymer industry (resins and lattices). In India, 10000 tonnes per annum are produced from fermentation ethanol by VAM Organic Chemicals Ltd. Acetaldehyde and acetic anhydride are converted at 90 °C with toluene-*p*-sulfonic acid as catalyst to vinyl acetate monomer and acetic acid as byproduct. Separation of products is achieved by distillation. 600 kg acetaldehyde and 1400 kg acetic anhydride are required to give 1000 kg vinyl acetate and 800 kg of acetic acid.[6]

Acetic acid esters have a considerable market as solvents. In Brasil, the market is increasing because ethyl esters may replace toxic cyclic solvents as toluol or benzol. Therefore, companies often synthesize esters from their products, namely ethanol, butanol and acetic acid giving ethyl acetate and butyl acetate as products. The technology for the production of ethyl acetate is still applied in Brasil (Cloroetil Solvents S.A.) and India (VAM Organic Chemicals Ltd.). The well known Tischtschenko process is currently not used on a technical scale, although an ongoing research project in Brasil is addressing this topic.[4] Somaiya Organo Chemicals Ltd produce butyraldehyde. The process utilises 600 kg of acetic acid and 600 kg of butanol to produce 1000 kg of butyraldehyde.

2.2.3 Other chemicals derived from fermentation ethanol. In India, considerable amounts of pyridine and β-picoline are produced from acetaldehyde, formaldehyde and ammonia. The production of β-picoline is especially attractive because further conversion to nicotinamide and nicotinic acid is possible.[6]

3 Research activities

There are a few major hurdles in the use of renewable resources like the availability of biomass at a constant quality the whole year over, the fractionation technology of lignocellulosic materials, limitations due to the metabolism of microorganisms, and the lack of integrated biomass based technologies. Recent research has tried to address these hurdles using different approaches. Of interest is the genetic engineering of microbial metabolism which yields various opportunities to produce new chemicals out of novel sugar resources (Fig. 6).

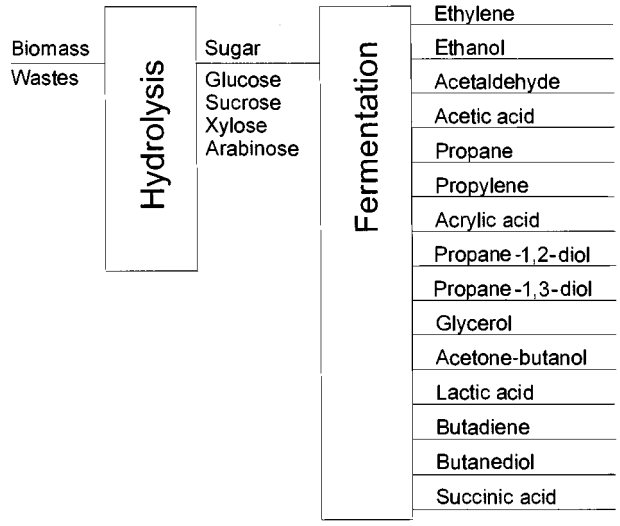

Fig. 6 Production of novel chemicals from lignocellulosic raw materials.

3.1 Raw material

Traditionally hexose sugars have been the major fermentation feedstocks. In Europe, the availability of these substrates is limited to sugar beet or sweet sorghum and starch hydrolysates

from corn, cereals or potatoes. In order to reduce the cost of fermented products it is essential to expand the range and form of raw materials to produce fermentation feedstocks. In addition, reductions in the cost of feedstocks could be achieved through the use of all the available sugars in the raw material sources. Therefore, the development of innovative techniques to hydrolyse hemicellulose and cellulose creates opportunities to use a range of non-traditional biomass resources as fermentation raw materials.

Fractionation of lignocellulosic materials may be achieved by various physical, chemical and biological methods like milling, extrusion, steam explosion or enzymatic hydrolysis. Combination of different methods may lead to sugar solutions with both pentose and hexose sugars from the hemicellulose and cellulose fraction of lignocellulosic materials.[7]

Developments in the field of plant breeding will not only increase the content of fermentable sugar but also will influence the processing of lignocellulosic materials. Research results on viable mutant plants with altered lignin synthesis capability are quite promising. This might allow a more extensive exploitation of plants.[8]

3.2 Microbiological approaches

Traditional fermentation processes have been based on hexose sugars. However, a large number of organisms have been screened and shown to be able to also use pentoses like xylose or arabinose. In general, these microorganisms have the disadvantage of low product tolerance.[9] Considerable work especially in the United States, UK and Sweden has been carried out to improve pentose fermentation.

On the other hand, genetic engineering has enabled scientists to broaden the spectrum of fermentatively produced chemicals. Due to the metabolic restrictions in microorganisms, only a few bulk products like ethanol, lactic acid or acetone–butanol can be produced *via* fementation. Changing fermentation technologies together with genetic engineering can broaden the product spectrum of microorganisms. Recombinant microorganisms with altered sugar metabolism are able to ferment sugar to chemicals, which the corresponding wild type strain does not produce. The following chapter will summarize research in these areas.

3.2.1 Ethylene from sugar based resources. A wide variety of fungi and bacteria have been found in soil and on the surface of fruits, that directly produce ethylene. These organisms are able to form ethylene from renewable resources like hydrolysates of organic biomass. In the last 20 years quite a lot of research has been done on different biosynthetic pathways of ethylene forming microorganisms. However, biotechnological production of ethylene in large industrial scale remains negligible.

In principle, two biosynthetic pathways for the production of ethylene in microorganisms have been described (Fig. 7). In one pathway, ethylene is produced *via* 2-oxoglutarate by an ethylene forming enzyme as in *Penicillium digitatum* and *Pseudomonas syringae*. This ethylene forming enzyme has been sequenced and was cloned and expressed in *Escherichia coli* and *Pseudomonas syringae* and *Pseudomonas putida*.[10] The ability of recombinant cells to synthesize the ethylene forming enzyme increased with specific activities 41 times higher than that of the ethylene forming enzyme in the parental *P. syringae*. In the second pathway, ethylene is produced *via* *S*-methyl 2-keto-4-thiobutyric acid, a deaminated derivative of L-methionine, by an NADH-Fe(III)EDTA oxidoreductase as in *Escherichia coli* strain B SPAO.[11]

Substrates for the fermentation are glucose, glycerol or L-methionine. Sakai *et al.*[11] succeeded in transferring the gene for the ethylene forming enzyme into the cyanobacterium *Synechococcus* sp. PCC 7942. The enzyme catalyses the conversion of atmospheric CO_2 to ethylene. Sakai *et al.*[11] reported a maximum specific ethylene forming activity of 323 nl ml^{-1} OD_{730}^{-1} h^{-1} a maximum ethylene formation of 16.4 μl ml^{-1} and a carbon recovery of 5.84% for ethylene (percentage of total carbon fixed incorporated into ethylene).

3.2.2 Ethanol from lignocellulosic raw materials. Genetic engineering could reduce the production costs for ethanol dramatically. Research is mainly focused on the fermentation of pentoses and other unusual sugars. The pentose sugars of importance are D-xylose and L-arabinose, which comprise up to 30% of the neutral carbohydrates derived from agricultural crop residues, wood and other plant materials. However, the conversion of pentose sugars is difficult to achieve because of the lack of suitable biocatalysts. Research activities in the field of simultaneous bioconversion of cellulose and hemicellulose to ethanol have been summarized recently.[12]

Activities in the United States focus on recombinant strains of *Zymomonas mobilis*, *E. coli* and on recombinant *Saccharomyces cerevisiae*. *Zymomonas mobilis*, a bacterium, has a high ethanol yield and product tolerance. Furthermore, it has considerable tolerance to inhibitors formed during hydrolysis of lignocellulosic materials. Zhang *et al.*[13] introduced four genes encoding xylose assimilation and pentose phosphate pathway enzymes into *Zymomonas mobilis* to enable it to grow on xylose as the sole carbon source with efficient ethanol production (xylose isomerase, xylulokinase, transketolase and transaldo-

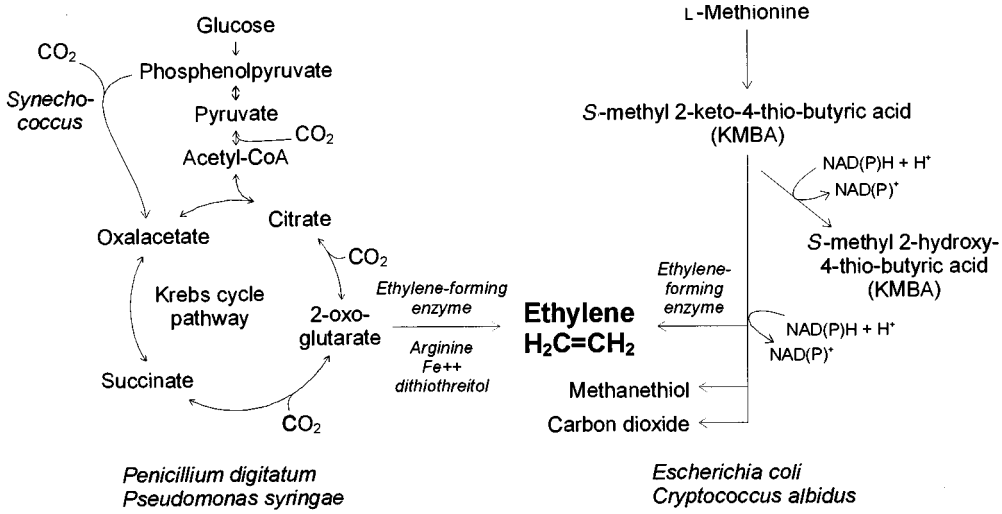

Fig. 7 Microbial synthesis of ethylene from renewable resources.

lase, see Fig. 8). Yields of ethanol produced were 0.44 g per gram of xylose consumed, which corresponds to 86% of the theoretical yield (5 moles of ethanol from 3 moles of xylose).

Ingram *et al.*[14] introduced the genes encoding for alchohol-dehydrogenase and pyruvate-decarboxylase from *Zymomonas mobilis* into *Escherichia coli* KO11, which enables fermentation of hemicellulose hydrolysates of agricultural wastes like bagasse, corn stover and corn hulls. Ethanol concentrations of over 40 g l^{-1} within 48 h could be attained, with yields ranging from 86% to over 100% of the maximum theoretical yield of 0.51 g ethanol per gram of sugar. BC International Corporation together with the US Department of Energy are currently building the first large scale biomass-to-ethanol plant in Jennings, La. based on this strain.[15]

Genetic modifications of *Saccharomyces* strains to enhance its sugar utilization range are reported by the insertion of genes encoding xylose reductase, xylitol dehydrogenase and xylulokinase.[16] Strain LNH-ST with genes integrated into the chromosome can co-ferment glucose and xylose with yields of 63.5% of theoretical yield on pretreated corn biomass and 70.4% on synthetic glucose–xylose mixtures.

In Europe, the company Agrol Ltd. (UK) focuses on the screening of thermophilic strains like *Bacillus stearothermophilus*,[17] which are naturally capable of utilizing pentoses. Genetic strain improvement is applied to lower byproduct formation by decreasing the activities of lactate dehydrogenase and pyruvate-formate lyase.

3.2.3 Acetaldehyde. The production of acetaldehyde has been investigated by applying different approaches. In the first, ethanol can be oxidized to acetaldehyde using *Candida utilis* or *Pichia pastoris*. Production of acetaldehyde by this method must be carefully regulated to limit the conversion of acetaldehyde to acetic acid. However, there is no significant advantage compared to the chemical oxidation described earlier.

Acetaldehyde can also be fermented directly from sugar. Changing the fermentation strategy from ethanol to acetaldehyde has some major advantages. First of all, acetaldehyde has a boiling point of 20.8 °C, which enables easy separation from the fermentation broth by stripping. Ethanol boils at 78.5 °C and separation makes up two-thirds of total production costs. On the other hand, acetaldehyde forms no azeotropic mixture with water which again reduces purification costs.

Direct fermentation of acetaldehyde with *Zymomonas mobilis* is described with two different strategies. Wecker and Zall[18] were mutating and screening for strains with decreased alcohol dehydrogenase activities (Fig. 9). Strain selection was done by adding allyl alcohol, which is normally converted by alcohol dehydrogenase to acrylaldehyde, a substance highly toxic to cells. Therefore only strains without alcohol dehydrogenase activities are able to grow in the presence of allyl alcohol making it easier to screen for mutants lacking alcohol dehydrogenase activity. On the basis of the amount of glucose utilized, the level of acetaldehyde production represents nearly 40% of the maximum theoretical yield.

Tanaka *et al.*[19] were investigating the performance of *Zymomonas mobilis* under various oxygen supply conditions. Aeration increases the activity of the NADH-oxidase and consequently the availability of NADH for the alcohol dehydrogenase is decreased. With optimised aeration rates, yields around 55% of the theoretical yield based on utilised glucose could be observed.

Acetaldehyde may also be produced with *Saccharomyces cerevisiae* using a well known process employed by the Germans during World War I. In this case, acetaldehyde is fixed by sodium sulfite and therefore can not act as hydrogen acceptor like in conventional ethanol fermentation. Instead of acetaldehyde dihydroxyacetone functions as the main hydrogen acceptor and becomes reduced to glycerol. Alcohol and acetaldehyde are separated by distillation, glycerol may be recovered after precipitation of sulfite by the addition of calcium oxide or calcium hydroxide followed by filtration. Technical glycerol is then obtained by distilling the supernatant liquor. 100 g of hexose theoretically yields 51 g of glycerol and 24.4 g of acetaldehyde, practical yields were about 20–25% glycerol, 30% alcohol and 5% acetaldehyde. It required almost 12 kg of refined sugar to produce 1 kg of dynamite glycerol on industrial basis. Losses of glycerol are mainly due to inefficient recovery processes.[20]

3.2.4 Acetic acid. The traditional biological production of acetic acid by a species of *Acetobacter* has been used almost exclusively for making vinegar. Because of the loss of one

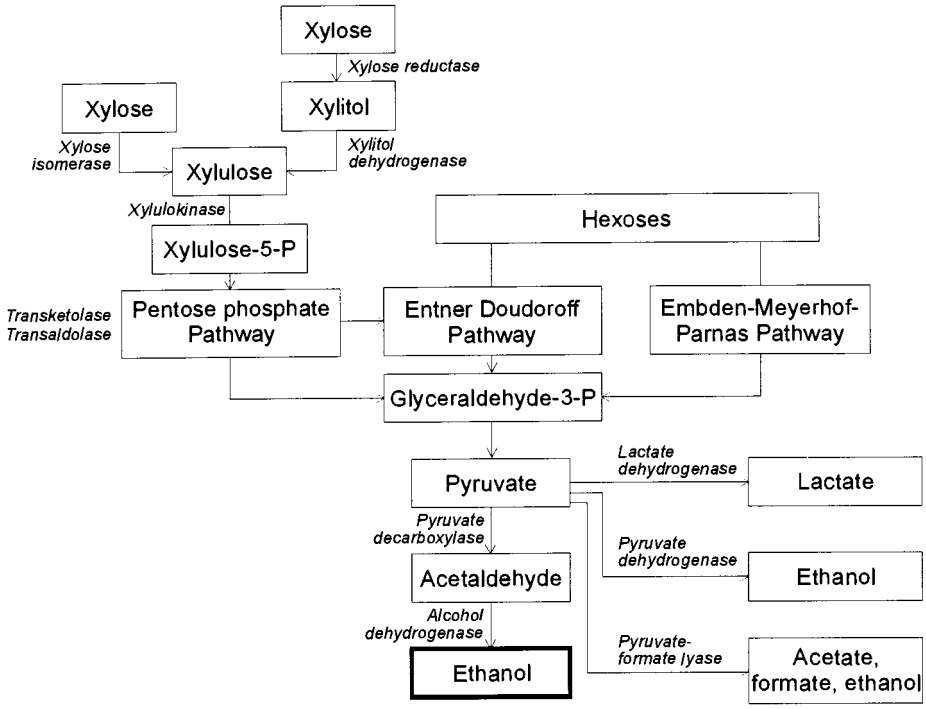

Fig. 8 Enzymes of interest for increasing biotechnological ethanol production.

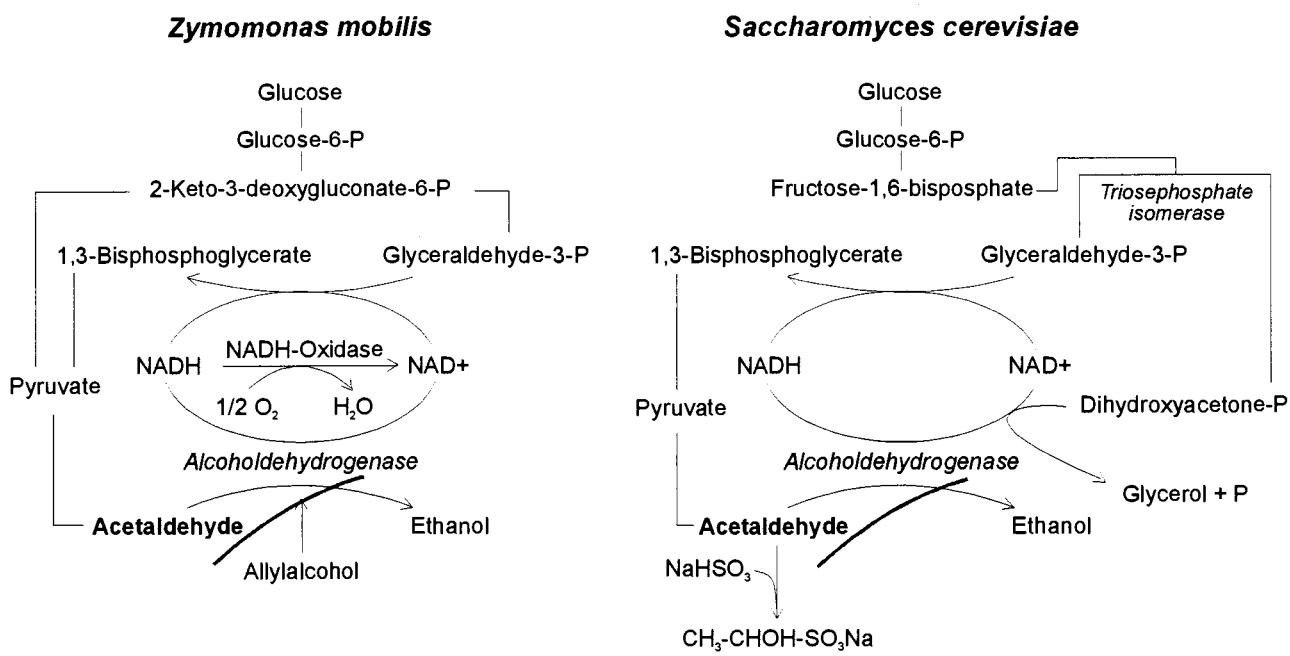

Fig. 9 Pathways for the production of acetaldehyde by *Zymomonas mobilis* and *Saccharomyces cerevisiae*.

Traditional production

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2$$
$$2\,C_2H_5OH + O_2 \rightarrow 2\,CH_3COOH$$
$$\text{Net reaction: } C_6H_{12}O_6 \rightarrow 2\,CH_3COOH + 2\,CO_2$$

Clostridium thermoaceticum

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow 2\,CH_3COOH + 2\,CO_2 + 8\,H^+ + 8\,e^-$$
$$2\,CO_2 + 8\,H^+ + 8\,e^- \rightarrow CH_3COOH + 2\,H_2O$$
$$\text{Net reaction: } C_6H_{12}O_6 \rightarrow 3\,CH_3COOH$$
$$2\,C_5H_{10}O_5 \rightarrow 5\,CH_3COOH$$

Fig. 10 Production of acetic acid by *Clostridium thermoaceticum*. 1 Mole glucose yields 3 moles of acetic acid, 2 mole of pentoses yield 5 moles of acetic acid.

carbon through CO_2 in the glucose to ethanol fermentation, the theoretical maximum yield of the whole reaction is 2 moles of acetic acid from one mole of glucose or only 0.67 g acetic acid per gram of glucose. In commercial practice, the actual yield is 0.50–0.55 g acetic acid per gram of glucose or roughly 75–80% of the theoretical yield.

Fermentation with *Clostridium thermoaceticum*, a spore forming thermophilic bacterium (optimum 55–60 °C), offers a significant advantage in terms of acetate yield compared to the conventional vinegar fermentations because this strain can, theoretically, produce 3 moles of acetic acid from 1 mole glucose (Fig. 10). In practice, 85% of the sugar may be converted to acetic acid. Furthermore, *Clostridium thermoaceticum* is strictly anaerobic and therefore, no costly aeration is required.

Additionally, *Clostridium thermoaceticum* is able to ferment various sugars like fructose, glucose and xylose. Unfortunately, the toxicity of acetate, pH optima and production rates do not suggest successful applications of this strain for industrial production of acetic acid. Clearly, new mutant strains must be obtained with improved properties for these applications, as demonstrated by Parekh and Cheryan.[21] Another possibility would be the isolation from nature of new strains or species of acetogens with properties suitable for industrial use.

Leigh *et al.*[22] isolated a new chemolithoautotroph, homoacetogenic, thermophilic, anaerobic microorganism *Acetogenium kivui* that oxidizes hydrogen and reduces carbon dioxide to acetic acid. The temperature optimum for growth is 66 °C and the optimum pH is 6.4. Suitable growth substrates include glucose, mannose, fructose, pyruvate and formate. *Acetogenium kivui* theoretically produces 3 moles acetic acid from 1 mole of glucose. Because of strong product inhibition, maximum acetate concentration is 30–40 g l^{-1}. In batch fermentation, 280 mM glucose are converted to 625 mM acetic acid in 50–60 h, which is a yield of 2.55 mol per mole of glucose (85% of theoretical).

3.2.5 Propane and propylene. Although propylene has to be considered as a byproduct of ethylene production, research on the biotechnological production of propylene has been carried out. According to Fukuda[23] C_3-hydrocarbon-producing strains are widely distributed. Among the 178 strains tested, 87 (49%) produced propane and 37 propylene from glucose media. The C_3-hydrocarbons are usually produced together with other hydrocarbons such that a mixture of propane, propylene, butane, butene, pentane, *etc.* is obtained. Unless the hydrocarbons are used for fuel purposes, some purification (usually distillation) will be required. Compared to C_2-hydrocarbons, the production rates are rather low. Highest productivity for propane is reported for *Cryptococcus albidus* (6.5 nl ml^{-1} h^{-1}) and *Brevibacterium ammoniagenes* (8.6 nl ml^{-1} h^{-1}), while the most efficient propylene producing strains are *Gliocladium roseum* (3.0 nl ml^{-1} h^{-1}) and *Schizosaccharomyces octosporus* (1.2 nl ml^{-1} h^{-1}) respectively.

Levy *et al.*[24] describe a process for the production of propylene from industrial waste streams. Propylene is produced from the electrolytic oxidation of butyric acid, which is formed by the suppressed-methane anaerobic fermentation of carbohydrates in waste water and recovered by liquid–liquid extraction. Besides propylene, methane and hydrogen may be recovered as byproducts. Yields are 15.1% propylene, 8.9% methane, 0.6% hydrogen and more than 50% CO_2 depending on waste sugar. Although yields seem to be quite low, very optimistic figures on return on investments are presented.

3.2.6 Acrylic acid. Although acrylic acid and chemicals associated with acrylics have a world market volume of almost 3×10^6 tonnes per annum, relatively few attempts have been made to produce them with microorganisms. Currently 100% of acrylic acid is produced from fossil fuels. The production from renewable resources is propagated *via* lactic acid fermentation and subsequent chemical conversion to acrylic acid. Un-

fortunately, the chemical conversion gives rather low yields due to decarbonylation, decarboxylation, and condensation reactions which mainly lead to acetaldehyde or penta-2,3-dione.[25]

Only a few microorganisms have been described that produce acrylic acid as a biochemical intermediate substance but observations of free acrylic acid in biological systems are rare. Anaerobic formation of acrylic acid is found in the direct reduction pathway of lactic acid of microorganisms like *Clostridium propionicum*. This conversion is a dehydration reaction. When this microorganism uses lactic acid as the energy source, the main metabolic products are propionic acid (2/3) and acetic acid (1/3). The propionic pathway may be blocked with 3-butynoic acid. Nevertheless, acrylate concentrations never exceeded 1% of the initial substrate concentration. These low yields are due to the enrichment of reducing equivalents like ferredoxin, rubredoxin and flavodoxin, which inhibit further growth of cells. These reducing equivalents can be regenerated by providing the cells with an external electron acceptor. Research activities in this area are limited and were summarized recently.[26]

3.2.7 Propanediol. The only industrial process for manufacturing propane-1,2-diol (propylene glycol) is direct hydrolysis of propylene oxide with water. Dipropylene glycol and tripropylene glycol are obtained as byproducts. Biotechnological research concerning propane-1,2-diol concentrates upon the formation of enantiomers, especially (*R*)-propane-1,2-diol. This compound can be used in organic synthesis, *e.g.* for the preparation of (*R*)-propylene oxide, optically active polymers and chiral crown ethers.

Different strategies are followed to obtain (*R*)-propane-1,2-diol from renewable resources *via* fermentation involving *Clostridium sphenoides* and several strains of *Thermoanaerobacterium thermosaccharolyticum*.[27] The formation of propane-1,2-diol starts from dihydroxyacetone, a common intermediate in sugar metabolism. Conversion to methylglyoxalate and subsequent reduction to lactaldehyde or hydroxyacetone and further reduction gives propane-1,2-diol as the end product (Fig. 11). As substrate, various sugars like glucose, mannose, xylose and cellobiose have been used.

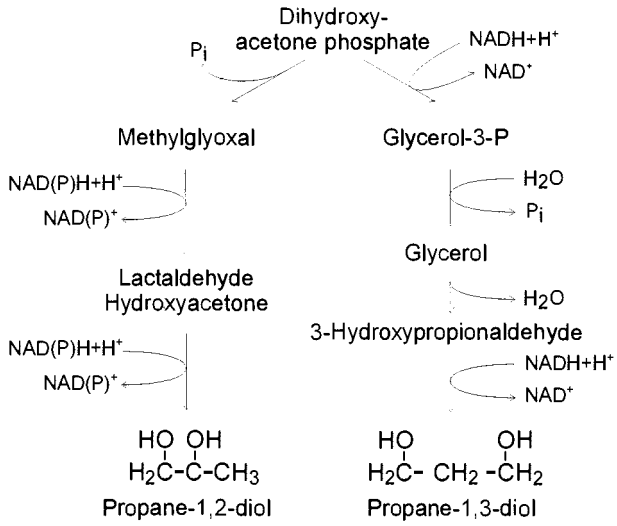

Fig. 11 Metabolic pathways to propane-1,2-diol and propane-1,3-diol (Cameron *et al.*[28])

Unlike propane-1,2-diol, propane-1,3-diol (1,3-PD) is produced on a much smaller scale. Due to the difficulties in manufacturing the product, the price is too high compared to other diols. As a result, the use of 1,3-PD is limited to applications requiring very specific performance characteristics. A potential large scale application represents the manufacturing of polyesters like poly(propylene terephthalate) PPT used in carpet fibers. Several microorganisms are known to ferment glycerol to 1,3-PD. The conversion of glycerol consists of three steps involving 3-hydroxypropionaldeyde (3-HPA, hydracrylaldehyde) as an intermediate. Due to the high price of glycerol, this fermentation is not economically attractive, however, it provides the basic knowledge for constructing recombinant microorganisms. Cameron *et al.*[27] cloned and expressed the genes for the conversion of glycerol to 1,3-PD from *Klebsiella pneumoniae* in *Escherichia coli* and *Saccharomyces cerevisiae*. As *S. cerevisiae* produces glycerol from glucose as a byproduct, these gene transfers enable the direct fermentation of 1,3-PD from glucose.

Theoretical product yields are 2 moles of propanediol per mole of glucose (0.84 g g^{-1}). As propanediol is more reduced than glucose, another compound has to be oxidised to maintain the overall electron balance. Assuming that the oxidized byproduct is CO_2 from glucose, the maximum theoretical yield is 1.5 mol mol^{-1} (0.63 g g^{-1}). Practical yields are lower but, nevertheless, DuPont and Genencor are working on the production of propane-1,3-diol from sugar.[28]

3.2.8 Glycerol. Glycerol is a component of all plants and animal fats and oils. However, it is not found in its free form but as a component of fatty acid esters. The glycerol content of fats and oils varies between 8 and 14%. In the USA 74% of the glycerol capacity is made up by production from fats and oils.

Fermentation of sugar (from molasses) by *Saccharomyces cerevisiae* in presence of sulfite (Neuberg's second type of fermentation) has been described earlier in this article (Section 3.2.3 and Fig. 9). Another way to produce glycerol with *Saccharomyces cerevisiae* but without sulfite has been described by Compagno *et al.*[29] The triosephosphate isomerase gene was inactivated by genetical engineering. Due to this inactivation, the accumulated dihydroxyacetone phosphate was reduced to glycerol. Glycerol was obtained in high molar yields (90%) as the major fermentation product. As the process is carried out in a medium consisting mainly of glucose and phosphate, problems with the recovery of glycerol have been reduced significantly.

Rehm[30] refers to investigations on the green algae species *Dunaliella tertiolecta* and *Dunaliella bardawil* which produce glycerol in a medium with high concentrations of NaCl. The possible advantages of these algae are that, CO_2 as a cheap and renewable resource is used as carbon source, solar energy is the main energy source, and protein as well as β-carotene may be obtained as valuable byproducts.

3.2.9 Lactic acid. Improvements on the economics of the process are mainly focused on broadening the substrate range, increasing the lactic acid tolerance, reducing the requirements for complex and cost intensive growth supplements and on product recovery. New processes include simultaneous saccharification and fermentation, thermophilic fermentation with *Bacillus stearothermophilus*, continuous fermentation and product recovery with membrane bioreactor systems and electrodialysis, and novel recovery technologies with ion exchange chromatography.[31]

3.2.10 Butanediol. Butane-2,3-diol has been known as a bacterial fermentation product since early this century. During World War II, fermentative butane-2,3-diol production was of great interest, mainly because it may serve as a precursor for the manufacture of buta-1,3-diene. However, none of the developed processes have been applied on a commercial scale. The interest in butane-2,3-diol production has been renewed because various sugar sources including both hexoses and pentoses from

lignocellulosic hydrolysates can be fermented. Furthermore, apart from butane-1,3-diol, a broad range of chemicals and products may be synthesized out of butane-2,3-diol namely, polyurethane, γ-butyrolactone or octane booster for gasoline.

Maximum theoretical product yields from 1 mol glucose are 0.67 mol butanediol and 0.33 mol CO_2, while maximum observed yields of both glucose and xylose range between 60 and 70% of the theoretical value. On a mass basis, this is equivalent to 0.30 to 0.35 g butanediol per gram of glucose. Besides CO_2, acetate, formate, ethanol and lactic acid are the major byproducts. Cao *et al.*[32] have reported yields of 0.31 g butanediol and 0.088 g ethanol per gram of corn cob cellulose which was hydrolysed by fungal cellulases.

3.2.11 Butadiene. Unfortunately, there is no method for the direct production of butadiene from renewable resources involving microorganisms as catalysts. Anyhow, there are some syntheses described for the chemical production of butadiene out of acetaldehyde and ethanol, which may be derived from fermentation processes, as highlighted earlier.

Butadiene from fermentation of butanediol has been described, especially in very old literature.[20] It is rather difficult to convert butane-2,3-diol to compounds with ethylenic double bonds by catalytic dehydration due to the strong tendency towards formation of the methyl ethyl ketone instead. The most successful process for the manufacture of butadiene from butane-2,3-diol is pyrolysis of the diacetate. The process is known to give butadiene of over 99% purity. The reaction is usually accomplished by passing the vapor of the diacetate through an unpacked tube at 585 °C to 595 °C. Yields of 82% are obtained in a single pass with a cumulative yield of about 87% of the theoretical yield.

According to McCutchan and Hickey[20] butadiene could also be produced from *n*-butanol, which was one of the driving forces for the industrial development of the acetone–butanol fermentation process at the beginning of the century. Unfortunately, no yields for the conversion of *n*-butanol to butadiene are available and data on the technical process are scarce.

3.2.12 Succinic acid. Succinic acid is of special interest because it may serve as an intermediate in the production of chemicals like butane-1,4-diol, tetrahydrofuran, γ-butyrolactone or adipic acid (precursor to nylon). Therefore, succinic acid has numerous potential uses in textile, plastics and resins, detergents and the food industry. It is currently produced by hydrogenation of maleic anhydride derived from petrochemical feedstocks.

Nghiem *et al.*[33] present a novel fermentation process for the production of succinic acid from renewable resources. The process is based on a recombinant *Escherichia coli* strain ATCC 202021 which can convert corn sugar into succinic acid. The process consists of two stages, the first is the growth phase under aerobic conditions, while the second is the main production phase under anaerobic conditions during which glucose is converted mainly into succinic acid with acetic acid and ethanol as byproducts. The developed process seems to be economically feasible and will be commercialised by Applied CarboChemicals, Inc. (USA).

4 Economical aspects

It has been demonstrated that various biotechnological processes for the production of chemicals from renewable resources are technically feasible, however, they remain uneconomical under current conditions. As a result, only a limited number of processes have made their way from laboratory scale to commercial industrial scale until now. At the same time, processes based on biomass lost economic competiveness as a direct result of cheaper petrochemical resources. Different factors are responsible for this development and these are discussed below.

4.1 Substrate costs

The cost of raw materials for fermentations can represent up to 70% of the total value of the fermentation product, particularly for commodity chemicals. Hence, substrate costs are the most critical costs in the production of bulk products from renewable resources. As stated earlier in this article, the availability of cheap substrates from wood, forest wastes, paper mill wastes, crop plants, agricultural residues, municipal wastes or algae is imperative for economically viable processes.

Changes in the price of raw materials can be achieved through the following strategies:

- Increasing the yield per hectare and/or producing crops with higher content of desirable ingredients
- Increasing the value of byproducts
- Fractionation of whole plant biomass by hydrolysis and natural lignin recovery
- Modifying crops or cultivation of new crops grown especially for industrial purposes
- Using wastes as raw material

Conventional fermentation processes often consider just one single product from the whole crop resulting in a scenario whereby the final product is burdened by the whole raw material cost. Biomass based plants have to convert all components of the starting material into useful products just as a petroleum refinery utilises all of the raw material to achieve low production costs. Hence, not only sugar has to be considered as raw material for fermentation, but also lignin or fibers as raw material for other industrial applications.[34]

The maximum allowable raw material costs for various chemicals can be calculated from the final product price and the corresponding fermentation yields as shown in Table 1. Product prices are taken from the Chemical Marketing Reporter.[35] The maximum sugar price (theoretical) refers to the maximum allowable price for substrate under theoretical yields, while the maxium sugar price (practical) equals the maximum allowable sugar price under observed product yields. At a given sugar price of approximately 0.4 Euro kg^{-1}, only the direct fermentation of acetic acid and lactic acid can be economically feasible. Economically viable processes can only be achieved if substrate

Table 1 Maximum allowable sugar prices for selected commodity chemicals.

Fermentation product	Process	Theoretical yield/kg kg^{-1}	Observed yield/kg kg^{-1}	Product price/ Euro kg^{-1}	Max. sugar price (theory)/ Euro kg^{-1}	Max. sugar price (pract.)/ Euro kg^{-1}
Ethylene	*via* ethanol-fermentation	0.32	0.27–0.28	0.55–0.60	0.18–0.19	0.15–0.17
Ethanol	direct fermentation	0.51	0.43–0.46	0.68	0.35	0.30–0.31
Acetic acid	*via* ethanol fermentation	0.70	0.54–0.58	0.77–0.88	0.54–0.62	0.42–0.51
Acetic acid	direct fermentation	1.00	0.85	0.77–0.88	0.77–0.88	0.65–0.75
Lactic acid	direct fermentation	1.00	0.95	1.48	1.48	1.41

Table 2 Major bioconversion parameters determining production costs (rec = recombinant, efficiency = observed yield/theoretical yield, nk = not known)

Product	Process	Auto-selective	Anaerobic metabolism	Pentose utilization	Commercial scale	Efficiency (%)
Ethanol	*Saccharomyces*	+	+	–	+	84–90
	Zymomonas rec.[13]	–	+	+	–	86
	E. coli KO11[14]	–	+	+	+/–	86–100
	Saccharomyces rec.[16]	–	+	+	–	70.4
	Bac. stearothermophilus[17]	+/–	+	+	+/–	80
Acetaldehyde	*Zymomonas mobilis*[18]	–	–	–	–	39
	Zymomonas mobilis[19]	–	–	–	–	55
Acetic acid	*Acetobacter*	+	–	–	+	75–80
	Cl. thermoaceticum[21]	+	+	+	–	85
Acrylic acid	*Cl. propionicum*	–	+	–	–	1
Propanediol	*E. coli* rec.[27]	–	+/–	+/–	–	12–16
Lactic acid	*Lactobacilli*	+	+	+/–	+	95
Butanediol	*Klebsiella oxytoca* ATCC 8724[32]	–	–	+	–	62.5
Succinic acid	*E. coli* ATCC 202021[33]	–	+	–	+/–	nk

costs are lower than the given maximum practical sugar price or if the yields are increased towards the theoretical yield.

4.2 Cost effective bioconversion parameters

Besides the corresponding product yields, product titers and volumetric productivities, other parameters have to be considered to end up with economically feasible bioconversion processes. First of all, all byproducts have to be considered as valuable products. This includes not only the fermentation end products, but also the applied microorganism itself. In most cases, microorganisms represent a valuable source of proteins for animal feed. Therefore, generally recognized as safe (GRAS) microorganisms should be promoted for industrial applications. Another critical cost parameter is the requirement of sterility of the substrate. While fermentations for lactic acid, ethanol or acetic acid can be performed without sterilization of the substrate, others like acetone–butanol–ethanol or ethanol fermentation with recombinant microorganisms require aseptic conditions, which catapults the cost of equipment and substrate pretreatment significantly. Furthermore, it makes a great difference whether the desired process requires aerobic or anaerobic conditions. If the process is aerobic, one is faced with additional costs for aeration and cooling equipment, thus increasing the overall production costs. Thermophilic strains growing at temperatures around 60 °C might help to overcome costs for cooling and sterilization. The major parameters determining the costs for bioconversion processes are summarized in Table 2 for a few selected processes.

4.3 Product recovery

Most biotechnological processes have the major disadvantage of low product concentrations. Therefore, separation of the product from the water stream contributes significantly to the overall production costs. Many products can be recovered by simple distillation, a method which has been optimized during the past years. Novel methods like pervaporation or electrodialysis enable continuous product separation simultaneously eliminating product inhibition.

5 Concluding remarks

Even today, the potential of microorganisms for the production of bulk chemicals is far from being fully exploited. Microorgansims may be applied for the production of typical metabolic end products like ethanol, lactic acid, acetone, butanol or butanediol. Some of these processes have been used during periods when fossil resources were in short supply and as such can still be resuscitated under changing circumstances namely, environmental legislative instruments. On the other hand, novel genetic engineering methods may increase the importance of microorganisms and their biosynthetic capabilities. New metabolic endproducts like acrylic acid, acetaldehyde or propanediol may be obtained by simple methods like mutation and advanced techniques of recombinant DNA technology.

However, the cost of feedstocks still remains one of the crucial points if biotechnological processes are to succeed. The fermentation of pentoses may lower the product costs significantly and research in this area is therefore justified. Current developments in ethanol production from pentoses demonstrate that metabolic engineering is worth further investigation and may even play an important role in the development of economic processes.

On the other hand, developments with thermophilic bacteria enable cheaper fermentations due to lower risks of contaminations and higher productivities and conversion rates. Some of these organisms have the capability of using pentoses and therefore genetic engineering may be avoided. Unfortunately, the potential of thermophilic microorganisms is still not yet fully recognized. Low product tolerances, high byproduct formation and high growth supplement requirements could be overcome by screening and mutation strategies.

Finally, process engineering has to be considered. New fermentation strategies may lead to new chemicals as demonstrated with acetaldehyde. The combination of product formation and product separation enables increased and sustained synthesis of toxic or inhibitory fermentation products. Parameters like process stability and process reliability have to be improved through long term experiments and up-scaling initiatives. Only a demonstration of process viability may convince potential industrial partners.

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