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Valorization of industrial waste and by-product streams *via* fermentation for the production of chemicals and biopolymers

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The transition from a fossil fuel-based economy to a bio-based economy necessitates the exploitation of synergies, scientific innovations and breakthroughs, and step changes in the infrastructure of chemical industry. Sustainable production of chemicals and biopolymers should be dependent entirely on renewable carbon. White biotechnology could provide the necessary tools for the evolution of microbial bioconversion into a key unit operation in future biorefineries. Waste and by-product streams from existing industrial sectors (*e.g.*, food industry, pulp and paper industry, biodiesel and bioethanol production) could be used as renewable resources for both biorefinery development and production of nutrient-complete fermentation feedstocks. This review focuses on the potential of utilizing waste and by-product streams from current industrial activities for the production of chemicals and biopolymers *via* microbial bioconversion. The first part of this review presents the current status and prospects on fermentative production of important platform chemicals (*i.e.*, selected C2–C6 metabolic products and single cell oil) and biopolymers (*i.e.*, polyhydroxyalkanoates and bacterial cellulose). In the second part, the qualitative and quantitative characteristics of waste and by-product streams from existing industrial sectors are presented. In the third part, the techno-economic aspects of bioconversion processes are critically reviewed. Four case studies showing the potential of case-specific waste and by-product streams for the production of succinic acid and polyhydroxyalkanoates are presented. It is evident that fermentative production of chemicals and biopolymers *via* refining of waste and by-product streams is a highly important research area with significant prospects for industrial applications.

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1. Introduction

The term "biorefinery" describes the process that entails refining of biomass in a commercial context for the production of fuels, chemicals, polymers, materials, food, feed and value-added ingredients. Klass[1] described biomass as "*all non-fossil-based living or dead organisms and organic materials that have an intrinsic chemical energy content*". In an industrial context, the term "refining" describes fractionation of a given raw material into various fractions that could be converted into commodity and speciality products aiming to maximise the efficiency of resource utilisation. There are several historical paradigms that describe the targets that should be fulfilled in order to establish successful refining concepts in a large scale. Corn and petroleum refining are two examples that demonstrate the innovation, capital investment, and research and development efforts required to refine crude and unexplored raw materials into several marketable products in a highly efficient and profitable manner.

Peckham[2] provides an insight on the market and technological forces coupled with innovative resourcefulness that led to the evolution of corn refining into a mature industrial sector. Corn refining started by simple starch extraction in 1948 by Thomas Kingsford, which was initially used as a laundry aid, food ingredient and sweetener production. Advances in corn refining gradually led to more efficient starch and glucose syrup production coupled to the commercialisation of new products, such as crystalline dextrose and corn oil. During the 20th century, corn refineries managed to resist competition through innovation in

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manufacturing a variety of derivatised starches and introduction of new technologies based on enzymatic hydrolysis for the production of a wide spectrum of sweeteners, characterised from different degrees of dextrose equivalence (DE), with high fructose corn syrups being the most important of these inventions. Nowadays, corn wet milling is one of the main fuel ethanol production processes in the USA. The advantages of using cereals and other agricultural products for fuel and chemical production lies on the available infrastructure and expertise in collection, distribution and processing. However, they do not provide sustainable renewable resources for widespread fuel and chemical production due to direct competition with food applications.

Petroleum refining is another example that started in the 1860s by producing kerosene through atmospheric distillation that was used as lighting fuel in oil lamps. This initial simple process evolved gradually to highly complex petroleum refineries producing fuels, chemicals, plastics and materials that have provided easy access to ample energy, and commodity and speciality products. This evolution was accomplished through a series of technological innovations including vacuum distillation, cracking, reforming and catalytic polymerisation among others. Apart from fuel production, petroleum refineries provide feedstocks for the production of base chemicals, specialities and consumer chemicals.[3] Base chemicals include petrochemicals (basic building blocks and intermediates), polymers and basic inorganics that are considered as commodity chemicals (high volume–low value products) and are used by the chemical industry or other industrial sectors (Fig. 1). The chemical industry relies on six basic chemicals or chemical groups including ethylene, propylene, the C_4 olefins (butadiene and butenes), the aromatics (benzene and toluene), the xylenes (*ortho*, *meta* and *para*) and methane. Speciality chemicals are low volume–high value products that constitute raw materials

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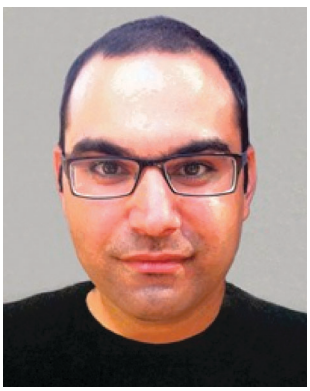

Anestis Vlysidis

Anestis Vlysidis is a Chemical Engineer graduated from the National Technical University of Athens. He did his PhD at the University of Manchester where he finished in 2011. His research was based on the development of sustainable biodiesel biorefineries for the production of biofuels and chemicals. Currently, he is a post-doctoral researcher at the Agricultural University of Athens in Greece. His research focuses on the utilisation of by-products from the pulp and paper industry, as well as agro-industrial residues for the production of value-added chemicals under the scope of life cycle analysis.

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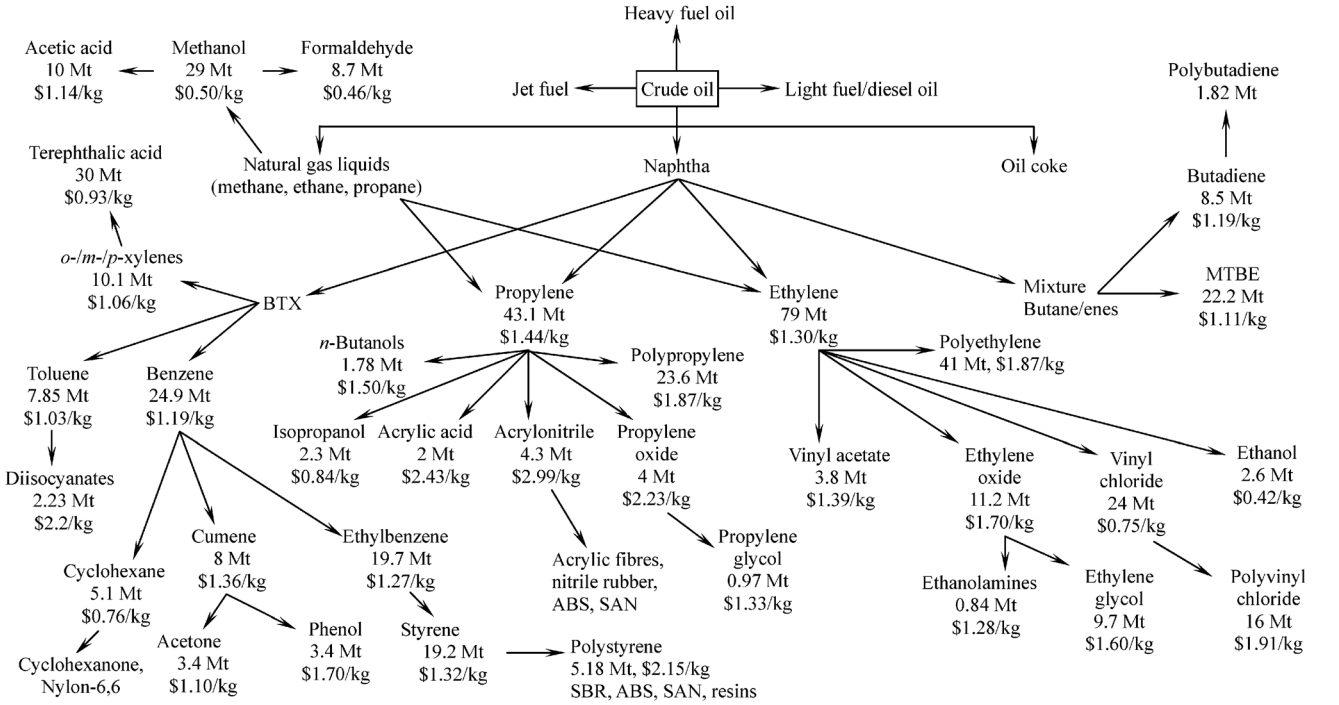

Fig. 1 Base chemicals and derivatives produced from petroleum (production capacities were taken from the journal *Chemical Engineering News*[4] and unit prices were taken from the *ICIS Indicative chemical prices*[5]).

for the production of pharmaceuticals, auxiliaries for industry, crop protection and pigments among others.[3] Consumer chemicals include products such as detergents, perfumes and cosmetics sold directly to consumers.

The establishment of petroleum-derived commodity and speciality chemicals received significant investment by international corporations and subsidies by governments to reduce their initially high prices in order to compete with traditional materials. Since the 1980s, strict environmental regulations enforced by governments has switched the rules of competition among petrochemical industries from the development of new chemicals and the investment on bigger and more efficient industrial facilities to the improvement of existing plants and the establishment of environmentally benign processing.[6] For instance, since 1990, fuel and power consumption in EU-27 chemical industry has been reduced by 27%, while energy efficiency was increased by 54% and greenhouse gas emissions were reduced by 49%.[3]

Similar analogies to corn and petroleum refining should be followed in the development of biomass refineries. Industrial

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Carol Lin graduated in Chemical and Materials Engineering from the University of Auckland, New Zealand, with a 1st class honours degree. Her PhD was carried out in the School of Chemical Engineering and Analytical Science at the University of Manchester, England. In collaboration with the Green Chemistry Centre of Excellence at the University of York, her research focused on novel wheat-based biorefining strategies for the production of succinic acid. Her current research interests focus on the valorization of food waste through conversion into commercially valuable products such as the production of bio-degradable polymers, biochemicals and biofuels.

investment and research has mainly focussed on biofuel production (*e.g.*, ethanol, biodiesel) to replace only a fraction (58.8×10^6 t oil equivalent) of the annual petroleum-derived fuels.[7] Despite the high volume of fuel required, the added value generated is very low. It gradually becomes evident that the creation of a sustainable and bio-based industry for chemical production will create higher added-value compared to current biofuel production processes. For instance, it has been recently reported that production of chemicals and polymer resins from sugars and biomass result in two to four times more added value, create six to eight times more employment and require less percentage of feedstock compared to biofuel production.[8] Therefore, renewable carbon should be utilised in an efficient, environmentally benign and profitable manner for integrated production of fuels and chemicals. However, the industrial implementation of bioethanol and biodiesel production was facilitated by existing raw materials (*e.g.*, corn, sugarcane, oilseed) and facilities (*e.g.*, corn wet milling plants) and the flexibility given by the utilisation of biofuels as blends with existing petroleum-derived fuels. Large-scale production of commodity chemicals and materials from biomass will require feedstock availability, flexibility and logistics, development of new technologies and unit operations, production of new building blocks, conversion of these building blocks into marketable products and significant investment to scale-up new processes. Sustainable production of chemicals should be coupled with biofuel production and/or extraction of value-added products, such as phytochemicals that could be used in high-value applications (*e.g.*, cosmetics) contributing significantly to the overall added-value of the whole process.

Feedstock supply will be a major problem in the bio-economy era as transportation of biomass resources involves significant costs. For this reason, bio-based industrial plants should be constructed in regions that renewable resources are easily accessible. The construction of new large-scale biorefineries will mainly target integrated production of fuels, chemicals and value-added products. Such facilities will require significant investment, technological innovation and viable solutions on transportation, storage and flexible utilisation of renewable resources. Local or regional production of bio-based chemicals and materials could be alternatively supported through integration of new technologies in existing industrial plants where waste or by-product streams could be used as renewable feedstocks. This synergistic approach could create significant added-value, require less capital investment, create new job opportunities, expand the market outlets of existing industrial sectors and reduce the environmental impact of existing plants. Furthermore, this approach could lead to a smoother transition from the petrochemical to the bio-economy era. For instance, in 2007 the largest three industrial sectors in EU-27 regarding added-value (*defined as the gross income from operating activities after adjusting for operating subsidies and indirect taxes*) were machinery and equipment, food and beverages, and chemicals including pharmaceuticals.[3] In addition, food and beverage, and chemical industries constitute the first and second subsectors in terms of investment in EU-27.[3] Besides the food and beverage industry, other industrial sectors (*e.g.*, pulp and paper, wood processing, biodiesel production) could evolve into advanced biorefineries through valorisation of waste and by-product streams. Given the fact that only 7% of worldwide petroleum consumption is currently used for chemical production, industrial waste and by-product streams from current industrial plants could provide significant quantities of renewable carbon for sustainable chemical production. Contrary to agricultural products, utilisation of industrial waste and by-product streams face problems associated with high water content (in many cases), inconsistent composition (in many cases), and lack of infrastructure and expertise for processing and conversion technologies.

Besides availability of renewable resources, another major problem of bio-based processes is the identification of platform chemicals and intermediates that could replace basic petrochemicals and their precursors. This is a very difficult task not only because there is a wide spectrum of alternatives, but also the development of technologies to convert renewable carbon into the selected building blocks is a relatively unexplored sector. In the last 10 years, specific research initiatives have focussed on the identification of potential core building blocks derived from renewable resources.[9–11] Such building blocks could be either already produced by the petrochemical industry or constitute novel products derived from biomass. Bio-based building blocks will be produced from renewable carbon through green chemical conversion routes or microbial bioconversions. It should be mentioned that the latter option, in many cases, results in low efficiencies/yields and research initiatives are required in order to enhance its commercial potential.

The aim of this review is to provide an insight on the potential restructuring of major industrial sectors into biorefineries where chemical production could be achieved *via* bioprocessing of current waste and by-product streams. Such integrated biorefineries should produce both speciality and commodity products to enhance market flexibility and economic viability. This review will emphasize on the potential of microbial bioconversion as a core unit operation for the production of bio-based chemicals and polymers as major commodities generated by such integrated biorefineries.

2. Fermentative production of value-added chemicals and biopolymers

Renewable carbon could be converted into chemicals *via* biotechnological or chemical routes. The final choice will be based on complete sustainability assessment, availability of resources and logistics of resource transportation. The scientific domains that describe the research involving chemical or biotechnological routes (exploiting microorganisms or enzymes) for the production of chemicals and materials from biomass are termed *Green Chemistry* and *White Biotechnology* (formerly *Industrial Biotechnology*), respectively. Although *Industrial Biotechnology* was initially exploited during World War I for chemical production (*i.e.*, Chaim Weizmann at the University of Manchester, United Kingdom, scaled-up the use of *Clostridium* for the production of acetone and butanol), it was outcompeted by petrochemical processes.

The incorporation of fermentative production of basic building blocks as unit operations in integrated biorefineries is dependent on the potential to produce the appropriate chemical structures from given resources, the development of wild-type or genetically engineered microbial strains producing the desired molecules at high efficiency, the effective downstream separation or conversion of molecules and the compatibility of developed processes with current industrial infrastructures. Important information required for the assessment of each platform molecule produced *via* fermentation includes current production capacities and future requirements, carbon and nitrogen requirements, available wild-type or engineered microorganisms, knowledge of metabolic pathways, and fermentation efficiency regarding final concentrations, yields and productivities. The low efficiencies/yields of many fermentation processes should be addressed in order to achieve commercial exploitation. Downstream separation efficiency depends highly on the efficient optimisation of fermentation processes (*e.g.*, low by-product formation, high concentration of desired product, low concentration of remaining nutrients). In addition, upstream processing of renewable resources will be highly dependent on the nutrient requirements for efficient microbial growth and product formation (*e.g.*, flexibility or specificity on carbon source utilisation, organic or inorganic nitrogen requirements, mineral and growth factors). This section presents recent accomplishments and future targets regarding production of bio-based chemicals and polymers *via* fermentation. It should be stressed that none of these bio-based chemicals and polymers is currently commercially produced from industrial waste and by-product streams.

2.1 Chemical production *via* fermentation

Fig. 2 presents building blocks that could be produced *via* fermentation. Some of them are currently produced from non-renewable resources. The importance of different building blocks is highly dependent on their functionality based on the presence of different functional groups (*e.g.*, carbon-to-carbon double bonds, amino groups, carboxyl groups, hydroxyl groups). For instance, esterification reactions for the production of polyesters are carried out between dicarboxylic acids (*e.g.*, succinic acid) and diols (*e.g.*, 1,4-butanediol). Current research focuses mainly on molecules with 2–6 carbons (C2–C6) and in the following sections important building blocks from each group are presented. This section mainly focuses on the fermentation stage and the main carbon sources that could be

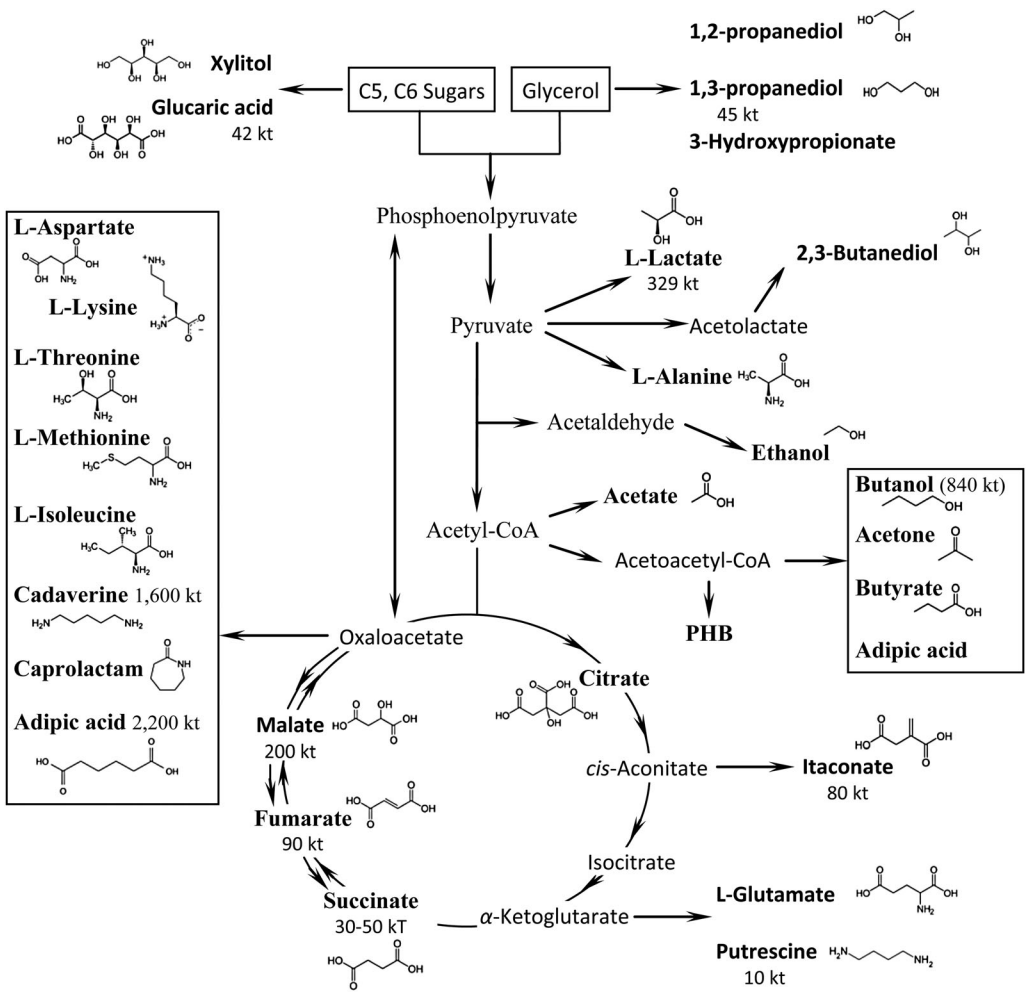

Fig. 2 Building blocks that could be produced *via* fermentation. Numbers next to biochemicals designate the total annual production in thousands of t.

used for the production of each chemical compound. Fermentation processes could be improved through adaptation or genetic engineering of microbial strains[12] and advancements in novel technologies, including consolidated bioprocessing of lignocelluloses, immobilization of biocatalysts, development of continuous production systems and integration of fermentation with downstream separation strategies.[13–16]

2.1.1 Ethanol (C2). Worldwide ethanol (CH_3CH_2OH) production was about 107 billion liters in 2012.[17] Besides an alternative to fossil fuels, ethanol is an important platform chemical for the production of ethylene and ethylene glycol through chemical catalysis that are used for the production of polyethylene and poly(ethylene terephthalate).

Ethanol can be produced by fermentation of various carbon sources, including sucrose-containing feedstocks, starchy crops and lignocellulosic materials. In general, the metabolic pathway involved in the ethanol fermentation is that one molecule of glucose is metabolized by microorganisms into two molecules of pyruvate, which will then be reduced to acetaldehyde by decarboxylase or pyruvate-formatelyase and acetaldehyde dehydrogenase. After that, it is further reduced to ethanol by the alcohol dehydrogenase coupled with NADH.[18] Theoretically, the glucose to ethanol production yield is 0.511 g g^{-1}. *Saccharomyces cerevisiae* and *Zymomonas mobilis* are two well-known microorganisms capable of producing ethanol from hexoses.[11] Although *Z. mobilis* (Fig. 3) shows better ethanol yield (up to 97%) than that of *S. cerevisiae* (90–93%) and also three to five folds higher productivity than that of *S. cerevisiae*, it is not the predominant microorganism used in industrial ethanol fermentation because of its narrow substrate consumption range and the extracellular formation of fructose oligomers levan and sorbitol when sucrose is used as carbon source leading to reduced ethanol production.[19] Also, the unacceptable biomass of *Z. mobilis* as animal feed constitutes a significant drawback of this strain when compared to *S. cerevisiae*.[19]

Research on ethanol production is focussed on the development of new processes and new strains based on the utilisation of C5 and C6 sugars from lignocellulosic biomass. For instance, engineered *E. coli* and *S. cerevisiae* strains have been developed for the production of ethanol from xylose with conversion yields of 0.48 g g^{-1} and 0.46 g g^{-1}, respectively.[20,21] Pretreatment of lignocellulosic resources is necessary in order to break down its recalcitrant structure and facilitate enzymatic hydrolysis.[22] Pretreatment may involve application of physical processes (*e.g.*, chipping, grinding and milling), physico-chemical processes (*e.g.*, autohydrolysis, steam explosion), and chemical processes such as acid and alkaline hydrolysis.[23] Acid hydrolysis is an effective and common pretreatment process and sulfuric acid is the most commonly used acid. However, different types of inhibitors, including acetic, formic and levulinic acids, furfural, aromatic compounds (phenolics) and extractives, are formed during acid hydrolysis reducing ethanol production yield.[24] Although some of the inhibitors were found to be removed with high efficiency by electrodialysis in laboratory scale, certain inhibitors such as furfural still need further removal.[24]

Novel fermentation processes are also under development for bioethanol production focusing on utilisation of novel raw materials and microbial strains or development of integrated fermentation with ethanol separation. *Arthrospira* (*Spirulina*) *platensis* biomass has been used as a glycogen accumulating halophilic cyanobacterium for direct production of bioethanol (6.5 g L^{-1} with a productivity of 1.08 g L^{-1} h^{-1}) using lysozyme and a recombinant amylase-expressing yeast strain.[25] Pervaporation is an established membrane technology that has been extensively studied as an ethanol separation process integrated with fermentation.[15]

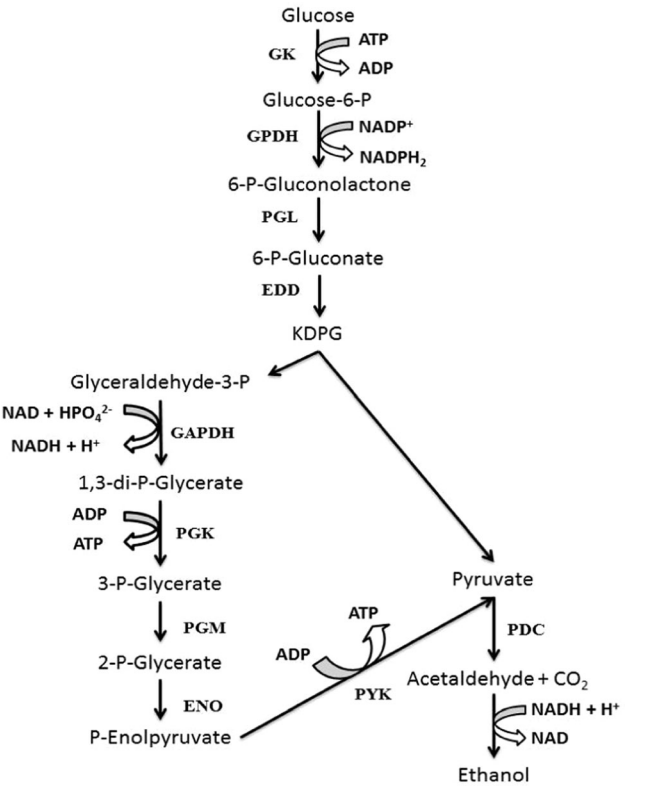

Fig. 3 Glucose metabolic pathways in *Z. mobilis* (GK: glucokinase, GPDH: glucose-6-phosphate dehydrogenase, PGL: phosphogluconolactonase, EDD: 6-phosphogluconate dehydratase, KDPG: 2-keto-3-deoxy-6-phosphogluconate, GAPDH: glyceraldehydes-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, PGM: phosphoglyceromutase, ENO: enolase, PYK: pyruvate kinase, PDC: pyruvate decarboxylase).

2.1.2 Lactic acid (C3). Lactic acid (2-hydroxypropanoic acid) is the most widely occurring hydroxycarboxylic acid in nature. It is a chiral molecule with two optical enantiomers, L(+) and D(−), which was first discovered in 1780 by Scheele in sour milk. Lactic acid industrial production started in 1881 in Littleton, MA, USA. Due to its versatile applications in the traditional food, pharmaceutical, textile, leather and chemical industries sector, together with the recently developed commercialization of lactic acid derived biopolymers, lactic acid annual production is expected to reach approximately 329 000 t by 2015.[26]

Lactic acid is either produced by chemical synthesis or microbial fermentation. Chemical synthesis is mainly based on the hydrolysis of lactonitrile (derived from acetaldehyde and hydrogen cyanide reaction) by strong acids, a route which

provides only the racemic mixture of D- and L-lactic acid. Likewise, lactic acid can be synthesized through base-catalyzed degradation of sugars, oxidation of propylene glycol, nitric acid oxidation of propylene and hydrolysis of chloropropionic acid among others,[27] but none of these routes have led to technically and economically viable processes.[28] Biotechnological production of lactic acid is superior to chemical synthesis regarding product specificity, usage of low cost substrates and lower consumption of energy. Lactic acid production is achieved *via* pyruvate with lactate dehydrogenase present in various lactic acid bacteria, including *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus*, and *Weissella.* The largest and most diverse genus of lactic acid bacteria is *Lactobacillus*, which includes species with different biochemical and physiological properties. Lactic acid bacteria, depending on their carbohydrate consumption metabolic pathways are further divided to obligatory heterofermentative microorganisms, among which the most common are *Lactobacillus brevis, L. fermentum, L. parabuchneri* and *L. reuteri* and to obligatory homofermentative, including *Lactobacillus acidophilus, L. amylophilus, L. bulgaricus, L. helveticus* and *L. salivarius*, which are used in the existing commercial production processes.[26] Besides lactic acid bacteria, some fungal strains such as *Mucor, Monilia*, and especially *Rhizopus* are also used for the production of lactic acid.

Lactic acid can be produced from a wide spectrum of carbon sources including starchy materials, many food industry by-products (*e.g.*, molasses, whey), agro-industrial residues and by-products (*e.g.*, lignocelluloses or hemicellulose hydrolyzates, cottonseed hulls, corn cob, corn stalks, wheat bran, brewer's spent grains) and various other renewable resources (*e.g.*, Jerusalem artichoke hydrolysates). Yun *et al.*[29] obtained 129 g L^{-1} lactic acid from rice and wheat bran, using *Lactobacillus* sp. RKY2, while Dumbrepatil *et al.*[30] reported the production of 166 g L^{-1} lactic acid from molasses by a mutant *L. delbrueckii* strain (Uc-3). A metabolically engineered *E. coli* strain produced 50 g L^{-1} of L-lactate from 56 g L^{-1} of crude glycerol,[31] while a fed-batch fermentation of glucose by another metabolically engineered *E. coli* strain showed a production of 138 g L^{-1} of D-lactic acid.[32] One of the highest yields was obtained by an engineered *Sporolactobacillus* strain, which produced 207 g L^{-1} D-lactate from 223 g L^{-1} of glucose with an optical purity of 99.3% in fed-batch fermentation supplemented with peanut meal as a nitrogen source.[33] Recently a newly isolated *Bacillus coagulans* C106, produced 215.7 g L^{-1} L-lactic acid from xylose in fed-batch mode, with a 95% lactic acid yield and 99.6% optical purity.[34]

Fermentation and separation of organic acids, including lactic acid, have been developed based on electrodialysis membrane processes involving monopolar or bipolar membrane configurations that separate organic acids from fermentation broths.[35] Mintian *et al.*[36] reported a lactic acid productivity of 8 g L^{-1} h^{-1} through the development of a continuous system that combined lactic acid fermentations with an electrodialysis membrane unit for lactic acid separation. The cost of industrial lactic acid separation by electrodialysis employing bipolar membranes would be 0.47 € per kg of produced acid at a production capacity of 5000 t.[37]

2.1.3 Propionic acid (C3). Propionic acid ($C_3H_6O_2$) is a monocarboxylic acid with broad industrial applications, including bactericides, fungicides, herbicides, pharmaceuticals, perfumes and emulsifying agents. The market for propionic acid is growing annually by 4% and requires an environmentally benign production process not depending on petrochemicals.[38,39] Propionibacteria produce propionic acid under anaerobic conditions but the formed propionic acid inhibits cell growth by disturbing the pH gradient across cell membrane even at low concentrations.[40] Research on propionic acid production mainly focuses on increasing acid-tolerance, yield and productivity. Zhang and Yang[41] have investigated immobilization of *Propionibacterium acidipropionici* in a fibrous-bed bioreactor as a mean to protect cells and increase their acid tolerance. In the same study, the carbon flow was directed towards propionic acid by knocking out the acetate kinase gene and reducing acetate production. Interestingly, the gene-knockout strategy did not only result in a high propionic acid concentration (104 g L^{-1}), but also in a "*global response*" such as a higher membrane-bound H^+-ATPase activity additionally contributing, by maintaining the intracellular pH gradient, to the increased acid tolerance. The propionic acid concentrations achieved in this study (104 g L^{-1} and 97 g L^{-1} when lactose and glucose were used as carbon sources) were around two times higher than concentrations obtained in other studies using non-modified and non-immobilized *P. acidipropionici* cells.[42–44]

Crude glycerol has been recently evaluated as a cheaper carbon source than glucose for propionic acid production (Table 1). The volumetric productivity during glycerol fermentations was 2 to 4 times higher than most fermentations carried out with sugarcane molasses or glucose as a carbon source.

Table 1 Concentration, productivity, and yield of propionic acid obtained from different carbon sources by *Propionibacterium freudenreichii* and *Propionibacterium acidipropionici*

Microorganism	Carbon source	Fermentation description	Concentration (g L^{-1})	Productivity (g L^{-1} h^{-1})	Yield (g g^{-1})	Ref.
P. freudenreichii CCTCC M207015	Hydrolysed sugarcane molasses	Fed-batch	92.0	0.36	0.46	45
P. freudenreichii CCTCC M207015	Glucose	Fed-batch in fibrous bed bioreactor	136.2	0.57	0.50	39
P. acidipropionici ATCC 4965	Sugarcane molasses	Batch	8.2	0.06	0.45	46
P. acidipropionici CGMCC 12230	Glycerol	Fed-batch	44.6	0.20	0.56	42
P. acidipropionici ATCC 4875	Corncob molasses	Fed-batch	71.8	0.28	—	43
P. acidipropionici ATCC 4875 (inactivated acetate kinase gene)	Glucose	Long-term fed-batch culture (~3 months) in fibrous-bed bioreactor	97.0	~0.07	0.53	41

In addition, the propionic acid production yield was higher when glycerol was used, but decreased with increasing initial glycerol concentrations.[42] The highest propionic acid concentration (136.2 g L^{-1}) has been achieved with immobilized *Propionibacterium freudenreichii* cells cultivated in fed-batch mode in a fibrous-bed bioreactor with glucose as carbon source.[39] Additionally, xylose and hemicellulose hydrolysates have also been used as substrates for propionic acid production.[43] The progress regarding increased productivity and acid-tolerance by using either genetically modified bacteria or immobilized cells in a fibrous-bed bioreactor may open the way for industrial biotechnological production of propionic acid.

2.1.4 1,3-Propanediol (C3). 1,3-Propanediol (PDO) ($C_3H_8O_2$) is a 3-carbon colorless diol that could be used in many chemical reactions, especially poly-condensations, resulting in the synthesis of poly-esters, poly-ethers and poly-urethanes.[47] PDO-derived plastics, besides their biodegradability, exhibit better product properties and higher light stability than those produced from 1,2-propanediol, 2,3-butanediol or ethylene glycol.[48] PDO can be used as monomer for the synthesis of polytrimethylene-terephthalate, a novel polymer with properties comparable to Nylon that can be used in carpets (Corterra) and textile fibers (Sorona).[48] PDO could be also used as a polyglycol-type lubricant and its addition can significantly improve the properties of various solvent systems (increased flexibility in blending ester quats), adhesives, laminates, resins (low intrinsic viscosity) and cosmetology products (long-lasting but not sticky moisturizing effect).[47,49]

Chemical synthesis of PDO is mainly achieved by two processes. In the "Degussa" process (now owned by "DuPont") propylene is catalytically oxidised to acrolein which is hydrated at moderate temperature and pressure to 3-hydroxypropionaldehyde followed by hydrogenation to PDO over a rubidium catalyst under, in most cases, high pressure.[49] The second process carried out by "Shell" is based on oxidation of ethylene to ethylene oxide followed by 3-hydroxypropionaldehyde production employing a "hydroformylation" reaction (also called "oxo synthesis") at high pressures (around 150 bar), extraction of the aldehyde from the organic phase with water and 3-hydroxypropionaldehyde hydrogenation using nickel as a catalyst under high pressure.[49] The conversion yield of PDO production is around 65% and 80% when acrolein and ethylene oxide are used as feedstocks.[49]

Fermentative production of PDO using wild-type strains can only be achieved from glycerol as carbon source.[48] Increased production of crude glycerol from biodiesel plants has rendered PDO an important target-molecule for glycerol biorefineries. PDO production from glycerol is carried out mainly by prokaryotes under strictly or partly anaerobic conditions, with (a significant) portion of glycerol being utilized as final electron acceptor. Glycerol assimilation occurs *via* an "oxidative" and a "reductive" branch. Portion of glycerol is transformed to dihydroxyacetone by NAD^+-dependent glycerol dehydrogenase. Dihydroxyacetone is then phosphorylated by dihydroxyacetone-kinase to enter glycolysis, resulting to the generation of the same end-products (*e.g.*, acetic acid, butyric acid *etc.*) as in sugar fermentation.[47,49] The quantities of reduced co-factors ($NADH_2$) that are synthesized can be re-generated *via* the "reductive" branch of glycerol assimilation pathway. Glycerol, which had not been metabolized, is dehydrated to 3-hydroxypropionaldehyde by a B_{12}-dependent dehydratase (GDHt) and then is reduced to PDO by a NAD^+-dependent oxidoreductase (PDOR) (reaction re-generating NAD^+). Thus, the pathway glycerol → PDO is not coupled with ATP production (thus, in this type of synthesis, besides PDO, other metabolites coupled with the generation of ATP are obligatorily synthesized) but only with $NADH_2$ recycling (thus, other pathways used for $NADH_2$ cofactors recycling, *i.e.* that of glycerol → lactate, are antagonistic to the pathway glycerol → PDO).

PDO production is mainly achieved by strains belonging to the genus *Clostridium*, the enteric group of bacteria such as *Klebsiella pneumoniae* and *Citrobacter freundii*, and lactic acid bacteria.[50–55] In the case of *Clostridium* sp. strains or enteric bacterial strains, glycerol can be utilized as the sole carbon and energy source for PDO production. Lactic acid bacteria can convert glycerol into PDO only in the presence of other carbon sources (*e.g.*, maltose, glucose, fructose, *etc.*) that are utilized for the production of ATP and biomass, whilst glycerol is utilized only as the final electron ($NADH_2$) acceptor of the process. Fig. 4 presents the PDO biosynthesis pathway of bacteria belonging to *Clostridium* sp. or to enteric group.

Depending on the bacterial strain used, PDO biosynthesis can be performed under micro-aerobic or even aerobic conditions,[50] while its production can be significantly influenced by fermentation mode, the anaerobic strategy imposed (*i.e.*, the anaerobiosis performed under the so called self-generated conditions or the anaerobiosis achieved after continuous N_2 sparging into the bioreactor), the purity of glycerol used, the utilization of sugars as co-substrates, the bioreactor geometry and the initial substrate concentration.[47,48,52,55] PDO production could be also feasible under completely non-aseptic conditions.[52,53] Maximum PDO concentrations up to ~90 g L^{-1} have been reported for natural strains cultivated in fed-batch cultures, while the respective values for continuous cultures are 40–50 g L^{-1} (Table 2). The maximum theoretical yield of PDO produced per unit of glycerol consumed, assuming that no antagonistic compounds are synthesized (*e.g.*, butyric acid for the case of *Clostridium* sp. strains) and no H_2 is released (thus, all intracellular $NADH_2$ quantities are recycled through the pathway

Table 2 Experimental results of natural PDO producing microorganisms using crude glycerol

Strain	Culture mode	PDO (g L^{-1})	Yield (g g^{-1})	Ref.
C. butyricum CNCM 1211	Batch	65.4	0.51	57
C. butyricum F2b	Continuous	48.1	0.55	58
	Batch	47.1	0.53	59
C. butyricum VPI 1718	Fed-batch	70.8	0.55	55
C. butyricum AKR102a	Fed-batch	76.2	0.51	60
Clostridium sp. IK 124	Fed-batch	80.1	0.56	61
K. pneumoniae DSM 2026	Fed-batch	53.0	0.40	62
K. pneumoniae TUAC01[a]	Fed-batch	66.3	0.45	50
K. oxytoca FMCC-197	Batch	50.1	0.39	52
C. freundii FMCC-B 294 (VK-19)	Fed-batch	68.1	0.40	53

[a] Pure glycerol.

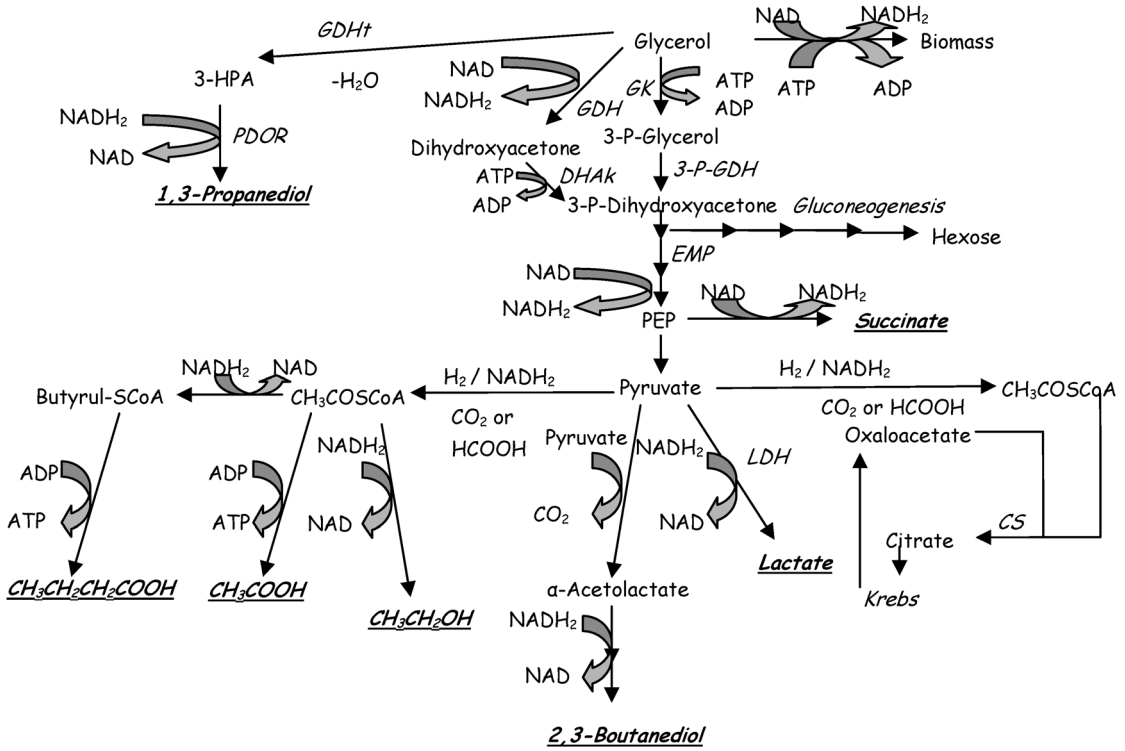

Fig. 4 Catabolic pathways of PDO production in *Clostridium* sp. and enteric group bacteria. EMP: Embden–Meyerhof–Parnas pathway; 3-HPA: 3-hydroxypropionaldehyde; GDHt: glycerol dehydratase; GDH: glycerol dehydrogenase; GK: glycerol kinase; DHAk: di-hydroxyacetone kinase; PDOR: 1,3-propanediol oxidoreductase; LDH: lactate dehydrogenase; Krebs cycle performed only in the bacteria of enteric group under micro-aerobic conditions.[47–49]

glycerol → PDO) is 0.72 mol mol^{-1} ($\sim$0.59 g g^{-1}).[49] The highest PDO production has been achieved by DuPont and Genencor International Inc., utilizing recombinant *Escherichia coli* strains with glucose employed as the sole substrate. Fed-batch cultivation of the genetically engineered *Escherichia coli* RJ8/pAH48/pKP32 strain on glucose resulted in a PDO concentration of 129 g L^{-1} with a conversion yield of 0.34 g g^{-1} and volumetric productivity of 1.7 g L^{-1} h^{-1}.[56]

2.1.5 3-Hydroxypropionic acid (C3)

3-Hydroxypropionic acid (3-HP) ($C_3H_6O_3$) is the third most important chemical in the list of the U.S. Department of Energy's top twelve value added chemicals from biomass.[9] 3-HP has a variety of applications, including crosslinking agent for polymer coatings, metal lubricants, antistatic agents for textiles and precursor for manufacturing of PDO, acrylic acid, methyl acrylate, acrylamide, ethyl 3-HP, malonic acid, propiolactone, and acrylonitrile.[63] In addition, 3-HP is a versatile agent used in the production of biodegradable polyesters such as poly(3-hydroxypropionate). The global market size of 3-HP was estimated at 3.6 $\times$ 10^6 t per year.[63]

Commercial 3-HP production is carried out by chemical processes, such as hydration of acrylic acid, hydrolysis of 3-hydroxypropionitrile and hydrolysis of β-propiolactone.[64] However, research is focused on the development of biotechnological synthesis of 3-HP due to the drawbacks of chemical processes. For instance, the rapid increase (up to 63%) of acrylic acid unitary cost has made 3-HP production *via* acrylic acid hydration an economically unfavourable process. The other two chemical processes involve the use of toxic cyanide and carcinogenic β-propiolactone rendering them unsuitable for mass-scale 3-HP production.[64]

Several microorganisms can produce 3-HP as an intermediate or end-product of their metabolism. For instance, *Lactobacillus collinoides* produce 3-HP and PDO.[65] *Chloroflexus aurantiacus* secretes 3-HP as an intermediate in the 3-hydroxypropionate cycle.[66] *Byssochlamys* sp., *Geotrichum* sp. and *Trichoderma* sp. are capable of degrading acrylic acid into 3-HP.[67,68] However, the low yield and productivities achieved by wild-type strains has diverted research towards the development of genetically engineered microorganisms (Table 3). Ashok *et al.*[69] reported that a recombinant strain of *Klebsiella pneumoniae* produced 22 g L^{-1} of 3-HP after 48 h fed-batch fermentation in the presence of potassium nitrate under anaerobic conditions. A recombinant strain of *Escherichia coli* expressing a B_{12}-dependent glycerol dehydratase

Table 3 Concentration, productivity, and yield of 3-HP obtained from different carbon sources and recombinant microorganisms

Microorganism	Carbon source	Concentration (g L^{-1})	Productivity (g L^{-1} h^{-1})	Yield (g g^{-1})	Ref.
K. pneumoniae	Glycerol	22	0.46	0.30	69
E. coli	Glycerol	38.7	0.53	0.34	70
E. coli	Glycerol	0.19	0.01	0.03	71

Table 4 Representative results for succinic acid production from various media and microbes

Microorganism	System	C-source	Yield ($g\ g^{-1}$)	Productivity ($g\ L^{-1}\ h^{-1}$)	Succinic acid conc. ($g\ L^{-1}$)	Ref.
E. coli SD121 *(mutant)*	Dual phase F-B	Glucose	1.13	1.55	116.2	76
A. succinogenes 130Z	B	Wheat hydrolysates	0.81	1.19	64.2	77
A. succinogenes mutant CGMCC1593	F-B	Cane molasses	—	1.15	55.2	78
A. succinogenes 130Z	B	Cotton stalk hydrolysates	1.23	0.62	15.8	79
	B	Corn stalks	0.66	0.56	17.8	
A. succinogenes 130Z	B	Waste bread	1.16	1.12	47.3	80
A. succinogenes 130Z	B	Cheese whey	0.57	0.44	21.2	81

B: batch, F-B: fed-batch.

(DhaB) and glycerol dehydratase reactivase (GDR) from *K. pneumoniae* and α-ketoglutaric semialdehyde dehydrogenase (KGSADH) from *Azospirillum brasilense* produced 3-HP with a yield of 0.34 $g\ g^{-1}$ and a productivity of 0.53 $g\ L^{-1}\ h^{-1}$ in fed-batch fermentation using glycerol as carbon source.[70] However, this process cannot be commercialised due to the use of expensive coenzyme B_{12}. Rathnasingh *et al.*[71] reported the feasibility of producing 3-HP from glucose through the malonyl-CoA pathway, which is B_{12}-independent. Recent results have shown that fermentative production of 3-HP is feasible but establishing this process on industrial scale requires significant improvements regarding 3-HP concentration ($>100\ g\ L^{-1}$), productivity ($>2\ g\ L^{-1}\ h^{-1}$) and substrate-to-product yield (>50%). Additional processing limitations that should be tackled are the toxicity of 3-HP, and the regeneration of NAD^{+}.[64]

2.1.6 Succinic acid (C4). Succinic acid ($C_4H_6O_4$) is a linear saturated di-carboxylic acid which appears as white crystals under standard conditions. It was first detected in amber which is known as *succinum* in Latin. Succinic acid or succinate, in its dissociate form, it is considered a highly promising building block for the production of various bulk and niche chemicals.[10] Although, it is mainly produced from petrochemical precursors, its production *via* fermentation is already implemented by a number of industries, such as Bioamber and Reverdia, while several other companies, such as Myriant, BASF and Purac, are constructing or are about to operate commercial-scale biosuccinic acid plants.[72]

Its global annual production is around 30 000 to 50 000 t, while its market price ranges from $2.4–3 per kg. The succinic acid market is expected to increase at an annual rate of around 19% in the following years.[72] At present, succinic acid has a high production cost and hence it is used as a niche product mainly in the pharmaceutical industry, in cosmetics and in the food and beverages as a flavour agent. The main target, though, is to expand the succinic acid market to commodity chemicals where it can be used as a building block for the production of various bulk/intermediate chemicals such as 1,4-butanediol and tetrahydrofuran, which are currently petroleum-derived. Its market is also expected to grow on the bioplastic sector and specifically in the production of polybutylene succinate and polyurethanes such as polyethylene succinate.[73]

Most of the natural succinic acid producers are bacteria isolated from the bovine rumen where succinic acid is produced and utilized to generate propionic acid which is used to supply energy and critical precursors for biosynthesis by oxidation.[74] The most widely used bacterial strain for succinic acid production are *Anaerobiospirillum succiniciproducens, Actinobacillus succinogenes, Mannheimia succiniciproducens*, *Basfia succiniciproducens* and mutations of *Escherichia coli*.[75] These microorganisms can metabolize a wide range of C5 and C6 sugars either in pure form or sugars derived from agricultural or industrial wastes, such as lignocellulosics, crude glycerol and wheat (Table 4).

Besides regulation and control of bioreactor operational parameters, succinic acid production is dependent on the availability of dissolved CO_2 and electron donors in the broth. The main branch metabolite (Fig. 5) which directs the carbon towards the C4 instead of the C3 pathway is phosphoenolpyruvate (PEP). The latter reacts with CO_2 with the help of PEP-carboxykinase and produces oxaloacetate (OAA). After the OAA production, the reductive pathway to succinic acid involves three more steps/reactions where two of those, OAA to malate and fumarate to succinate require an electron donor. The production of more reduced products, like succinic acid, is favoured in the presence of electron donors, like NADH, against other by-products such as acetic and formic acid. Glycerol and sorbitol are more reduced feedstocks than other carbon sources, like xylose and glucose, and hence they produce more electrons during their catabolism (Fig. 5). The available electrons are utilised for the production of more reduced end-products such as succinic acid.[82,83]

Succinic acid production could be improved by implementing integrated separation of succinic acid and by-products that are generated during fermentation. Meynial-Salles *et al.*[13] developed an integrated membrane-bioreactor-electrodialysis process using the strain *A. succiniciproducens* that led to the production of

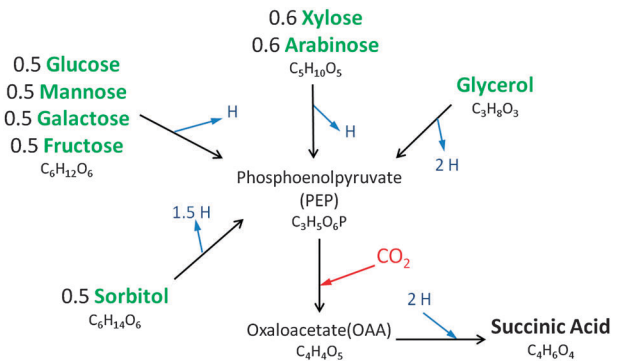

Fig. 5 The significance of electron donors and carbon dioxide in the succinic acid metabolic pathway.[82]

83 g L^{-1} of succinic acid in the permeate stream with an average succinate yield of 1.35 mol mol^{-1} and a productivity of 10.4 g L^{-1} h^{-1}.

2.1.7 Malic acid (C4). Malic acid ($C_4H_6O_5$), named from the Latin word ''malum'', is a dicarboxylic acid with an asymmetric carbon, having D-(−)- and L-(+)-isomers, and an intermediate of the tricarboxylic acid cycle. Malic acid is mainly used as an acidulant (holds about 10% of this market) and taste enhancer in beverages, candy and food. It is also used in several non-food applications, including cosmetics, pharmaceuticals, medicine, metal cleaning, paints and in the production of plastics.[84] Together with succinic acid and fumaric acid, malic acid was considered among the most important platform chemicals by Werpy and Petersen.[9] The annual production of malic acid is estimated to approximately 200 000 t per year.[85] It is commercially derived either by chemical synthesis (*via* hydration of maleic or fumaric acid) resulting in a DL racemic mixture or by an enzymatic process (using fumarase) from fumaric acid, yielding L-malic acid.[84]

L-malic acid can be produced *via* fermentation by species of the fungus *Aspergillus*, by yeast strains (*e.g. Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*) and by genetically engineered *Escherichia coli* strains. The highest malic acid production (113 g L^{-1}) was achieved from glucose in batch cultures of *Aspergillus flavus*.[86] However, the potential aflatoxin production, which disqualified *A. flavus* as a producer of food-grade chemicals, together with the oxygen transfer and morphology problems, hindered the industrialization of the specific fermentation process. Hence, in order to avoid toxin production problems recent research focussed on other strains. West[87] used *Aspergillus niger* ATCC 10577 (a non-toxin producing strain) and yielded 19 g L^{-1} malic acid from thin stillage. Zelle *et al.*[88] used a genetically engineered *S. cerevisiae* strain to produce 59 g L^{-1} of malic acid from glucose. Zhang *et al.*[89] reported the production of 34 g L^{-1} malic acid within 72 h of a two-stage fermentation (aerobic cell growth followed by anaerobic malate production) using the genetically engineered strain *E. coli* XZ658. The most promising result was reported by Taing and Taing[90] using the strain *Zygosaccharomyces rouxii* V19 that produced 74.9 g L^{-1} malic acid from glucose when it was supplemented with 0.5% glutamic acid. It is claimed that the production yield could be further improved, by optimizing the fermentation process, favoring the commercial production of malic acid.

2.1.8 Fumaric acid (C4). Fumaric acid ($C_4H_4O_4$) is a dicarboxylic acid that was included in the original list of the 12 most promising building blocks derived from biomass published by Werpy and Petersen.[9] It was removed from the revised list due to limited research innovation on its production *via* fermentation.[10] In 2007, the worldwide production capacity was in the range of 90 000 t per year based on isomerisation of maleic acid derived originally from *n*-butene.[91] The main current applications of fumaric acid include the food industry as acidulant and the chemical industry with major applications in resins and plasticizers production among other products.[91]

In the 1940s, fumaric acid was produced on an industrial scale *via* fermentation in the USA.[91] Fermentative production was replaced by petrochemical synthesis due to high production costs resulting from relatively low fumaric acid concentration (less than 70 g L^{-1}) and productivity (less than 1 g L^{-1} h^{-1}) achieved *via* fungal fermentation. Fermentative production of fumaric acid has been predominantly investigated with fungal strains of *Rhizopus arrhizus* or *Rhizopus oryzae*. In aerobic cultures, *Rhizopus* strains produce lactic acid or fumaric acid, while under anaerobic conditions these strains may produce more ethanol than organic acids. The main difference between *R. oryzae* strains that produce fumaric acid or lactic acid is the presence of one (ldhB) or two (ldhA and ldhB) lactate dehydrogenase genes, respectively.[92] Fumaric acid production by *R. oryzae* is achieved through the reductive TCA cycle, located in the cytosol, *via* three metabolic stages beginning from pyruvate (Fig. 6). Due to pyruvate requirements for both the oxidative TCA cycle leading to fungal growth and the reductive TCA cycle leading to fumaric acid production, two-stage (*i.e.*, fed-batch)

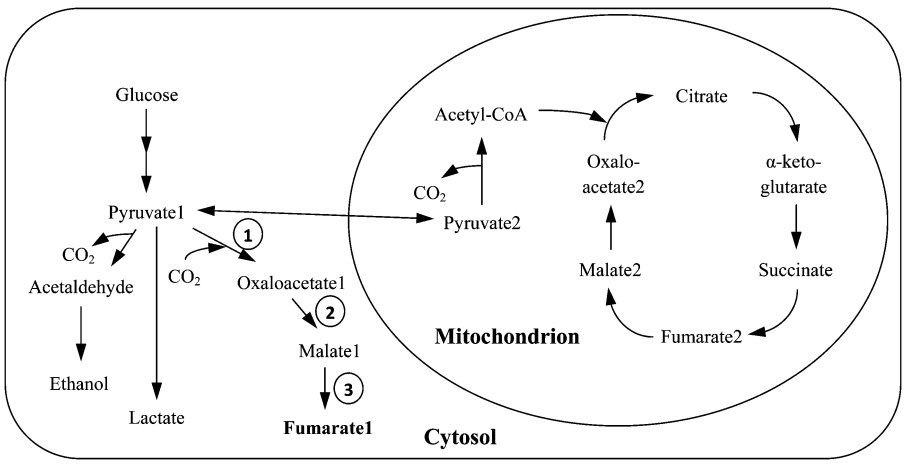

Fig. 6 Production of fumaric acid in *R. oryzae* is achieved in 3 steps: (1) production of oxaloacetate involving CO_2 fixation with pyruvate carboxylase; (2) production of malate employing malate dehydrogenase; and (3) production of fumaric acid employing fumarase.[93]

fermentations should be developed involving fungal growth on nutrient-complete media in the first stage followed by fumaric acid production *via* nitrogen or phosphorus limitation. The theoretical glucose to fumaric acid production yield is 1.29 g g^{-1} when adequate amount of CO_2 is utilised. However, the yield achieved is lower due to by-product formation (*e.g.*, lactic acid, ethanol) and parallel operation of the TCA cycle.

Fumaric acid production by *Rhizopus* strains can be achieved mainly with glucose and starch hydrolysates, while sucrose, molasses and xylose result in lower efficiency compared to glucose.[91] Glycerol utilisation for fumaric acid production should be investigated further. Ling and Ng[94] achieved the highest fumaric acid concentration (130 g L^{-1}) and glucose to fumaric acid conversion yield (1 g g^{-1}) using $CaCO_3$ as neutralising agent and controlling dissolved oxygen at different levels during fungal growth (80–100%) and fumaric acid production (30–80%) stages. Cao *et al.*[95] reported the highest productivity (4.25 g L^{-1} h^{-1}) using immobilised fungal cells coupled with fumaric acid separation *via* adsorption. Scale-up of fumaric acid production by *Rhizopus* strains is hindered by difficulties to control fungal morphology, oxygen transfer rate, low solubility of fumaric acid salts, use of appropriate neutralising agents (*e.g.*, $CaCO_3$, Na_2CO_3, $(NH_4)_2CO_3$, $MgCO_3$), use of appropriate nitrogen sources and C/N ratios. The utilisation of different neutralising agents results in fumarate salts with varying, but relatively low, solubilities in water that leads to viscous solutions with low oxygen transfer rate and low final fermentation efficiency. Adding methanol or fatty acid esters may increase fermentation efficiency.[96,97] In general, the best approach to increase fumaric acid production is after the optimisation of medium composition to apply cell immobilisation or creation of small-size pellets that can be recycled. Fumaric acid production could be also enhanced by integrating fermentation with downstream separation using adsorption on selective resins or cooling crystallization.

Recent research has focused on improving fumaric acid production by metabolically engineered yeast or *Rhizopus oryzae* strains.[98,99] Ferreira *et al.*[100] suggested the potential implementation of zygomycete belonging fungal strains in biorefining schemes for the production of fumaric acid or lactic acid, enzymes, lipids, chitosan and animal feed.

2.1.9 2,3-Butanediol (C4). 2,3-Butanediol (BDO) is an odorless, colorless and transparent liquid at normal temperature with a chemical formula of $C_4H_{10}O_2$ and three stereo-isomer forms: dextro- [L-(+)-] and levo- [D-(−)-] forms that are optically active, as well as an optically inactive *meso*-form (Fig. 7). The levo-isomer

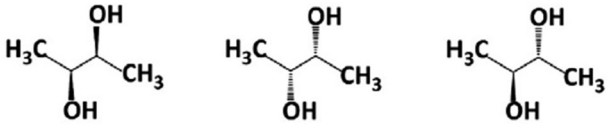

Fig. 7 Stereo-isomers of 2,3-butanediol.[102]

form of BDO has a low freezing point of −60 °C which makes it interesting for commercial use as an antifreeze agent. Both the levo- and dextro-isomers are excellent chiral components for asymmetric synthesis and are used in pharmaceutical, agro-chemical, fine chemical and food industries.[101] For instance, methyl-ethyl-ketone, the dehydration product of BDO can be used as an excellent organic solvent for resins and lacquers, while it can also find applications as liquid fuel having a higher heat of combustion than ethanol.[101,102] BDO can be converted to 1,3-butadiene, which is used as a basic chemical for the production of synthetic rubbers, polyesters and polyurethanes. Industrial 1,3-butadiene production *via* biomass conversion was carried out during World War II.[102] BDO can also be dehydrogenated to form two high added-value compounds, namely acetoin and diacetyl. Acetoin is used as an aroma carrier in flavors and essence, while diacetyl is important for the organoleptic quality of dairy products, such as cheese, butter and fermented cream, and is most widely known as a flavoring compound with a pungent buttery aroma. Likewise, BDO can also be ketalized with acetone to produce a ''tetra-methyl'' compound, which is a potential gasoline blending agent similar to the commonly used methyl *tert*-butyl ether.[102]

BDO synthesis is part of a mixed acid fermentation pathway (Fig. 8) during anaerobic or micro-aerobic growth of different wild-type microorganisms that mostly belong to bacteria of the enteric group (*e.g., Klebsiella pneumoniae*, *Enterobacter aerogenes*, *K. oxytoca*) and to the species *Paenibacillus polymyxa*, *Serratia marcescens* and *Bacillus amyloliquefaciens*.[101,102] According to the metabolic scheme presented in all microorganisms capable to synthesize BDO, 1 mole of pyruvic acid after decarboxylation and condensation with 1 mol of pyruvate yields in the synthesis of 1 mol of α-acetolactate (reaction catalyzed by α-acetolactate synthase). Thereafter, under anaerobic conditions, α-acetolactate is converted into acetoine, while in the presence of oxygen it is subjected to decarboxylation to yield in the synthesis of diacetyl. Finally, the dehydrogenase of 2,3-butanediol (BDH) converts acetoine into BDO.[103] The gene encoding the synthesis of the enzyme BDH, namely *bdhA*, was identified in 2008.[103] In addition to BDO, other undesirable end-products are formed, such as

Table 5 Experimental results of BDO producing microorganisms growing under various fermentation configurations

Microorganism	Carbon source	Culture mode	BDO (g L^{-1})	Yield (g g^{-1})	Ref.
K. pneumoniae SDM	Pure glucose	Fed-batch	150.0	0.43	105
K. oxytoca FMCC-197	Commercial glucose	Batch, shake flask	32.1	0.43	52
Serratia marcescens H30	Sucrose	Fed-batch	139.9	0.41	106
K. oxytoca	Molasses	Continuous, cell-recycling	118.0	0.42	107
K. pneumoniae CICC 10011	Jerusalem artichoke	Fed-batch	84.0	0.32	108
K. pneumoniae G31	Pure glycerol	Fed-batch	70.0	0.53	104
E. aerogenes FMCC-10	Waste glycerol	Shake flasks	22.0	0.40	51

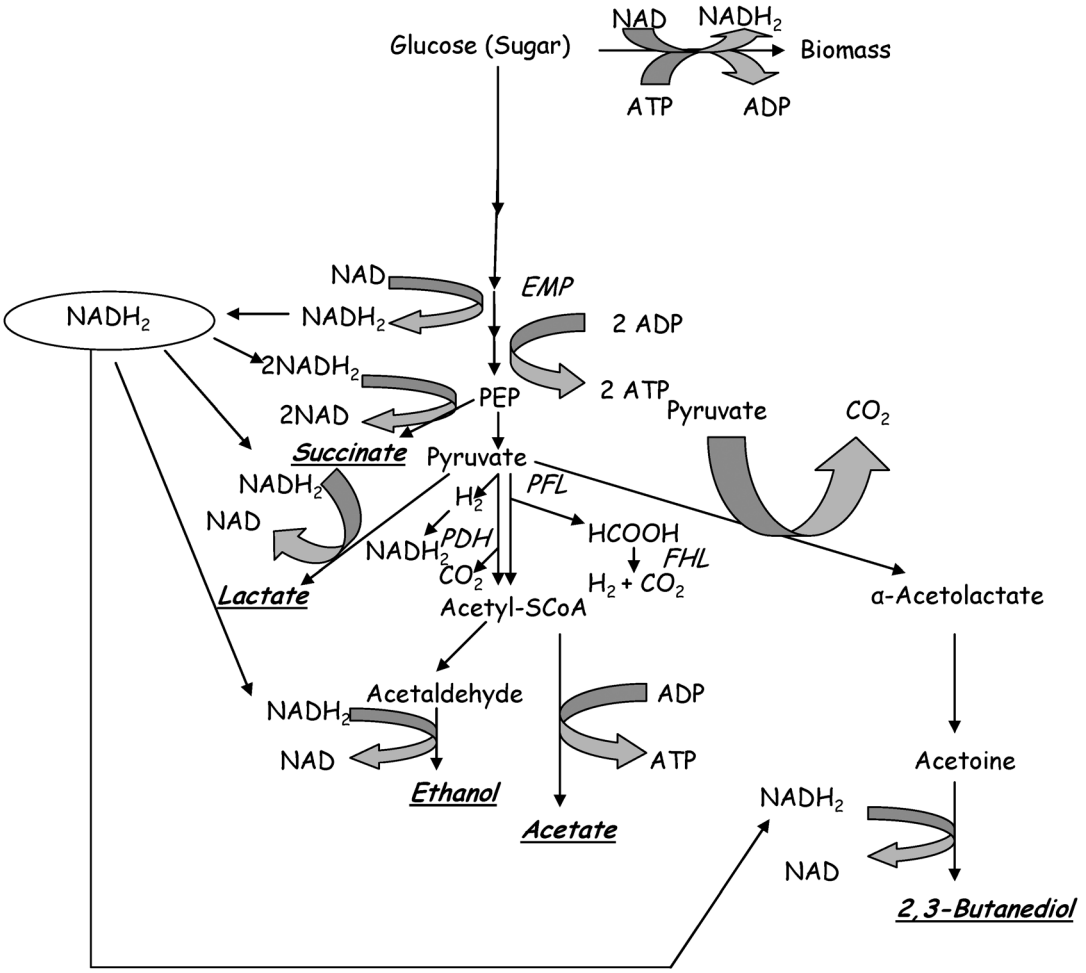

Fig. 8 Catabolic pathways of BDO production in enteric group bacteria. EMP: Embden–Meyerhof–Parnas pathway; PDH: pyruvate dehydrogenase; PFL: pyruvate formate lyase; FHL: formate hydrogenolyase.[47–49]

ethanol, acetate, lactate, formate and succinate, depending on the microorganisms and the cultivation conditions applied. Given that the production of BDO is coupled with $NADH_2$ co-factors recycling, pathways antagonistic to $NADH_2$ recycling (like, *i.e.*, hexose → lactate or hexose → ethanol) are antagonistic to the synthesis of BDO. The maximum theoretical hexose to BDO conversion yield is 0.50 g g^{-1}.[103]

BDO production is influenced by dissolved oxygen (the synthesis of BDO is enhanced under essentially low dissolved oxygen tensions – see: Celińska and Grajek[103]), pH (the maintenance of pH in slightly acidic conditions – see: Petrov and Petrova[104]) and incubation temperature. BDO can be produced from many renewable resources including starch hydrolysates, molasses, whey permeate, crude glycerol, hydrolysates from Jerusalem artichoke, and wood and corn cob hydrolysates. Table 5 presents representative literature-cited results regarding BDO production *via* fermentation.

2.1.10 Butyric acid (C4). Butyric acid ($CH_3CH_2CH_2COOH$) is a four-carbon aliphatic organic acid with various applications, including cosmetic, polymer, chemical, food and pharmaceutical manufacturing.[109] It is used for the production of textile fibers, photographic films and eyeglass frames manufacturing. In 2008, petrochemical production of butyric acid, based on oxidation of butyraldehyde, was approximately 500 000 t.[110]

More than ten microorganisms belonging to the genera *Clostridium, Butyrvibrio, Butyribacterium, Eubacterium, Fusobacterium, Megasphera*, and *Sarcina* can be used for the production of butyric acid from various renewable resources, including glucose, xylose, fructose, glycerol, lignocellulosic raw materials, molasses, potato starch and cheese-whey.[109,110] The microbial strain *Clostridium tyrobutyricum* has been predominantly used for butyric acid production under anaerobic conditions. Butyric acid can be produced with similar efficiencies from either glucose or xylose. Microbial cell immobilization in fibrous-bed bioreactors (Table 6) improves butyric acid production.[110–114] Fermentation of cane molasses containing mixed sugars (glucose, fructose and sucrose) with *C. tyrobutyricum* resulted in a high volumetric productivity of 3.22 g L^{-1} h^{-1}.[113]

In butyric acid fermentations, inhibition by metabolic products is significant.[111] In order to reduce acetic acid production, Liu *et al.*[112] used mutants strains of *C. tyrobutyricum* ATCC 25755, but acetic acid concentration was similar to that in the wild type. Creating efficient engineered or adapted strains could solve these problems. For instance, fed-batch fermentation carried out on glucose by an adapted strain of *C. tyrobutyricum*

Table 6 Butyric acid production from various media using fed-batch cultures of *Clostridium tyrobutyricum* either as suspended cultures or immobilized on fibrous-bed bioreactors

Microorganism	Carbon source	Butyric acid concentration (g L^{-1})	Productivity (g L^{-1} h^{-1})	Yield (g g^{-1})	Ref.
C. tyrobutyricum ATCC 25755	Glucose	86.9	1.1	0.46	110
C. tyrobutyricum ATCC 25755	Corn fibre hydrolysate	—	2.91	0.47	111
C. tyrobutyricum ATCC 25755 mutants	Glucose	43	0.37	0.47	112
C. tyrobutyricum ATCC 25755	Cane molasses	55.2	3.22	0.46	113
C. tyrobutyricum ZJU 8235	Jerusalem artichoke hydrolysate	60.4	1.14	0.38	114

immobilized in a fibrous bed bioreactor led to around 87 g L^{-1} butyric acid.[110]

2.1.11 Putrescine (C4) and cadaverine (C5)

Putrescine (1,4-diaminobutane, $NH_2(CH_2)_4NH_2$) and cadaverine (1,5-diaminopentane, $NH_2(CH_2)_5NH_2$), first described in 1885 by Ludwig Brieger, are important monomers for the production of polyamides with many industrial applications. Contrary to other biotechnologically produced polymers, *e.g.*, polyhydroxyalkanoates and polylactic acid, polyamides exhibit improved material properties and open the way for high-value products, such as nylon-4,6 that can be derived *via* polycondensation of putrescine with adipic acid.[115–118] Cadaverine can be used in the synthesis of polyamides with succinic acid and sebacic acid, respectively. The current annual production of polyamides of 3 500 000 t is based mainly on the utilization of petrochemical feedstocks. Despite the enormous economic potential of biotechnological production of polyamides, only few studies describing their microbial production are available. Most of the studies described were conducted with engineered bacterial strains, where the degradation pathway for putrescine and cadaverine was deactivated, in order to design "*superior*" production strains.

Putrescine can be produced by bacteria from arginine: (1) *via* the agmatine deiminase pathway and (2) *via* the ornithine decarboxylase pathways. The first pathway, where arginine is first decarboxylated to agmatine and afterwards deiminated to putrescine, occurs predominantly in bacteria of the genus *Lactobacillus*.[119,120] The second pathway involves deimination of arginine to form ornithine followed by conversion of ornithine to putrescine by decarboxylation.[119,121,122]

In the case of putrescine production, Qian *et al.*[123] developed an engineered *Escherichia. coli* strain by amplifying the gene of putrescine production (*argC-E*) and deleting the genes (*speE, speG, pupa, argI, and argR*) responsible for degradation and by-product formation pathways. Results showed a putrescine concentration of 24.2 g L^{-1} with a productivity of 0.75 g L^{-1} h^{-1}. A similar approach was employed by Schneider and Wendisch[122] leading to the development of an engineered strain of *Corynebacterium glutamicum* that produced 6 g L^{-1} putrescine with a yield of 0.12 g g^{-1} from glucose and a productivity of 0.1 g L^{-1} h^{-1} in batch shake flask cultures. However, this process suffered from the costly supplementation with L-arginine. Schneider *et al.*[124] improved the engineered *C. glutamicum* strain by fine-tuning *argF* expression through modifications of the promoter, resulting in 19 g L^{-1} putrescine concentration with a yield of 0.16 g g^{-1} from glucose and a productivity of 0.55 g L^{-1} h^{-1} in fed-batch culture.

Cadaverine can be produced by bacteria *via* direct decarboxylation of lysine.[117,125] In order to produce cadaverine from renewable resources, Buschke *et al.*[117] introduced successfully genes into *C. glutamicum* for conversion of hemicellulose fractions. Qian *et al.*[125] engineered an *E. coli* strain in such a way that the cadaverine degradation was inactivated and the gene encoding lysine decarboxylation overexpressed. The final strain produced 9.61 g L^{-1} cadaverine with a productivity of 0.32 g L^{-1} h^{-1}.

2.1.12 Itaconic acid (C5). Itaconic acid ($C_5H_6O_4$) is another decarboxylic acid that was initially included in the top 12 most promising platform chemicals reported by Werpy and Petersen[9] but it was removed from the revised list published by Bozell and Petersen.[10] Reduction of itaconic acid leads to the production of 3-methyl succinic acid, a precursor of chemicals such as methyl butanediol, butyrolactone and tetrahydrofuran that can be used as solvents and polymers. Other interesting reactions include amidations to itaconic amides which have uses as polymers, specialty chemicals and reductive amidations to give pyrrolidinone. Itaconic acid has also several potential uses as a monomer or co-monomer in polymers.[126]

The fungal strain *Aspergillus terreus* NRRL 1960, isolated at the Northern Regional Research Laboratory (NRRL)[127], is the most widely studied strain. Low cost substrates have been widely employed as carbon or nitrogen sources. Itaconic acid production by *Aspergillus terreus* TN-484 using raw corn starch resulted in concentrations up to 60 g L^{-1} using 140 g L^{-1} of initial corn starch concentration.[128] Promising results were reported by Jarry and Seraudie[129] regarding itaconic acid production (49.6 g L^{-1}) using *A. terreus* NRRL 1960 cultivated on glycerol. Levinson *et al.*[130] reported the production of approximately 30 g L^{-1} itaconic acid from 80 g L^{-1} glucose using *Pseudozyma antarctica* NRRLY-7808 cultivated under nitrogen-limited growth conditions in flask fermentations. Chandragiri and Sastry[131] used *Ustilago maydis* NCIM 983 for the production of 68.4 g L^{-1} itaconic acid from 80 g L^{-1} glucose concentration.

2.1.13 Xylitol (C5). Xylitol ($(CHOH)_3(CH_2OH)_2$) is a polyol and a five-carbon sugar alcohol widely used in the food and pharmaceutical industries due to its tooth-friendly nature, capability of sugar substitute for insulin-independent diabetic patients and as a food sweetener.[132] In 1970s, xylitol was first produced from birch trees in Finland by chemical methods. Since then, due to its sweetening power similar to that of sucrose and also beneficial properties, the production of xylitol has attracted global interest. The global production of xylitol is expanding and estimated currently up to 60 000 t per year. Traditional production of xylitol is achieved by chemical

hydrogenation of D-xylose, which is generally obtained from wood sources in the presence of nickel catalyst at high temperature and pressure with a yield of 50–60%. In light of the wide range of its application, its international market has been expanding constantly.

Xylose is metabolized to xylitol by specific microbial strains in a sequential catalytic activity of xylose reductase and xylitol dehydrogenase enzymes.[132] Yeasts are regarded as the most robust xylitol producing strains with *Candida* strains being the most extensively studied. These strains are capable of maintaining the redox balance during xylitol accumulation. Fermentative production of xylitol can be achieved with free or immobilized cell systems cultivated on pure xylose or xylose-based hydrolysates. Kwon *et al.*[133] employed *C. tropicalis* cultivated on xylose to produce 182 g L^{-1} xylitol at a productivity of 12 g L^{-1} h^{-1} and a conversion yield of 0.85 g g^{-1}. Zhang *et al.*[134] employed a recombinant strain of *Kluyveromyces marxianus* cultivated on corn cob hemicellulose hydrolysates to produce 9.1–11.3 g L^{-1} xylitol at a conversion yield of 0.49–0.63 g g^{-1}.

2.1.14 Microbial oil—glycerol (C3) and fatty acids (C14–C22). In recent years, there is growing interest on the production of microbial oils and fats (or "single cell oils, SCO") using oleaginous microorganisms cultivated on several types of renewable carbon sources. SCO are mainly composed of neutral fractions, principally triglycerides and to a lesser extent steryl-esters.[135–139] Unable to integrate into phospholipid bilayers, storage microbial lipids cluster to form the hydrophobic core of the lipid or oil bodies.[138] The fatty acid composition of SCO depends on the oleaginous microorganism and the cultivation conditions used (Table 7). Conventional uses of SCO include:

- Biodiesel production when the fatty acid composition is similar to vegetable oils.[140]
- Medical and dietetical uses when SCO contain rare polyunsaturated fatty acids, such as γ-linolenic acid and arachidonic acid.[135]
- Cocoa-butter substitutes when the fatty acid composition is similar to the one of cocoa-butter.[137,138]

SCO could be potential substitutes for natural oils and fats as feedstock for chemical production. Natural oils and fats are currently converted into free fatty acids, glycerol, fatty acid methyl esters and fatty alcohols that are subsequently converted into many end-products, including fatty acid esters, fatty amines, fatty acid ethoxylates, soaps and various glycerol derivatives, with a spectrum of applications such as biodiesel, surfactants, lubricants, waxes, cosmetics, paints and chemical feedstocks among others.[144] Natural fats and oils are gradually replacing petroleum in the production of fatty alcohols.[145] New developments in olefin metathesis allow direct polymerisation or functionalisation of fatty acids and their derivatives by introducing a variety of functional groups.[146] Advancements in the field of lipid biotechnology will improve microbial production of chemicals, lipid modification, fatty acid functionalisation, and synthesis of novel fatty compounds.[145,147] Chowdhury *et al.*[148] reported the efficient production of bio-lubricants (octyl-esters) *via* a two-step process involving *Candida rugosa* lipase-mediated hydrolysis of waste cooking oil to free fatty acids followed by Amberlyst 15H esterification of free fatty acids with octanol.

SCO production can be achieved by many microalgae, yeast (*e.g. Cryptococcus*, *Lipomyces*, *Rhodosporidium*), fungi (*e.g. Mortierella*, *Cunninghamella*) and bacteria that accumulate triglycerides at more than 20% (w/w) of their total dry weight.[149] SCO accumulation is achieved using either sugars (or similarly metabolized substrates such as glycerol) or hydrophobic compounds (*e.g.* triglycerides, free fatty acids) as carbon sources. Lipid accumulation from sugars is achieved in a two-stage fermentation under conditions of nitrogen (or other nutrient) limitation and in the presence of excess carbon that cause rapid reduction of growth rate and in effect gradual reduction of carbon assimilation rate. This results in the preferential channeling of the carbon flux towards lipid neo-synthesis. This is the so-called "*de novo*" lipid accumulation process (Fig. 9). Under these conditions, oleaginous microorganisms produce large amounts of TCA cycle intermediates, such as citric acid and iso-citric acid, which are not further catabolized *via* the TCA cycle. In fact, nitrogen exhaustion leads to a rapid decrease of the concentration of cellular AMP, which is further cleaved in order for nitrogen to be offered to the microorganism. Cellular AMP concentration decrease alters the Krebs cycle function; NAD^+-(and in various cases $NADP^+$-isocitrate) dehydrogenase, that is allosterically activated by intracellular AMP, loses gradually or abruptly its activity[150] and the carbon flow, hence, is directed towards the accumulation of intra-mitochondrial citric acid. Then, citric acid is secreted inside the cytoplasm, in order to be cleaved by ATP-citrate lyase, a key enzyme showing the

Table 7 Representative fatty acid composition of SCO produced by various oleaginous yeast strains and comparison to a number of technical oils

Microorganism	Total lipid content (%, w/w)	Fatty acids (%, w/w of total lipids)					Analogous (similar) technical profile
		C16:0	C16:1	C18:0	C18:1	C18:2	
Candida curvata D	58	32	—	15	44	8	Palm/Palm olein
Candida 107	42	44	5	8	31	9	Palm
Cryptococcus albidus	65	12	1	8	73	12	Olive oil
Lipomyces starkeyi	63	34	6	5	51	3	Palm/Palm olein
Lipomyces starkeyi	68	55.9	1.8	13.8	25.8	0.1	Cocoa butter
Trichosporon pullulans	65	15	—	2	57	24	Canola/olive
Yarrowia lipolytica	43	15	3	11	47	21	Chicken fat
Rhodosporidium toruloides	67.5	20	0.6	14.6	46.9	13.1	Lard

The fatty acid composition is taken from Wynn and Ratledge,[141] Angerbauer *et al.*[142] and Li *et al.*[143]

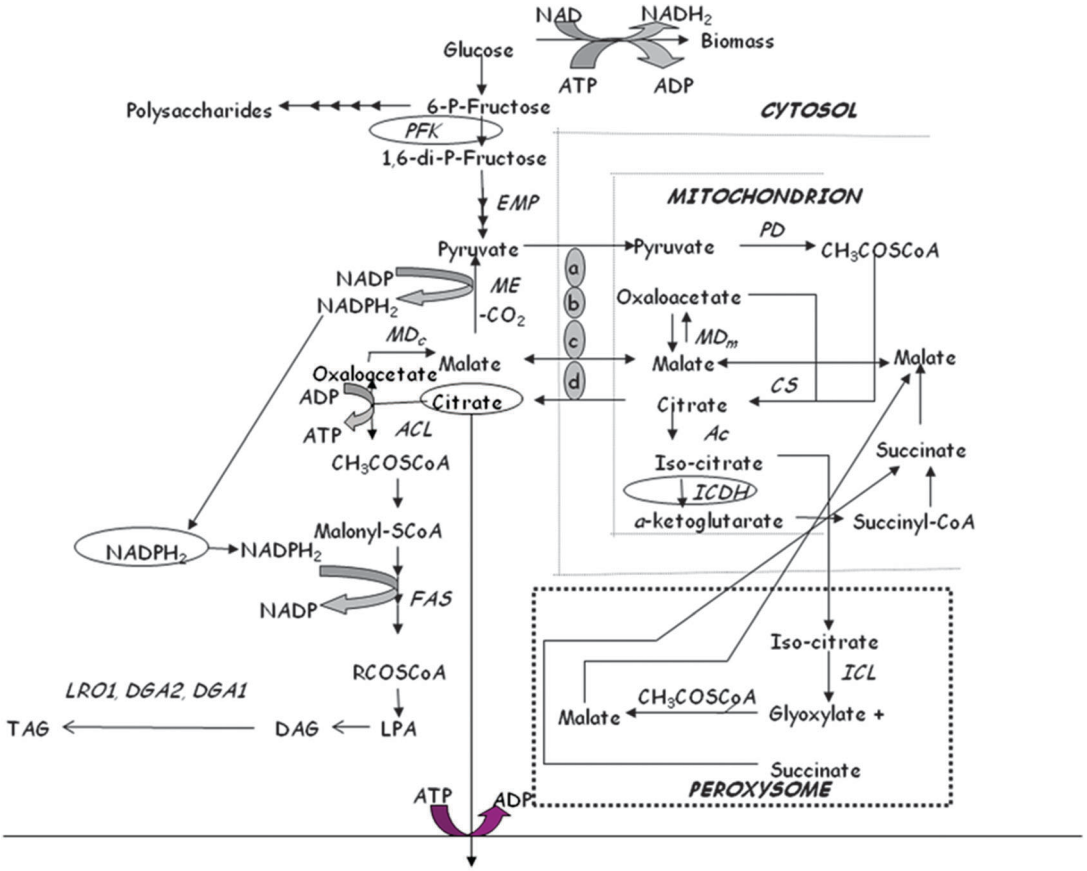

Fig. 9 Pathways involved in the breakdown of glucose by microbial strains capable of producing SCO, polysaccharides and/or citric acid in nitrogen-limited conditions. LPA: lysophosphatidic acid; DAG: diacylglycerols; TAG: triacylglycerols; TRSP: citric acid transporting system; a–c: systems transporting pyruvic acid from cytosol to mitochondrion and inversely; d: system transporting citric and malic acid from cytosol to mitochondrion and inversely; ACL: ATP-citrate lyase; FAS: fatty acid synthetase; ICDH: iso-citrate dehydrogenase; MD_c: malate dehydrogenase (cytoplasmic); MD_m: malate dehydrogenase (mitochondrial); ME: $NADPH^+$-malic enzyme; PD: pyruvate dehydrogenase; CS: citrate synthase; ICL: iso-citrate lyase; PFK: phospho-fructokinase; DGA1: diacylglycerol acyltransferase; DGA2: phospholipid diacylglycerol acyltransferase; LRO1: lysophosphatidic acid acyl transferase; EMP: Embden–Meyerhof–Parnas pathway.[135,136,139,140]

oleaginous nature of the microorganism, into acetyl-CoA and oxaloacetate, and acetyl-CoA, by virtue of the action of fatty acid synthetase, generates cellular fatty acids and subsequently triglycerides that are the most common form of lipophilic compounds found in the oleaginous microorganisms.[136–138,140] In the non-lipid producing microorganisms, nitrogen exhaustion provokes secretion of the previously hyper-synthesized citric acid into the growth medium (case of the fungus *Aspergillus niger* and many of the strains of the yeast *Yarrowia lipolytica*) or results in a block in the level of 6-phospho-fructokinase (with mechanisms similar to the ones related with the decrease of activity of NAD^+-isocitrate dehydrogenase), leading to the accumulation of (intra-cellular) polysaccharides.[135,137] Specifically, in the case of the yeast *Y. lipolytica*, citric acid and storage lipids can be synthesized sequentially or in parallel.[136]

Lipid accumulation from hydrophobic substances (like triglycerides, soap-stocks, *etc.*) is achieved *via* hydrolysis of culture triglycerides with (extra-cellular or cell-bound) lipases and fatty acids are incorporated inside the microbial cells or mycelia with various incorporation rates.[138] While only a restricted number of microorganisms can grow on triglycerides (these that can synthesize lipases) much more microbial cells can present growth on media composed of free fatty acids as the sole carbon source.[138] Previously incorporated from the substrate, fatty acids will be either partly or completely assimilated for growth needs [by virtue of the process of β-oxidation, into smaller chain acyl-CoAs and finally acetyl-CoA-reactions catalyzed by various acyl-CoA oxidases (Aox)] or will be accumulated as storage materials, as they are or after having been subjected to enzyme-catalyzed modifications (*e.g.*, desaturation or elongation reactions), therefore, there is no biosynthesis through acetyl-CoA condensation, and, thus, this process of lipid accumulation is called "*ex novo*".[138] It is interesting to state that individual fatty acids present in the substrate will be removed from the medium (and hence incorporated inside the microbial cell) at different rates and the fatty acid composition of the medium can be significantly altered as a function of fermentation time.[136,138] On the other hand, "*new*" fatty acid profiles that did not previously exist in the substrate fat are likely to be produced in the intracellular level (this is the so-called "*fat biomodification process*").

In the *ex novo* lipid accumulation process, lipid production is a growth associated process occurring simultaneously with cell growth, being entirely independent from nitrogen exhaustion from the culture medium.[136]

A plethora of (pure or raw agro-industrial) substrates can be used as carbon sources by oleaginous microorganisms, such as pure sugars, sugar-based renewable materials or sugar-enriched wastes (*e.g.*, cheese-whey, molasses), lignocellulosic sugars, vegetable oils, crude industrial saturated fatty acids, pure fatty acids, waste cooking oils, (biodiesel-derived) glycerol and mixtures of hydrophilic substrates with fats or oils.[137,140,151] Optimum SCO production is achieved in fed-batch fermentation in which total dry weights, SCO contents and productivities of 50–185 g L^{-1}, 40–60% (w/w) and up to 0.8 g L^{-1} h^{-1} have been reported, respectively.[140]

2.2 Biopolymer production *via* fermentation

The term *biopolymer* or *natural polymer* defines macromolecules that are produced in nature *via* polymerisation reactions catalysed by enzymes. They can be categorised in three groups: (1) polymers extracted from natural resources, (2) polymers produced *via* chemical synthesis using monomers or intermediate molecules derived from biomass or produced *via* fermentation, and (3) polymers synthesised by microorganisms. Biopolymers can be characterised as bio-based, renewable, biocompatible and/or biodegradable, properties defining the sustainable nature of such products. Biopolymers can be used as bioplastics (*e.g.*, packaging or structural materials), biomaterials (referred mainly to medical applications) or biocomposites depending on their properties. The scientific domain covered by *white biotechnology* focuses either on production of building blocks that could be polymerised *via* chemical synthesis or on biopolymers accumulated intracellularly or secreted extracellularly by wild-type or engineered microorganisms.

Bacteria can produce four major families of biopolymers including polysaccharides, polyesters, polyamides and polyanhydrides (such as polyphosphates).[152] Some representative biopolymers are glycogen, xanthan, alginate, dextran, cyanophycin, polyhydroxyalkanoate (PHA), cellulose and poly-γ-glutamate. The biosynthesis and even the properties of bacterial biopolymers are controlled by complex regulatory pathways responding to external stimuli in order to serve a wide spectrum of biological functions, such as carbon and energy reserve under nutrient limiting conditions or as a product of a cell protection mechanism.[152]

The development of cascade processing will be a major breakthrough in the establishment of bioplastics that could lead to efficient processes, competitive with petroleum-derived plastics.[153] Cascade processes of bioplastics will be based on the collection of post-consumer plastics after use and re-utilisation in various applications. For instance, bioplastics could be hydrolysed into monomers that could be used as building blocks for chemical production.

This section presents PHA and bacterial cellulose as representative biopolymers produced by microorganisms.

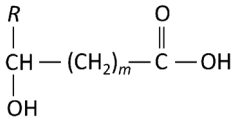

Fig. 10 Generic chemical formula of hydroxyl alkanoates in which m = 1–4 and R represents either H or alkyl side groups of varying chain lengths (C_1–C_{13}).

2.2.1 PHA. PHA is a family of biopolyesters accumulated intracellularly in the cytoplasm, as inclusions (or granules), by many microorganisms for carbon and energy reserve purposes. PHA production is usually triggered under the presence of an excess carbon source and a limiting nutrient (*e.g.*, nitrogen, phosphorus or oxygen) that is necessary for microbial growth. This wide family of polyesters contain monomers of 3-, 4-, 5- or 6-hydroxy alkanoic acids (Fig. 10).

Depending on the number of carbon atoms in the chemical formula of hydroxyalkanoic acid monomers, PHA can be divided into short-chain length (3–5 carbon atoms), medium-chain length (6–14 carbon atoms) (Fig. 11) and long-chain length (more than 14 carbon atoms). Despite the wide diversity of hydroxyalkanoic acids (more than 150 different hydroxyalkanoic acids have been identified[154]), the most widely studied PHA contain mainly 7 monomers (m = 1 in all cases): 3-hydroxybutyrate (3HB, R = CH_3), 3-hydroxyvalerate (3HV, R = CH_2CH_3), 3-hydroxyhexanoate (3HHx, R = $CH_2CH_2CH_3$), 3-hydroxyoctanoate (3HO, R = $(CH_2)_4CH_3$), 3-hydroxynonanoate (3HN, R = $(CH_2)_5CH_3$), 3-hydroxydecanoate (3HD, R = $(CH_2)_6CH_3$) and 3-hydroxydodecanoate (3HDD, R = $(CH_2)_8CH_3$). PHB and P(3HB-*co*-3HV) are the most well-studied members of the PHA family. Several publications have covered adequately the historical overview, research conducted and industrial applications related to PHA.[154–159]

In recent years, PHA production from pure carbon sources has been replaced by research initiatives utilising hydrolysates from renewable resources or waste and by-product streams from various industrial sectors (Table 8). This is feasible due to the wide diversity of carbon sources (Fig. 12), including glucose, fructose, sucrose, xylose, lactose, glycerol, ethanol, fatty acids, amino acids, acetic acid and butyric acid among others, that can be used for PHA production by wild-type, adapted or genetically engineered microorganisms. PHA production can be achieved by various bacterial strains as secondary metabolites under nutrient liming conditions (*e.g., Cupriavidus necator*, *Bacillus*

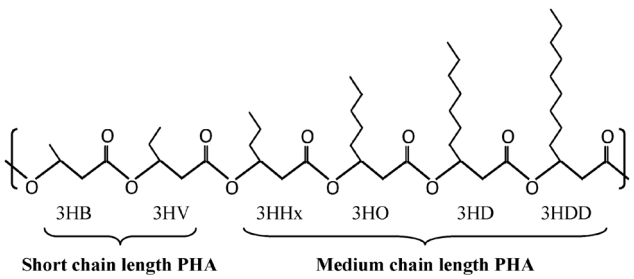

Fig. 11 Classification of common PHA based on the number of carbon atoms in their molecular formula.[154]

Table 8 PHA production from various waste and by-product streams

By-product or waste stream	Strain	Type of PHA	PHA ($g\ L^{-1}$)	PHA content (%)	Productivity ($g\ L^{-1}\ h^{-1}$)	Ref.
Crude glycerol	*Cupriavidus necator* DSM 545	PHB	38.1	50	1.1	161
Crude glycerol & precursors	*Cupriavidus necator* DSM 545	P(3HB-*co*-4HB)	10.9	36.1	0.17	162
		P(3HB-*co*-4HB-*co*-3HV)	16.7	36.9	0.25	
Crude glycerol and rapeseed meal hydrolysates	*C. necator* DSM 545	P(3HB-*co*-3HV)	10.9	55.6	0.12	163
Waste rapeseed oil & precursor	*C. necator* H16	P(3HB-*co*-3HV)	105	76.1	1.46	164
Sugar cane bagasse hydrolysates[a]	*Ralstonia eutropha*	PHA	6.3	56.5	—	165
Glucose, xylose and sugar cane bagasse hydrolysate	*Burkholderia sacchari* IPT 101	PHB	60	58	0.5	166
Whey	*Escherichia coli* CGSC 4401	PHB	168	87	4.6	167
Hydrolyzed whey	*Haloferax mediterranei* DSM 1411	P(3HB-*co*-3HV)	12.2	72.8	0.09	168
Hydrolyzed whey & precursors		P(3HB-*co*-3HV-*co*-4HB)	14.7	87.5	0.14	
Saccharified waste potato starch	*Ralstonia eutropha* NCIMB 11599	PHB	94	55	1.47	169
Sugar cane molasses and urea	*Bacillus megaterium* BA-019	PHB	30.5	42	1.27	170

[a] Detoxification approach to combine PHA production with removal of major organic inhibitors including formic acid, acetic acid, furfural and acid soluble lignin.

Table 9 Current and potential PHA production on an industrial scale[173]

PHA type	Manufacturer	Capacity (t)	Price € per kg
PHB	Mitsubishi Gas Chemical Company Inc. (Japan), Telles (US), PHB Industrial Company (Brazil), Biomer Inc. (Germany)	50–50 000	1.5–5
P(3HB-*co*-3HV)	Biomer Inc. (Germany), Tianan Biologic, Ningbo (China)	50–10 000	—
P(3HB-*co*-3HHx)	P&G (US), Lianyi Biotech (China), Kaneka Corporation (Japan)	1000–50 000	13
P(3HB-*co*-4HB)	Tianjin Gree Bio-Science Co./DSM	10 000	3–4

megaterium, *Pseudomonas cepacia*) or as primary metabolites during growth (*e.g., Alcaligenes latus*), halophilic bacteria (*e.g., Halomonas boliviencis*) and haloarchae (*e.g., Haloferax mediterranei*). The metabolic pathways involved in the production of different PHA are quite diverse depending on the microorganism, the carbon source and the precursor used (Fig. 12). The most common pathway refers to the utilization of carbohydrates or acetate for the production of PHB, the synthesis of which is regulated at the enzymatic level.[160] PHB synthesis is catalysed by the action of three enzymes beginning from acetyl-CoA: (1) 3-ketothiolase catalyses the condensation of two acetyl-CoA molecules into acetoacetyl-CoA, (2) NADPH-dependent acetoacetyl-CoA reductase catalyses the reduction of acetoacetyl-CoA into (*R*)-3-hydroxybutyryl-CoA and (3) PHB synthase catalyses PHB synthesis accompanied by the release of CoA.[160] PHB synthesis is regulated by the intracellular concentrations of acetyl-CoA and free CoA. Therefore, low TCA-cycle activity and inhibition of citrate synthase is necessary in order to enhance PHB synthesis. This can also be supported by the fact that increasing intracellular concentrations of NADH and NADPH enhance PHB production and reduce citrate synthase activity.[160]

The application of biorefinery schemes and the utilization of waste and by-product streams are essential in order to develop cost-competitive PHA production. However, low-cost production should coincide with the production of PHA with desired properties that should be regulated during fermentation. The combination of these targets is a very difficult task and for this reason PHA production on an industrial scale is still limited, despite the intensive research and development that has been conducted in the last 30 years. In recent years, research has focused on the identification of wild-type strains or engineering of existing strains that can produce PHA co- and ter-polymers[162,168] with desired properties when cultivated on crude resources. Based on the properties of PHA, in particular the composition and type of monomers and the molecular weight, they can be processed to a wide range of end-products including films and sheets, fibres, molded articles, laminates and coated articles, elastics, adhesives, non-woven fabrics, synthetic paper products and foams.[171,172] Table 9 presents current and potential commercial scale PHA manufacturers.

In the frame of cascade processing, PHA-derived articles (*e.g.*, packaging materials) could be collected and hydrolysed into the respective monomers that could be used as building blocks in the chemical industry. Enzymatic hydrolysis of PHA by lipase or intracellular and extracellular depolymerises produced by several bacterial strains is feasible and the recovery of 3HB units is economically viable.[174]

2.2.2 Bacterial cellulose. Plant cellulose is the most abundant biopolymer on earth ($\sim 1.5 \times 10^{12}\ t\ y^{-1}$) that can be used in various applications, but it cannot be produced in a pure form (due to the presence of lignin, hemicelluloses and other molecules). Cellulose biosynthesis can be carried out by algae (*Phaeophyta*, *Rhodophyta*, *Chrysophyta*), some fungi, Gram-negative bacteria (*e.g., Acetobacter*, *Achromobacter*, *Aerobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, *Sarcina*) and the Gram-positive bacterium *Sarcina ventriculi*. The most efficient producers of cellulose are *Acetobacter hansenii*, *A. pasteurianus* and mainly *A. xylinum*.[175]

Bacterial cellulose has the same chemical structure $(C_6H_{10}O_5)_n$ as that of plant cellulose but has different physical and chemical

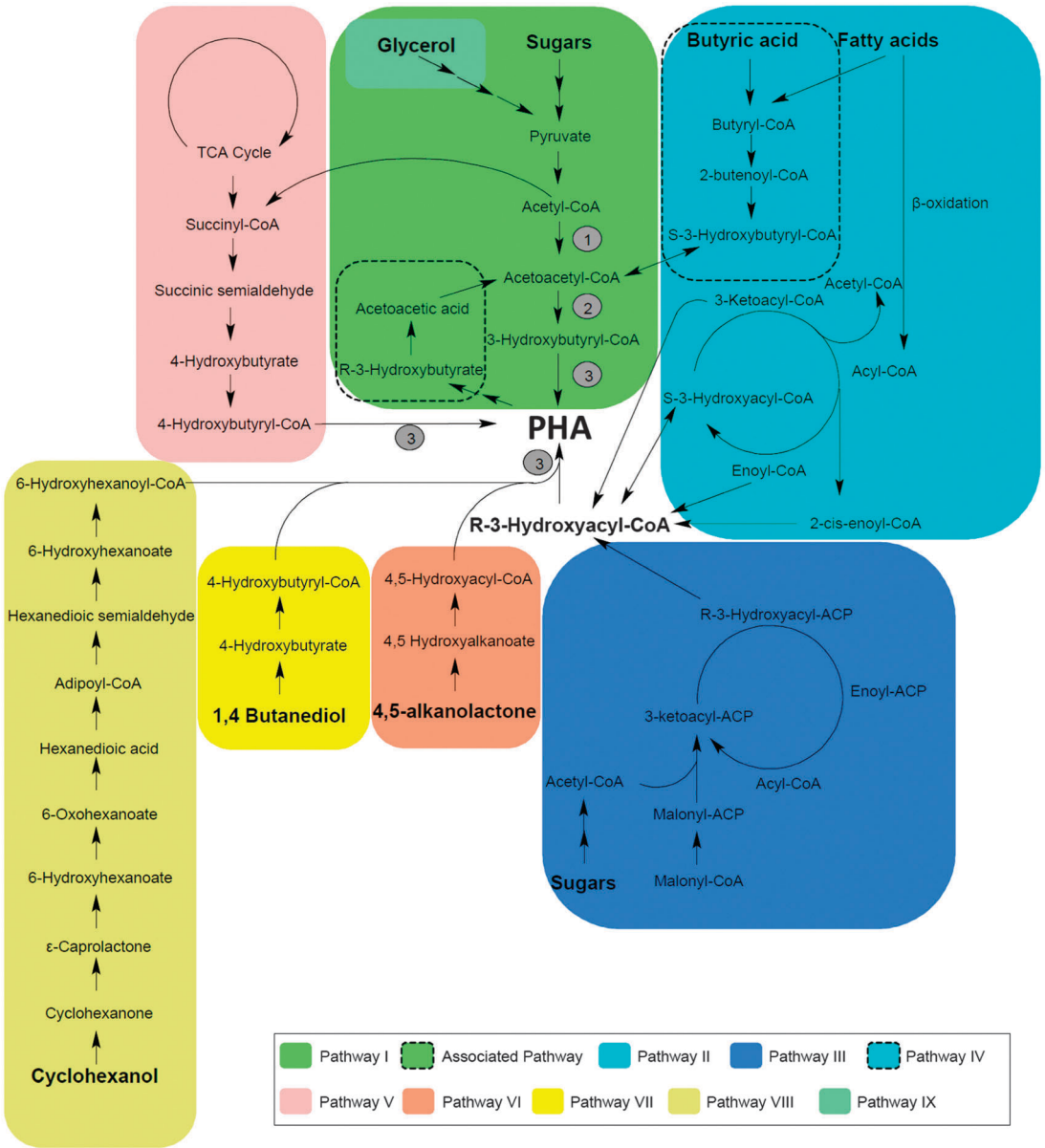

Fig. 12 PHA biosynthetic pathways. The most common pathway in *C. necator* for the production of PHB requires 3 enzymes: (1) 3-ketothiolase, (2) NADPH-dependent acetoacetyl-CoA reductase, and (3) PHB (or PHA in other cases) synthase.[154]

properties. It is produced at high purity, it exhibits a higher degree of polymerization and crystallinity index and has higher tensile strength and water holding capacity than that of plant cellulose, making it appropriate in applications that plant cellulose is considered unsuitable.[176] Bacterial cellulose can be used in pharmaceutical and medical applications, especially in the area of wound healing. In particular, Biofill produced two products (Bioprocess and Gengiflex), as dressings for extensive wounds, while another bacterial cellulose preparation (Prima Cel_{TM}) produced by Xylos Corp has already been applied in clinical tests to heal ulcers and wounds.[175] Regarding food applications, bacterial cellulose (accepted as GRAS in 1992) is used when lack of flavour interactions, foam stabilization, and stability over wide pH range, temperature and freeze–thaw conditions are required. The first application of bacterial cellulose in the food industry was in the calorie-free food "*nata de coco*" in the Philippines, while its involvement in ice creams, as a low-calorie additive, thickener, stabilizer and texture modifier seem to have great potential.[176] Bacterial cellulose is also suitable as a reinforcing agent for paper and fibers, made from glass, carbon, phenol resin and silicon, while it is already used in the production of headphones and loudspeaker membranes (Sony Corp.)[177]

The mechanism of cellulose biosynthesis by the "model microorganism" *A. xylinum* occurs between the outer and plasma membranes of the cell by the action of cellulose synthase, which catalyzes the addition of uridine diphosphate glucose (UDP glucose) to the cellulose chain end. The polymerization rate is limited by the rate of the cellulose complex

crystallization, after which bacterial cellulose exits the cell as an elementary fibril, forming a 3-D network.[177]

Commonly a 60–80% of the utilized carbon source is converted into crude polymer in high yielding fermentations. Bacterial cellulose production can be achieved with many carbon sources including glucose, sucrose, fructose, maltose, xylose, starch and glycerol. High yields of bacterial cellulose can be achieved through the addition of various nutrient supplements. Son *et al.*[178] achieved a four times higher bacterial cellulose concentration (15.2 g L^{-1}) through the addition of ethanol in cultures with *Acetobacter* sp. A9. Keshk and Sameshima[179] observed an increase in bacterial cellulose production (10.1 g L^{-1}) in cultures of *Gluconacetobacter xylinus* IFO 13773, when the glucose-based fermentation medium was supplemented with lignosulfonate. Bae *et al.*[180] reported the production of 12.8 g L^{-1} bacterial cellulose by *A. xylinum* BPR 2001 cultivated in a corn steep liquor-fructose medium supplemented with an agar concentration of 0.4% that was around 60% higher than the bacterial cellulose concentration (8 g L^{-1}) achieved without agar addition. In another recent study, different additives including agar, carboxymethylcellulose, microcrystalline cellulose and sodium alginate were added into fermentation medium in agitated culture to enhance bacterial cellulose production by *A. xylinum* ATCC 700178. The addition of carboxymethylcellulose led to the highest bacterial cellulose concentration (8.2 g L^{-1}) compared to the control fermentation (1.3 g L^{-1}).[181]

Many researchers reported the implementation of several support materials in order to enhance bacterial cellulose production. For instance, a 2.5-fold higher bacterial cellulose concentration (7.05 g L^{-1}) has been achieved with a plastic composite support biofilm reactor.[182] Research has also focused on the utilisation of industrial by-product streams. For instance, glycerol utilisation by *Gluconacetobacter* sp. RKY5 led to the production of 5.6 g L^{-1} bacterial cellulose.[183] Bae and Shoda[184] produced 7.8 g L^{-1} of bacterial cellulose using molasses in fed-batch cultures of *A. xylinum* BPR2001. The highest bacterial cellulose concentration (28 g L^{-1}) has been reported by Vandamme *et al.*[185]

3. Major industrial waste and by-product streams

In the bio-economy era, fermentative production of chemicals and biopolymers should rely on the utilization or renewable carbon sources. The following sections present waste and by-product streams produced by major mature industrial sectors. It is envisaged that such renewable carbon sources could be utilised for fermentative chemical and biopolymer production. Potential biorefinery concepts combining fermentative production of chemicals and production of value-added products *via* fractionation of the original resource are also presented.

3.1 Food industry waste streams

In 2011, the global petroleum consumption reached 88×10^6 barrels per day that corresponds to 12×10^6 t oil consumption per day.[7] If it is considered that only 7% of annual petroleum consumption (3×10^8 t per year) is required for chemical production,[6] then the 1.3×10^9 t of food waste generated worldwide per annum constitute a renewable resource for chemical production that at least in terms of capacity should not be neglected.[186] However, direct comparison is misleading because the carbon content of food waste streams is lower due to the high water content of many food waste streams. For instance, despite the high quantities of whey produced annually from the dairy industry, the high water content (more than 90%) requires pre-treatment steps including ultrafiltration membranes to separate protein isolates and evaporation to concentrate the remaining lactose that could be used as carbon source in fermentation processes.

Food wastes are generated through the whole food supply chain in the following three major stages: (1) production and storage of raw material (mainly in third world countries), (2) industrial processing, and (3) municipal waste disposal including domestic consumption and out-of-date or unconsumed products disposed from restaurants, supermarkets and various catering services. In recent years, food waste valorisation is gaining momentum and could play a pivotal role in the bio-economy era regarding chemical and biopolymer production. Recent studies[80,187–192] and actions (Cost Action TD1203, http://costeubis.org/; Innovation and Technology Commission in Hong Kong ITS/323/11 and ITS/353/12, http://www.itf.gov.hk/l-eng/prj_search_index.asp) focus on the development of advanced biorefining concepts for the production of chemicals, materials and fuels from food supply chain waste. This section will cover valorisation of food processing waste streams for fermentative production of chemicals and polymers *via* biorefinery concepts. Valorisation of municipal food waste streams *via* fermentation is not covered in this review mainly because of the differences in processing strategies, such as logistics, collection, fractionation based on type of waste, quality issues, high heterogeneity and need for construction of new plants among others, compared to industrial food waste streams where integration of new processing lines in existing industrial facilities is feasible.

3.1.1 Food processing waste streams. The approach followed to describe the waste and by-products streams generated by the food industry in EU-27 has been adopted from the AWARENET report.[193] The food industry could be divided into 5 major sectors. Table 10 presents the major types of food processes and the average amount of waste and by-product streams generated per kg of raw material utilised. It can be observed that the waste and by-product streams generated by each process are quite variable. The majority of these streams is currently utilised as animal feed, composting, fertiliser and for the production of value-added products. Waste and by-product streams are also landfilled, but this approach should be avoided. Furthermore, the processes presented in Table 10 generate also significant quantities of wastewaters with significant organic loading. Utilisation of carbon and nutrient sources from such waste and by-product streams for fermentative production of chemicals and biopolymers will require restructuring of current treatment or valorisation processes and optimisation of resource utilisation.

Table 10 Clasification of food processes and respective yield of food waste and by-product generated per kg of raw material utilised[193]

Food sector	Production processes	% of wastes and by-products
Fish sector	Fish canning	30–65
	Fish filleting, curing, salting and smoking	50–75
	Crustaceans processing	50–60
	Molluscs processing	20–50
Meat sector	Beef slaughtering	40–52
	Pig slaughtering	35
	Poultry slaughtering	31–38
Dairy sector	Milk, butter and cream production	Negligible
	Yoghurt production	2–6
	Fresh, soft and cooked cheese	85–90
Wine sector	White wine production	20–30
	Red wine production	20–30
Vegetable sector	Fruit and vegetable juice production	30–50
	Fruit and vegetable processing and preservation	5–30
	Vegetable oil production	40–70
	Corn starch production	41–43
	Potato starch production	80
	Wheat starch production	50
	Sugar production and sugar beet	86

In addition, the effluents generated could be used as process water in fermentation processes as a means for saving and re-cycling water usage. Utilisation of sea water is another option to minimise the usage of fresh water.[194] Integrated biorefineries could take advantage of anaerobic digestion of effluents from fermentation and food processes in order to generate energy for the plant (*i.e.* decrease of fossil fuel utilisation) and inhibitor-free process water that could be re-utilised in fermentation processes. Finally, the CO_2 generated from breweries or wineries could be utilised in fermentation processes for the production of succinic and fumaric acids.

Table 11 presents the quantities of major food products in EU-27. The seven most important food products in terms of production capacities are: (1) dairies and cheese making, (2) meat-related products, (3) oils and fats, (4) grain mill products, (5) beer, (6) sugar, and (7) bread, fresh pastry goods and cakes. It is obvious that the quantities of food products produced only in EU-27 countries is significant and if we take into consideration the percentage of the initial raw materials that is produced as waste and by-product streams then we can realise how significant this renewable resource could become for chemical and material production. It should be stressed though that in the quantities mentioned in Table 11 it is also included the quantities of by-products (*e.g.*, molasses, whey) traded in EU-27 in 2012. Therefore, it is difficult to estimate precisely the amount of wastes and by-products generated that would be directly available for chemical and biopolymer production.

Table 12 presents the water, carbohydrate, protein and oil content of major food processing wastes and byproducts. Carbohydrates or oils could be used as carbon sources, while proteins could be used as nitrogen sources in fermentation processes. However, the utilisation of food processing wastes

Table 11 Quantities of specific food production sold in EU-27 in 2012[195,196]

NACE code	NACE code description	Total quantity (10^6 t)
10.11	Processing and preserving of meat	37.5
10.12	Processing and preserving of poultry meat	15.2
10.13	Production of meat and poultry meat products	17.7
10.20	Processing and preserving of fish, crustaceans and molluscs	5.3
10.32	Manufacture of fruit and vegetable juice	10.5
10.41	Manufacture of oils and fats	57
10.51	Operation of dairies and cheese making	80
10.52	Manufacture of ice cream	3
10.61	Manufacture of grain mill products	56.9
10.62	Manufacture of starches and starch products	18.7
10.71	Manufacture of bread; manufacture of fresh pastry goods and cakes	26.4
10.72	Manufacture of rusks and biscuits; manufacture of preserved pastry goods and cakes	8
10.73	Manufacture of macaroni, noodles, couscous and similar farinaceous products	5
10.81	Manufacture of sugar	31.9
10.82	Manufacture of cocoa, chocolate and sugar confectionery	8.8
10.83	Processing of tea and coffee	6.5
11.02	Manufacture of wine from grape	14.4
11.05	Manufacture of beer	50

Table 12 Composition of major food processing and municipal wastes and byproducts[188]

Type of food waste	Water content (%)	Carbohydrate content (%)	Protein content (%)	Oil/fat content (%)
Carbohydrate-rich wastes				
Molasses, beet	23	65.1	6.7	—
Spent grains from breweries	80–83	9–11.6	3.2–4.6	1.5–2.4
Whey	92.7	4.9	0.9	0.9
Apple pomace	3.9–10.8	48–62	2.9–5.7	1.2–3.9
Orange waste (peel, pulp and seeds) (dry basis)	79	47	6.5	—
Cassava pulp (dry basis)	6.8	69.9	1.55	0.12
Waste bread (whole wheat and white bread)	33–43	41–51	8–13	3
Rice flour (*e.g.*, waste streams from confectionary industries originally produced as food for infants)	5	86.1	7.3	1.1
Wheat bran (crude)	11	64.5	15.5	4.2
Pear pulp (dry matter)		62.8	5.1	1
Tomato pomace (dry basis)		25.4–50 (fiber)	15.4–23.7	5.4–20.5
Grape pomace without seeds (dry basis)	58.2–78.9	12.5–48.8	11.0–11.4	4.47–5.19
Lees from sherry wine		4.1 (sugars)	15.1	5.4
Potato peel (dry basis)	85	69.7	8	2.6
Potato tuber	83.3	12.5	2.6	0.1
Protein- and/or fat/oil rich wastes				
Municipal meat waste (dry basis)	41		24.6	69.9
Municipal fish waste (dry basis)	73.9		57	19.1
Soybean meal	10	29.9	42	4
Linseed meal	8	38	36	0.5
Yeast from breweries	5	39.5	43	1.5
Yeast, hydrolysate	5.5	—	52.5	—
Corn steep liquor	50	5.8	24	1
Dried distillers soluble	8	45	26	9
Fish meal (anchovy), 65%	8	—	65	3.8
Blood	86	—	12	0.3
Meat and bone meal	8	—	50	8
Pharmamedia (derived from cottonseed embryo)	1	24.1	59.2	4
Peanut meal and hulls	9.5	23	45	5
Slaughterhouse waste	74	—	9	14

only for fermentative production of chemicals and biopolymers may not be cost-competitive. For instance, utilisation of olive pulp waste streams for ethanol production will be economically viable only if it is combined with generation of other value-added products.[197] For this reason, optimisation of feedstock utilisation should be carried out *via* the development of biorefinery concepts. In this way, high-value phytochemicals should be initially extracted followed by production of fermentation media with optimised nutrient utilisation. For instance, some phytochemicals like hesperidin and limonene in orange peel residues have been successfully extracted by microwave treatment.[198] In addition, various flavonoids (*e.g.*, flavanones, flavonols, flavanols, anthocyanidins) are present in various fruits, including berries, grapes and apples, and can be extracted from the waste and by-product streams generated during their processing.[199] The rest of the components (*e.g.*, surplus protein) should be used in other market outlets.

Food processing wastes contain mono-, di- or polysaccharides that could be directly used or converted into directly assimilable carbon sources *via* enzymatic hydrolysis. Protein and phytic acid hydrolysis is also required. This means that after extraction of value added products, the production of fermentation media is dependent on the conversion of these macromolecules into assimilable nutrients. Production of enzyme consortia can be achieved *via* solid state fermentations in solid food waste streams (*e.g.*, wheat milling by-products).

In recent years, research has focused on the optimization of biorefinery development based on food waste streams using various fractionation and valorization strategies depending on the type of waste.[199–201] A generic processing scheme has been proposed by Oreopoulou and Tzia[202] for fractionation of fruit pomace leading to the isolation of carotenoids, antioxidants, dietary fiber and pectin. Olive mill wastewaters are under investigation for integrated recovery of bioactive molecules and biotechnological production of ethanol, organic acids and enzymes.[203] Fractionation of whey could be developed for the production of functional isolates and a lactose-rich stream that could be used in various fermentations.[204]

3.2 Wood processing industry

One major industrial domain that generates vast amounts of by-products is the wood processing industry. Approximately 31% of the land worldwide is covered by forests corresponding to a total forestry area of over 4000 Mha with the majority being natural forests, while plantations cover only 7% of the total forest land. Although deforestation (*e.g.*, the change of a forest to arable land) has remained at high levels, the rate of deforestation has dropped from 16 Mha per year in the 1990s to 13 Mha per year in the 2000s.[205]

The world's consumption of industrial round wood in 2010 has been estimated by FAO in 1 534 211 000 m^3, where the five leading consumption countries are USA, China, Canada, Brazil and Russia. From the processing of this round wood, a volume of 131 388 000 m^3 of wood residues is generated.[205] Industrial round wood is the wood that enters the forest

Table 13 Percentage of wood residues production of each continent and the top wood residue producing countries in 2010.[205]

Continent	Percentage of global production (%)	Country	Wood residue production (m^3)
North America	16.6	Canada	8 774 000
		USA	13 000 000
South America	13.9	Brazil	13 958 000
Asia	23.2	China	15 300 000
		Japan	6 294 000
		Malaysia	5 599 000
Europe	43.9	Austria	3 828 000
		Finland	4 782 000
		France	7 747 000
		Germany	2 830 000
		Poland	5 252 000
		Romania	2 342 000
		Russia	7 900 000
		Sweden	5 000 000
Oceania	1.8	Australia	2 300 000
Africa	0.6	South Africa	657 000

processing industry (*e.g.* sawlogs, veneer logs, pulp wood *etc.*) and the residues are primarily produced from the sawing of the wood and they are mainly rejects from sawmill like sawdust, trimmings, edgings and veneer rejects. Bark, and wood chips and particles are not included as wood residues. The latter is used as feedstock in the pulp and paper industry, while the first one has no proper market in the wood processing industry and is usually burnt for heat purposes. Only a small proportion of this residue is utilized for the production of chemicals.[206] Most of the wood residues are generated in Europe (43.9%), while China, USA, Brazil and Canada are the leading wood residues producing countries (Table 13).

Fig. 13 shows the correlation of global production of wood residues with industrial round wood in the period 2007–2011. It can be estimated that 11.5–13 m^3 of industrial round wood generates around 1 m^3 of wood residues. The main utilisation of wood residues is heat and power generation either on-site (*e.g.*, providing the heat for timber drying) or in other plants as

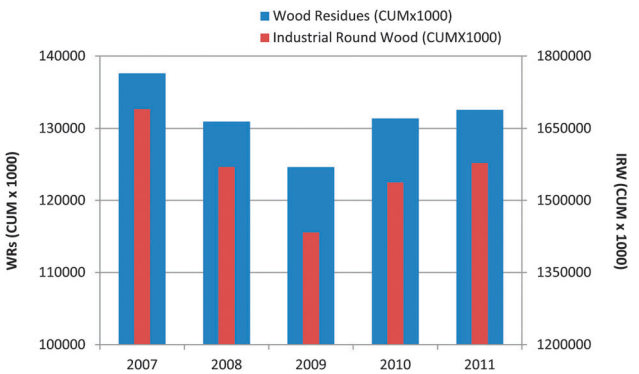

Fig. 13 Production of wood residues and consumption of industrial round wood.[210]

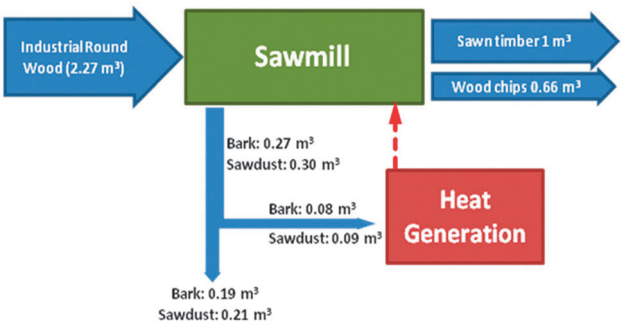

Fig. 14 Overall material balances for the production of 1 m^3 of dried sawn timber.[207]

solid fuel. Sawdust is primarily used as a raw material for the production of particle boards and fiberboards.[207] It can also be used as a feedstock in the pulp industry, however, it shows poorer quality compared to pulpwood.[207] Sawdust, saw trimmings, wood shavings and cutter chips are the main feedstocks used in the wood pellet industry that is a growing industrial sector in recent years.[208] There are other less obvious uses for wood residues that can also account for the residues/wastes of the wood furniture manufacturing industry, such as animal bedding (*i.e.*, horse, cattle or poultry bedding), playground surfaces and landscape and garden mulches composting.[209]

Fig. 14 presents a typical sawmill production process. For the production of 1 m^3 of dried sawn timber, a typical sawmill uses 2.27 m^3 of round wood (including bark). Wood chips, bark and sawdust are also produced. Wood chips (0.66 m^3) can be considered as a co-product and can be used directly as a raw material to the pulp industry. A portion of bark and sawdust can be burnt to produce heat for the various applications of the plant (*e.g.*, drying of the timber). The rest which is approximately 0.19 m^3 of bark and 0.21 m^3 of sawdust (if an equal proportion of sawdust and bark is used for heat provision) remains as by-product.[207] Therefore, there are significant quantities of wood residues available as feedstock for chemical and material production.

Wood residues have similar composition to the inner part of a tree, composed mainly of cellulose (33–51%, db), hemicelluloses (20–30%, db) and lignin (21–32%, db). The differences in the composition are mainly dependent on the tree species. Wood residues from the forest industry have favourable physical properties as they have low moisture content (less than 20%) and they are, generally, a clean solid uniform material without impurities.

3.2.1 Wood residue as feedstock for chemical production *via* fermentation. Wood-based chemicals and fuels can be obtained by extraction, hydrolysis and thermal treatment. Non-structural wood components can be extracted either as exudates or by applying solvent extraction techniques. The most well-known extracted components are rosin, turpentine, waxes and steroids, phenolic compounds from the group of flavonoids and tannins, several carbohydrates like arabinogalactan and natural rubber. Removal of some of the above extractants is not new as it originates from ancient times, however most of

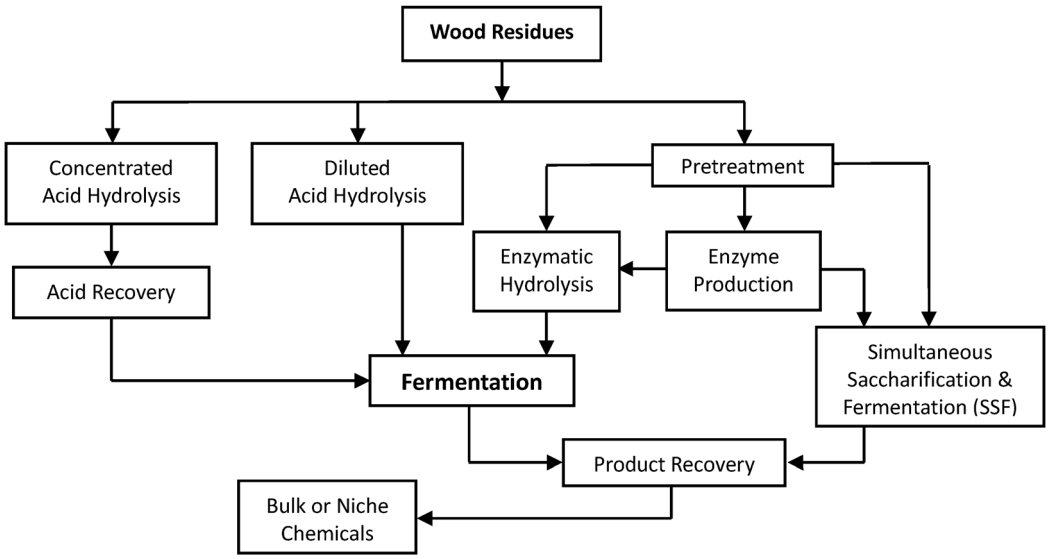

Fig. 15 Simple schematic diagram for biochemical production from wood residues.[211]

the above chemical routes were replaced by petrochemical processes.[206]

Cellulose and hemicelluloses contained in wood residues can be converted to C5 and C6 sugar monomers by hydrolysis either in acidic conditions (concentrated or diluted acid hydrolysis) or by combined chemical and enzymatic treatment. The obtained sugars can be used as carbon sources for chemical and biopolymer production *via* fermentation. The biochemical production from wood biomass constitutes a promising but also a complex valorising method of this waste stream and mainly includes alternative processing options presented in Fig. 15.[211] The most crucial step is to obtain the sugars from the polysaccharides in a cost-efficient way. Moreover, the efficiency of the pretreatment step of wood residues, if an enzymatic hydrolysis is applied, affects all the following steps and therefore it also plays a crucial role on the economics of the whole process. Process intensification can be applied here by combining the hydrolysis step with the fermentation of sugars, a process also known as simultaneous saccharification and fermentation.[212]

Acid hydrolysis can be carried out by using either concentrated or diluted acids, mainly phosphoric, nitric, sulfuric or hydrochloric acid. Concentrated acid hydrolysis can result in higher yields ($\sim$90% of the theoretical glucose yield); however it causes equipment corrosion and results in high energy requirements for acid recovery.[211] In the case of dilute acid hydrolysis, there is a low need for acid and hence its recovery is not necessary as long as it will not inhibit the fermentation process. Main disadvantages of dilute acid hydrolysis are the low yields (50–60% of the theoretical glucose yield) and the need for high temperatures which can degrade further the sugars obtained from hemicelluloses.[211]

Pretreatment of the lignocellulosic biomass is essential when enzymatic hydrolysis is applied in order to facilitate the penetration of the enzymes into the wood structure for the cleavage of cellulose. A pretreatment method should efficiently hydrolyse the hemicellulose content to C5 and C6 sugars, decrease the crystallinity of the cellulose and increase its pore size.[213] Other pretreatment methods of lignocellulosic material include steam explosion (with or without of acid catalyst), addition of an alkali or an organic solvent, wet oxidation pretreatment methods and biological techniques or combination of the above.[213–216] Enzymatic hydrolysis is performed under mild conditions using cellulases which are primarily produced by fungi, most important being *Trichoderma*, *Aspergillus* and *Penicillum* species.

Fermentation of the produced sugars can be implemented simultaneously with enzymatic hydrolysis or separately. The main advantage of conducting hydrolysis and fermentation in separate unit operations is that each unit is executed under its optimal conditions while the benefits of a simultaneous hydrolysis and fermentation process, apart from the reduced capital investment, is the direct consumption of the produced sugars by the fermenting microorganisms and hence there is negligible end-product inhibition of the enzymes. So far, research has focused mainly on the production of ethanol *via* fermentation from the hydrolysates of lignocellulosic compounds by using various microorganisms including *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Escherichia coli*.[211,217,218] Fewer papers have investigated the bioproduction of other chemicals from wood residues such as lactic acid, 2,3-butanediol, butanol, fumaric and succinic acid. Theoretical ethanol yield for different tree species ranges from 403–425 L per t of dry matter for hexoses and 25–57 L per t for pentoses.[213] Typical good yields are 0.4 to 0.5 g ethanol per g of sugars while ethanol productivity can reach high values (>1 g L^{-1} h^{-1}) when immobilized cells are employed. However, ethanol concentrations hardly ever exceed 25 g L^{-1}.[217]

Rodríguez-López *et al.*[219] reported a concentration of 9.84 g L^{-1} fumaric acid at a glucose to fumaric acid conversion yield of 0.44 g g^{-1} when *Rhizopus arrhizus* DSM 5772 was cultivated in a 2 L bioreactor using hydrolysates from *Eucalyptus globulus* wood chips mixed with a synthetic medium at a ratio of 85/15 (w/w). Kim *et al.*[220] investigated the production of succinic acid in batch and continuous cultures of *Mannheimia succiniciproducens*

MBEL55E using NaOH treated wood hydrolysates. The final succinic acid concentrations, yield and productivities achieved in batch and continuous cultures were 11.73 and 7.98 g L^{-1}, 0.56 and 0.55 g g^{-1}-sugars (glucose and xylose), and 1.17 and 3.19 g L^{-1} h^{-1}, respectively. Lee *et al.*[221] reported the production of 23.8 g L^{-1} succinic acid in less than 33 h using *Anaerobiospirillum succiniciproducens* cultivated on wood hydrolysates supplemented with 10 g L^{-1} of corn syrup. Lactic acid production up to 108 g L^{-1} with productivity up to 1.7 g L^{-1} h^{-1} has been produced *via* fermentation from wood hydrolysates.[222,223]

3.3 Pulp and paper mills

Another major industrial sector that generates vast amounts of lignocellulosic wastes is the pulp and paper industry. The world's annual wood pulp production in 2011 was approximately 173 × 10^6 t and the world's top producers were USA and Canada in North America (67 × 10^6 t), Brazil in South America (15 × 10^6 t) and Sweden and Finland in Europe accounting for approximately 21 × 10^6 t of wood pulp.[210] Wood pulp can be produced by four different methods: The chemical wood pulp which is the most widely applied technology accounting to 75.6% of the total produced pulp, the mechanical wood pulp which is produced at around 17%, the semi-chemical pulp (4.9%) and the dissolving wood pulp process (2.5%).[224]

The chemical wood pulp can be produced by two different methods, the kraft or sulfate process and the sulfite process. The first one is an alkaline method where the woodchips are cooked in a solution of sodium hydroxide (NaOH) and sodium sulfide (Na_2S), while the latter is an acidic method that employs calcium sulfite ($CaSO_3$) or magnesium sulfite ($MgSO_3$) for delignifying wood particles. For the production of 1 t of chemical pulp approximately 2.5 t of wood under bark are required.[224] The main production stages are similar for the two chemical pulping processes (Fig. 16). Industrial round wood is initially debarked and then cut down to small chips. The bark is usually burnt for the production of energy and steam, while wood chips enter the digester to start the cooking process together with the cooking chemicals (white liquor) where the delignification of the wood (*i.e.*, the separation of lignin from cellulose) occurs. During the digestion process, wood particles are delignified and cellulose fibres are formulated. The generated pulp is then washed in the pulp washing unit to remove all impurities, such as cooking chemicals and dissolved organic substances. Finally, the pulp is bleached and dried.

The liquid wastes from a pulp and paper industry are generated during the cooking and the pulp washing process where vast amounts of water are needed to clean the cellulose fibres and to recover the cooking chemicals and the dissolved organic compounds derived from wood such as lignin and hemicelluloses (Fig. 16). The liquid waste stream from the kraft process is usually called black liquor while the one from the sulfite process is called spent sulfite liquor (SSL). The amount of used water and hence the amount of produced wastes can vary significantly among pulp mills. Improved process design on the pulp washing and implementation of water integration techniques can result in decreasing considerably the overall water consumption.[224,225]

Most of the chemical pulp mills apply the kraft method while only a 10% employ the sulfite method. In the latter, the generated SSL accounts for 8–9 m^3 for the production of 1 t of pulp,[226] while in the kraft process approximately 7 t of weak black liquor are generated from 1 t of pulp.[227] In both cases, the liquid waste stream has a solid content of around 10–20% and it is processed through multiple evaporation steps to increase its solid content to 60–75%.

The composition of the liquid waste stream produced from the chemical pulping process depends primarily on the cooking method (kraft or sulfite method) but also on the cooking conditions and the raw material (*e.g.* tree species). The spent liquors contain more than 50% of the wood substances (*e.g.* lignin and hemicelluloses) and various inorganic soluble salts from the cooking chemicals. Moreover, the concentration of the different compounds present in the spent liquors depends mainly on the level of evaporation resulting in the well-known thick black liquor or spent sulfite liquor.

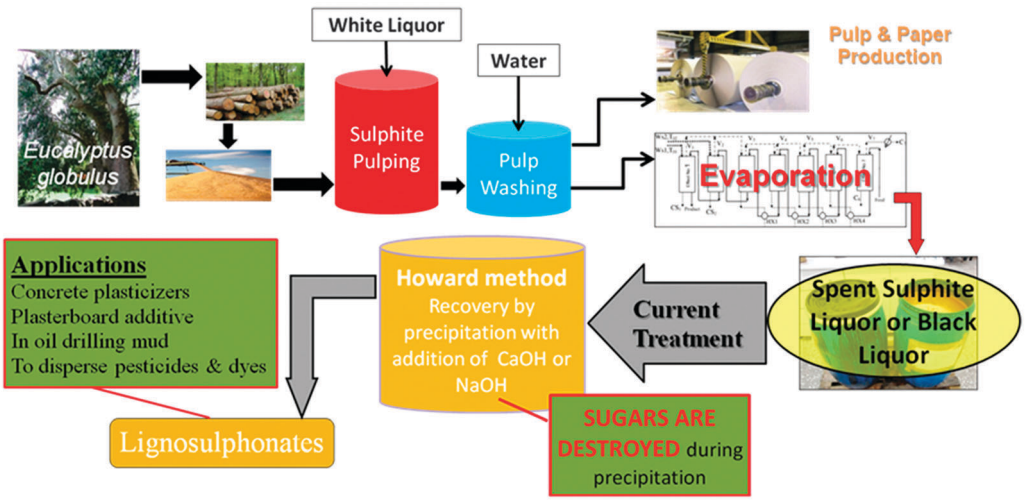

Fig. 16 A simplified schematic diagram for chemical pulp production.

The main compounds of spent liquors from pulp mills are lignin or lignosulfonates, in the case of sulfite process, and sugar monomers which have resulted from the decomposition of hemicelluloses during the digestion process. These monomers are mainly C5 and C6 sugars, namely xylose, arabinose, mannose, galactose and glucose. Furthermore, other compounds are contained in the spent sulfite liquor or black liquor in lower amounts such as furfural, formic acid, 5-hydroxymethylfurfural and levulinic acid which have resulted from the decomposition of the sugars during the cooking process. Acetic acid is also produced from the degradation of hemicelluloses. A small amount of lignin is also decomposed during the cooking process to smaller molecules, *e.g.*, phenolic compounds.[228] In the thick spent sulfite liquor or black liquor, lignin concentration varies between 30–45% (w/v), while the content of sugars ranges form 90–200 g L^{-1}. Acetic acid content ranges between 3–7 g L^{-1}, while 5-hydroxymethylfurfural and furfural are contained in lower amounts (<0.4 g L^{-1}). Phenolic compounds are usually between 1–2% (w/w) of dry solids.

Spent sulfite liquor or black liquor are currently treated as a waste and most of the pulp mills burn their thick spent liquor in boilers to produce the heat and energy required for the operation of the plant and also to recover a percentage of the chemicals used for the cooking of the wood.[224] Some pulp mills separate and recover the lignin content as this material has a market value and can be used directly as an end-product or can be converted into renewable materials and chemicals.[229] Lignosulfonates can be recovered by adding caustic lime, ultrafiltration or ion-exchange and they can be used as concrete plasticizers and in the oil drilling mud. However, in most of the pulp and paper mills, the sugars contained in the spent liquors are not utilised at all and are usually destroyed during the recovery of the lignosulfonates and/or the cooking chemicals. Spent sulfite and black liquors could be regarded as raw materials for the production of chemicals and biopolymers *via* fermentation and other value-added products (*e.g.*, lignosulfonates, phenolics).

3.3.1 Valorisation of spent liquors from pulp and paper industry *via* fermentation. Pulp and paper industries can be upgraded in advanced biorefineries (Fig. 17) by producing a variety of products through the valorization of their liquid wastes. Spent sulfite liquor and black liquor are the only lignocellulosic material accessible in vast amounts which has been already hydrolyzed. Cellulose fibres are produced as the main product, while from pulp's liquid wastes several co-products can be generated, such as lignosulfonates or lignin, phenolic compounds as antioxidants, and chemicals from the bioconversion of C5 and C6 sugars.

Bioethanol is already produced in pulp industrial plants. According to IEA Bioenergy, 10 ML y^{-1} of ethanol are produced in Sweden only.[230] Moreover, several research studies have been carried out examining the valorization of the C5 and C6 sugars contained in the spent sulfite liquor or black liquor for the production and optimization of bioethanol production.[226] However, this liquid waste contains several impurities like acetic acid, furfural, lignin and phenolic compounds which

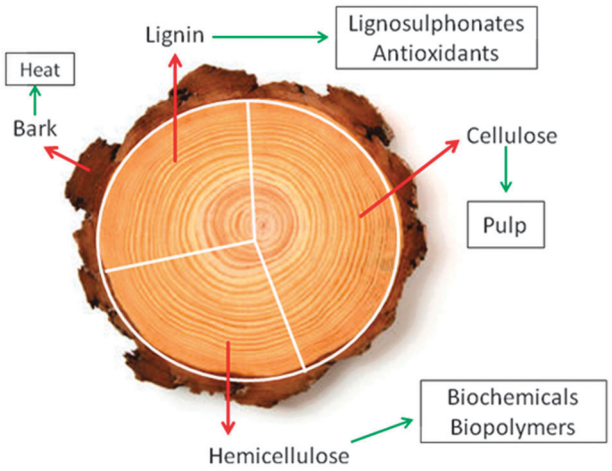

Fig. 17 Restructuring the traditional pulp and paper industry into a biorefinery.

act as inhibitors on microbial growth and therefore a pretreatment step is usually necessary prior to fermentation. Overliming with $Ca(OH)_2$ and ion-exchange resins are two of the most applied purification techniques.[226,231] Typical ethanol yields from pretreated SSL range from 0.3 to 0.48 and final ethanol concentration varies from 5 to 18 g L^{-1}.[226,232]

The utilization of spent sulfite liquor or black liquor for chemical and biopolymer production has not been studied in detail. Research focused on fermentation of wood hydrolysates for chemical production could be adapted in the case of spent liquors. Frazer *et al.*[233] reported the production of 19.1 g L^{-1} BDO at a yield of 0.48 g g^{-1} and a productivity of 0.2 g L^{-1} h^{-1} when wood hydrolysates were pretreated with $Ca(OH)_2$ and IR-120 Plus resin. Pretreated wood hydrolyzates have been used for the production of 32.7 g L^{-1} xylitol using *Candida guilliermondii* FTI 20037.[234] The acetone–butanol–ethanol fermentation from wood pulping hydrolysates resulted in the production of 17.7 g L^{-1} acetone–butanol–ethanol using the bacterial strain *Clostridium beijerinckii*.[235] Bacterial cellulose production (0.75 g L^{-1}) was recently evaluated using industrially derived hardwood spent sulfite liquor from *Eucalyptus globulus*.[236] Recent research at the Agricultural University of Athens on succinic acid production from spent sulfite liquor using the bacterial strains *Actinobacillus succinogenes* and *Basfia succiniciproducens* has resulted up to 9.5 g L^{-1} succinic acid when the medium contained a lignosulfonate concentration of 45.9 g L^{-1}.

3.4 Biodiesel production plants from oil crops and algae

Biodiesel (fatty acid methyl esters, FAME) production *via* transesterification of triglycerides that are either contained in vegetable oils and animal fats or produced by oleaginous microorganisms (*e.g.*, algae, yeast, fungi) constitute a constantly growing industrial application that could evolve into advanced biorefineries for the production of fuel, chemicals, materials, energy and high-value products. In 2021, worldwide biodiesel production from edible vegetable oils is expected to increase up to 30×10^6 t that will increase the share of vegetable oil utilisation for biodiesel

Table 14 Worldwide production of major oilseeds, oil and meal in 2012/2013[240]

Oilseed	Total production ('000 t)	Oil production ('000 t)	Meal production ('000 t)	Total oilseed used for oil extraction ('000 t)	Average oil content in oilseed (%)
Soybean	267 606	43 004	181 075	224 079	19.2
Rapeseed	61 130	24 138	35 806	59 944	40.3
Cottonseed	45 320	5282	15 780	21 062	25.1
Sunflower	36 360	14 060	14 933	28 993	48.5
Palm kernel	14 678	6413	7677	14 090	45.5
Palm	—	55 293	—	—	—
Coconut	—	3747	—	—	—

production to 16% from 12% in the period 2009–2011.[237] Ongoing research is exploring the ability of oleaginous microorganisms to accumulate intracellularly triglycerides in order to develop biodiesel production plants that do not compete with food production. Biodiesel production from algae is currently under investigation in pilot-scale facilities, while Solazyme Inc. has received a loan from Brazil's national development agency for the construction of a plant producing oils from sugar-consuming algae.[238] In October 2012, Neste Oil inaugurated in Finland the first pilot plant in Europe for the production of microbial oil from waste and residues using oleaginous yeast and fungi.[239] Increasing biofuel production from oil crops and oleaginous microorganisms will coincide with increasing production of by-products. Sustainable valorization of by-products will lead to the development of advanced biorefining schemes. The main by-product streams from biodiesel plants are crude glycerol and various meals remaining after oil extraction.

3.4.1 Oilseed meal valorisation perspective. Table 14 presents the worldwide production of major oil crops, oils and meals in 2012/2013. The regions that produce the highest quantities of major oilcrops are North America (87.2×10^6 t soybean), European Union (19.1×10^6 t rapeseed), Former Soviet Union – 12 (17.6×10^6 t sunflower) and South East Asia (12.7×10^6 t palm kernel and 49.6×10^6 t palm oil).[240] The annual production of vegetable oil is expected to increase up to 28% for the period 2012–2021, which is higher than the respective increase of annual oilcrop production due to the contribution by palm and coconut oil.[237] The expected increase of vegetable oil utilisation will be distributed for food consumption (64%) and biofuel production (33%). Rapeseed and sunflower are expected to remain the main oilseeds for biodiesel production, but palm oil consumption is expected to increase two-fold. By 2021, the annual production of oilseed meal is expected to increase up to 23% corresponding to approximately 315×10^6 t.[237]

The composition of the residue that remains after oil extraction from oil crops is dependent on the cultivar, region, cultivation conditions and the processing employed after mechanical extraction of oil. For instance, dehulling and complete oil removal *via* oil extraction may alter significantly the composition and final applications of the meal. Table 15 presents the composition of various fractions derived from rapeseed and sunflower seeds. The rapeseed and sunflower meals produced from dehulled seeds has improved quality than whole rapeseed meal due to the significantly lower quantities of lignin and fiber. However, dehulling is highly dependent on the potential applications of hull and the added-value that results from the fractionation process. For instance, in the case

Table 15 Composition of rapeseed and sunflower meals[241]

Component	Seeds	Kernels	Hulls	Meal from seeds	Meal from dehulled seeds	Meal content taken from literature
Composition of rapeseed						
Dry matter (DM, %)	91.9	93.4	14.3	93.3	93.5	89.4–91.3[a]
Oil (%, DM)	47.7	53.3	12	1	1	2.4–2.7[a]
Protein kjeldahl (%, DM)	21.8	24.5	15.2	40.7	44.2	35–38.8[a]
Crude fibre (%, DM)	8.1	2.7	32.3	15.2	6.4	11.6[a]
Ash (%, DM)	4.7	4.7	6.6	8.5	9.7	6.3–7.4[a]
NDF (%, DM)	14.9	5.27	50.7	30	11.2	—
ADF (%, DM)	11.2	3.3	41.8	22.5	6.7	—
ADL (%, DM)	5.9	0.3	23.1	11.1	0.9	—
Composition of sunflower						
Dry matter (DM, %)	92.8	90.5		88.8	90.5	90.3–96.2[b]
Oil (%, DM)	48	61.25	2.5	2.2	1.2	1.3–3.2[b]
Protein kjeldahl (%, DM)	16.7	20.6	6.2	31.9	52.6	20–40[b]
Crude fibre (%, DM)	17.3	2.4	57.6	28.1	6.2	14–25[b,c]
Ash (%, DM)	3.5	3.6	3.2	7.05	9.24	5.75–7.47[b]
NDF (%, DM)	26.6	5.4	83.9	45.1	13.7	—
ADF (%, DM)	19.5	2.7	64.9	32	7	—
ADL (%, DM)	6.3	0.4	22.3	10.5	0.9	—

[a] Taken from Lomascolo *et al.*[242] and Wang *et al.*[243] [b] Taken from Kachrimanidou *et al.*[244] [c] Dietary fiber.

of sunflower, the extent of dehulling is dependent on the amount of hull that is required to generate energy for the industrial facility *via* combustion and by the extent of oil removal during dehulling.[241] Besides energy generation, sunflower hull has been evaluated for the production of biomaterials (*e.g.*, particleboard panels, bricks, tiles), hemicelluloses and organic amendment for the soils.

Oilseed meals are rich sources of protein, carbohydrates, mineral and phenolic compounds. The carbohydrates contained in oilseed meals are mainly pectins, pentosans and cellulose. Oilseed meals contain significant quantities of phenolic compounds that could be used as natural antioxidants. Domínguez *et al.*[245] reported that the main phenolic compounds (up to 70%) in sunflower meal are chlorogenic and cafeic acids, whereas cinnamic, *p*-coumaric, *p*-hydroxy-benzoic, vanillic and sinapic acids are contained in lower quantities. Rapeseed meal contains approximately 5.3–6.9 mg total phenolics per g meal.[242]

Edible oilseed meals can be utilised directly as animal feed or can be hydrolysed for the production of protein hydrolysates, whereas non-edible oilseed meals can be mainly used as biocontrol agents, fertilisers and for energy generation *via* combustion.[246] There are major concerns on the use of many oilseed meals as animal feed due to the high content of dietary fibre and the presence of antinutritional compounds, as in the case of rapeseed meal that is a rich source of glucosinolates. For this reason, extraction of protein isolates of high purity from oilseed meals could be carried out in order to be used as a more efficient feed additive.

Conventional biodiesel plants could be restructured into advanced biorefineries by utilising oilseed meals for the production of antioxidants, enzymes, vitamins, antibiotics, edible mushrooms, biogas and bio-oil.[246] Oilseed meals could be utilised for the production of nutrient-rich fermentation supplements that could substitute for commercial nutrient preparations. Rapeseed and sunflower meals could be converted into fermentation media using a two-stage bioprocess based on the production of crude enzyme consortia *via* solid state fermentation using a fungal strain of *Aspergillus oryzae*.[243,244] Such nutrient supplements have been successfully employed for the production of P(3HB-*co*-3HV)[163,244] and microbial oil.[247] A combination of chemical pre-treatment using dilute sulfuric acid and enzymatic hydrolysis with commercial enzymes could lead to the production of various fermentable sugars (*e.g.*, glucose, fructose, sucrose and arabinose) from rapeseed meal for the production of 23.4 g L^{-1} succinic acid using the bacterial strain *Actinobacillus succinogenes*.[248] Galactose, sucrose, raffinose, stachyose, and mixtures of these sugars extracted from soybean meal and soy solubles have been evaluated for the production of succinic acid by engineered *Escherichia coli* strains.[249]

3.4.2 Crude glycerol – carbon source for fermentation. Glycerol is the co-product produced during transesterification of TAGs with methanol resulting in approximately 1 kg of glycerol per 10 kg of biodiesel generated. Therefore, the expected increase of worldwide biodiesel production from edible vegetable oils to 30×10^6 t by 2021 will result in the generation of around 3×10^6 t of glycerol. The glycerol produced during transesterification is separated from FAME, neutralized and distilled to remove methanol and most of the water. The resulting stream, called crude glycerol, is of varying purities depending on the technology employed for biodiesel production and the nature of the oil used. Usually crude glycerol streams contain mainly glycerol (77–90%) mixed with various impurities including water (5.3–14.2%), methanol (up to 1.7%), residual fatty acids and corresponding esters, and either NaCl (4.2–5.5%) or K_2SO_4 (0.8–6.6%) depending on the catalyst used in transesterification reactions.[48,250,251] Glycerol is also produced as by-product during bioethanol production where the "*thin stillage*" that remains after ethanol separation *via* distillation contains approximately 2% (w/v) glycerol.[82] As mentioned earlier, glycerol can also be produced during oleochemical production using various sources of triglycerides. Traditional glycerol markets cannot absorb this glut of glycerol, and hence, new ways to add value to this material are in great need.[252]

Crude glycerol streams could be used as carbon sources in various fermentations for the production of many chemicals and biodegradable polymers including PDO, succinic acid, BDO, PHA, SCO, ethanol, citric acid, polyols, itaconic acid and dihydroxyacetone.[51–53,55,58,59,83,150,244,251,253]

3.4.3 Algae meal. Utilisation of algae for biodiesel production will generate significant quantities of defatted algal biomass that could be used in integrated biorefineries for the extraction of value-added products and the production of fermentation media. Creating value-added products is essential because producing only biofuels from algal biomass will be most probably not a cost-competitive option.[254] The main components of algae are: lipids (15–60%), protein (20–60%), polysaccharides (10–50%) and nucleic acids (3–5%).[254] Microalgal biomass containing starch and proteins provides a suitable resource for the production of carbon and nitrogen rich hydrolysates.[255,256] It should be noted that the algal composition produced in the plant influences process economics, as increasing lipid content reduces other value-added components.[254] Future biorefineries based on oleaginous microorganisms (*e.g.*, algae and yeast) should optimise the composition of microbial biomass in order to maximise profit depending on the market outlets and product prices.

Phototrophic microalgal strains are grown on inorganic nitrogen and phosphorous sources, carbon dioxide and light in photo-bioreactors. The biomass productivity, however, is restricted by light limitation. Eriksen[256] estimated that the productivity of heterotrophic species cultured under oxygen limitation in a 3 L bioreactor is 35 times higher than the productivity of phototrophic species cultured under light limitation in a 3 L photo-bioreactor. An increase in light intensity as an approach to overcome the restriction in productivity often leads to photo-oxidation of the photosynthetic apparatus and to a decrease in growth rate.[256] Therefore, in order to produce large quantities of algal biomass large photobioreactors are needed, making processes, however, infeasible.

Table 16 Ethanol and lipid production using media derived from microalgal biomass

Microorganism	Biomass treatment	Conversion of starch to sugar [%]	Product, yield	Ref.
Diatomaceous algae and *Rhodosporidium toruloides* Y4	Starch hydrolysis in algae by *in situ* enzymes	Up to 75	Lipid production by *R. toruloides* Y4	257
Chlamydomonas reinhardtii and *Saccharomyces cerevisiae*	Enzymatic liquefaction and saccharification	57	Ethanol, 0.24 g g^{-1} biomass	258
Chlorococum sp. and *Saccharomyces bayanus*	Supercritical carbon dioxide extraction of lipids	—	Ethanol, 0.38 g g^{-1} lipid-extracted microalgae debris	260

Heterotrophic algae can be grown in ordinary stirred bioreactors. Their cultivation on low-cost feedstocks is essential to facilitate industrial implementation.

Theoretically, every microorganism that grows heterotrophically may grow on a hydrolysate of microalgal biomass. Microalgae have resistant cell walls and therefore, a pretreatment is necessary to convert carbohydrates and proteins into directly assimilable nutrients. The majority of recent studies utilise pretreated microalgal biomass for the production of ethanol. Another approach is the cultivation of oleaginous microorganisms (Table 16) on hydrolysed defatted microalgal biomass for the production of lipids.[257] Furthermore, amino acids from algal proteins could be used as precursor in putrescine and cadaverine production. Pretreatment methods of microalgal biomass include mainly enzymatic hydrolysis.[258] Jeon *et al.*[259] used ultrasonication of *Scenedesmus obliquus* biomass to improve the bioaccessibility of carbohydrates in microbial fermentations. They found that sonication duration of 15 min at 45 °C increases the fraction of dissolved carbohydrates by 32%.

Optimisation of utilisation of algae biomass components for fermentation media production would result in remaining protein for other applications (Fig. 18). Proteins usually isolated from oilseeds find application as anitoxidants, food and feed, and cosmetics. Furthermore, various heterotrophic algae produce value-added products, such as xanthophylls (lutein, zeaxanthin, violaxanthin, and neoxanthin) used in the food industry to color skins of meat and egg yolk[261,262] and phycocyanin, a blue appearing phycobiliprotein that can be used in fluorescent probes, as food additive and antioxidant.[263] According to Fig. 18, all by-product streams (*e.g.*, carbohydrates, proteins, polyunsaturated fatty acids, minerals, glycerol) will be upgraded to value-added products as far as possible. The organic by-product streams that cannot be used for this purpose will be used for combined heat and power production.

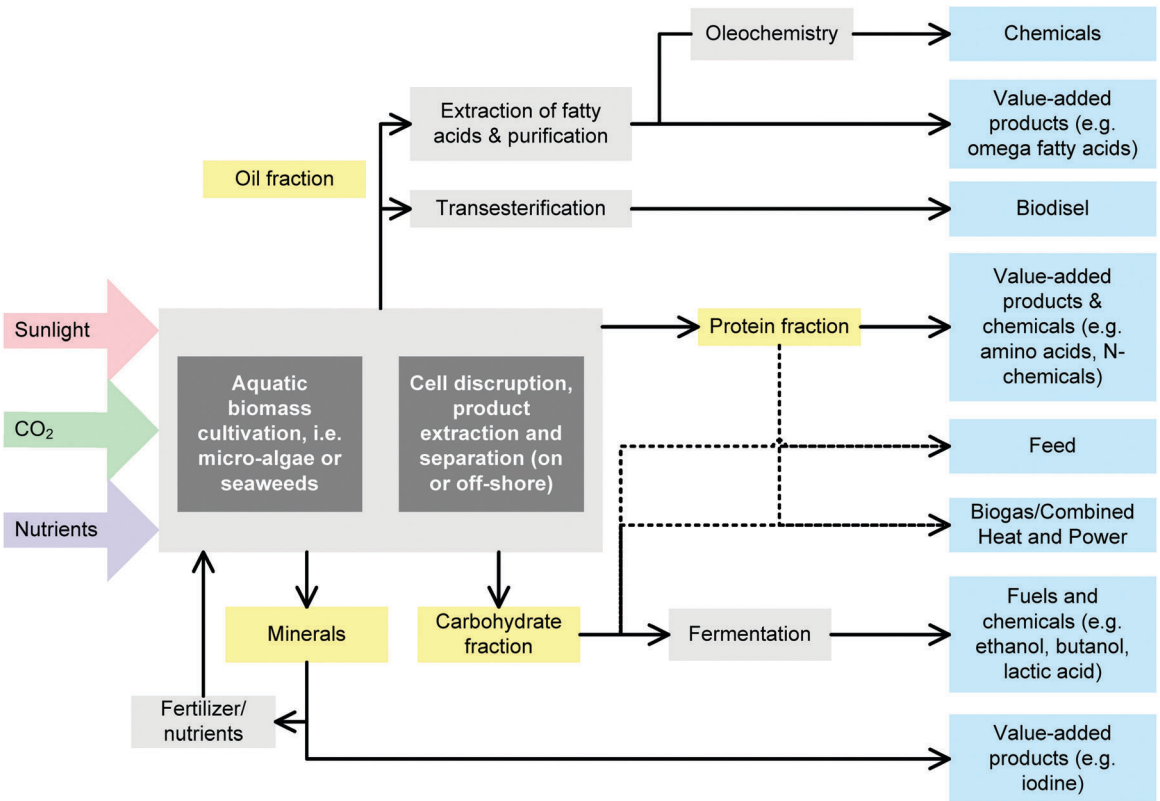

Fig. 18 Description of an algae-based biorefinery.[264]

Table 17 Major ethanol producers and feedstocks utilised

Region	Raw materials[a]	Million gallons[b]
USA	Corn (98%), sorghum (2%)	14 887
Brazil	Sugarcane (100%)	5557
Europe	EU-27: wheat (48%), sugar beet (29%)	1179
Asia	China: corn (70%), wheat (30%)	952 (China: 555)
Canada	corn (70%), wheat (30%)	449

[a] Balat and Balat.[265] [b] 2013 ethanol industry outlook.[266]

3.5 Bioethanol production plants

Ethanol can be used both as fuel and platform chemical as it was mentioned in Section 2.1.1. Table 17 presents major bioethanol producing countries and regions and the predominant feedstocks used. Fuel ethanol production processes are reviewed in several literature-cited publications.[265] It can be produced by three types of feedstocks: (1) starchy crops, (2) sugar crops, and (3) lignocellulosic biomass. The by-products generated from bioethanol production plants could be utilised for the development of novel biorefineries and various processing schemes are currently under development depending on the initial raw material used.

3.5.1 Distillers dried grains with solubles (DDGS). A comprehensive review regarding DDGS has been presented by Liu and Rosentrater.[267] Corn and wheat (Table 17) are the main cereal grains used for fuel ethanol production. Dry and wet milling processing schemes are mainly employed. Corn wet milling processes are employed in the USA for ethanol production, the successful implementation of which can be attributed to the generation of value-added co-products such as oil, corn gluten meal and corn gluten feed. Dry milling of cereal grains is the most widely used process employed for bioethanol production that generates DDGS, a by-product stream consisting of fibres, protein, lipids, minerals and vitamins. In 2012, the US ethanol industry produced 31.6×10^6 t DDGS from 114×10^6 t of corn.[266] The cost-competitiveness of bioethanol production from cereal grains is highly dependent on the revenue derived from DDGS.

The composition of DDGS is highly dependent on the raw material and the processing method employed for ethanol production (Table 18). DDGS is currently used as animal feed. Although the protein content in DDGS is three-fold higher compared to the original grain, the amino acid analysis shows that it is still considered an incomplete animal feed regarding the amino acid requirement of optimised feed rations.[268,269] Other potential uses of DDGS are production of biocomposites[270] and generation of bioenergy *via* thermochemical conversion and anaerobic digestion.[267]

Restructuring of conventional cereal-based bioethanol plants could be achieved either through fractionation of cereal components prior to ethanol fermentation as in the case of corn wet milling processes or by fractionation of DDGS. An advanced wheat-based biorefinery concept has been developed for the production of bioethanol and valuable co-products, including gluten, bran and yeast.[271] This continuous biorefinery concept involved upstream fractionation of wheat into starch-, gluten- and bran-rich fractions combined with optimisation of wheat component utilisation for on-site production of hydrolytic enzymes and ethanol. Similar wheat-based biorefinery schemes have been developed for the production of biodegradable polymers (*e.g.*, PHB) and platform chemicals (*e.g.*, succinic acid).[77,272]

The development of wheat fractionation prior to ethanol fermentation demands the construction of new industrial plants. Current industrial plants employing dry milling of wheat or corn could be converted into advanced biorefineries by fractionating DDGS into various fractions with different applications. The oil content of corn-based DDGS could be used for the production of biodiesel.[273] The carbohydrates (Table 19) present in DDGS could be fractionated and hydrolysed into C5 and C6 sugars for fermentative production of chemicals. In addition, the significant quantities of protein and minerals (Table 19) contained in DDGS could provide sufficient quantities of nutrients to formulate fermentation media for any microbial bioconversion. Optimisation of nutrient requirements for fermentation could be combined with extraction of surplus quantities of protein and value-added components (*e.g.*, β-glucan) for the production of valuable co-products to improve the cost-competitiveness of the biorefinery.

3.5.2 Integration of lignocellulosic residue valorisation in current ethanol production plants. Current industrial facilities for ethanol production from sugar cane and cereals could be converted into integrated biorefineries through the valorisation of lignocellulosic residues (*e.g.*, cereal straws and sugar cane bagasse). Nowadays, about 1.5×10^9 t of cereal straw is produced each year.[274] Furthermore, the worldwide sugar cane bagasse production reached 344 thousand t in 2009.[275] The process of ethanol production from lignocellulosic feedstocks

Table 18 Composition of DDGS derived from different corn- and wheat-based processes[267]

Constituent (% dry matter basis)	Corn-based DDGS		Wheat-based DDGS
	Mean	Range	
Dry matter	88.9–90.5	87.1–92.7	—
Protein	27.4–31.4	25.8–33.3	32.3–39.3
Oil	10.7–12	9.1–14.1	4.98–7.66
Ash	4.4–5.8	3.7–8.1	—
Starch	4.9–5.3	3.2–5.9	0.4–6.3
Total carbohydrate	52.1–56.5	—	—
Crude fiber	8.8–10.2	8.3–10.6	5.56
Acid detergent fiber	15.9–16.8	11.4–20.8	—
Neutral detergent fiber	38.8–42.1	33.1–49.1	48.1

Table 19 Representative carbohydrate and mineral content of corn-based DDGS[267]

Constituent	Composition (% dry basis)	Mineral	Range of composition (mg g^{-1})
Glucan	21.2	K	6.7–12.4
Cellulose	16.0	P	5–9.9
Starch	5.2	Mg	2.1–3.8
Xylan	8.2	S	3.3–11
Arabinan	5.3	Na	0.6–5.1
Total carbohydrate measured in DDGS	59.4	Ca	0.1–7.1

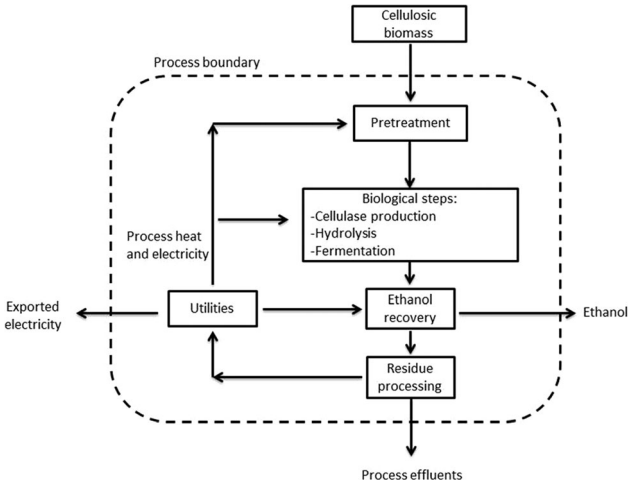

Fig. 19 Simplified process flow diagram for the production of cellulosic ethanol.[277]

involves pretreatment, enzymatic hydrolysis, sugar fermentation and product recovery (Fig. 19). Cellulose is utilised for the production of ethanol, but hemicelluloses and lignin, which are currently used as fuels to generate steam and electricity, could be utilised for the production of chemicals. Zhang[276] stressed that the effective co-utilization of lignin and hemicelluloses could increase 6.2 fold the revenues of lignocellulosic biorefineries.

Hemicellulose, in which xylans are the most abundant polymer, can be converted into sugar-rich hydrolysates by dilute acid hydrolysis (Table 20). Hemicellulosic hydrolysis could be also carried out using enzyme consortia. The efficient hydrolysis of xylan requires various enzymes such as *endo*-β-1,4-xylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, ferulic acid esterase, and *p*-coumaric acid esterase. Many microorganisms, such as *Penicillium capsulatum* and *Talaromyces emersonii*, can degrade xylan with their enzyme systems.[278] The sugar-rich hydrolysate can be utilized by microorganisms to produce various chemicals. It is reported that xylitol can be obtained from detoxified hemicellulose hydrolysate by *Candida guilliermondii*. The yields ranged from 0.57 g g^{-1} to 0.79 g g^{-1}.[234,279]

The remaining lignin-rich residue is currently employed for the production of heat and electricity. However, lignin can be converted into valuable products by a novel biorefinery approach. Previous investigation indicated that lignin in the remaining solids can be extracted with high purity.[281] In a fermentative approach, lignin polymer can be degraded by enzymes, enzyme-secreting fungi and bacteria into various products like β-aryl ether, di-aryl ether and biphenyl. Vares *et al.*[282] produced lignin peroxidase, manganese peroxidase, glyoxal oxidase, and laccase by *Phlebia radiate* using solid state fermentation. Fermentative degradation of lignin could be a novel process to provide a renewable source for aromatic and phenolic compound production.

Table 20 Composition of hemicellulosic hydrolysates (g L^{-1}) produced *via* dilute acid hydrolysis[280]

Residue	Xylose	Glucose	Arabinose	Acetic acid	Furfural	HMF
Sugar cane bagasse	46	3	5	10	0.6	<0.1
Sugar cane bagasse	18.5	1.2	1.7	6.5	<0.1	<0.1
Sugar cane bagasse	26.4	5.5	2.1	5.5	<0.5	<0.1
Sugar cane bagasse	18.5	5.1	—	3.7	2	<0.1
Corn cobs	35.3	3.2	4.6	3.7	0.3	<0.1
Corn stover	22.5	2	3.6	2.3	0.3	<0.1
Rice straw	16.2	6	2.2	0.63	—	—
Rice straw	16.4	4.4	2.4	1.4	0.41	<0.1

4. Techno-economic evaluation of fermentation processes

Economic evaluation of a biotechnological project is an assessment of its potential financial return and it is performed in order to quantify the expected profitability as the result of various choices such as plant size, pricing, alternative raw materials, total number and types of products (bio-refining approach), available processes (*e.g.*, different microorganisms, alternative downstream separation schemes based on fermentation outcomes) or engineering features (batch *vs.* continuous operation, recycling *etc.*). Needless to say that the challenge is to complete successfully such a task despite the fact that, in most cases, information about the biotechnological project is limited, vague or involves high level of uncertainty. Successful commercialization of fundamental research strongly depends on unbiased and systematic completion of a preliminary economic study which results in unambiguous recommendations about the potential financial return. Equally important is the use of economic evaluation as a tool to supply feedback to fundamental research in terms of defining medium and long term research objectives and milestones.

The important types of cost estimates that are usually performed are the total capital investment that includes the equipment cost, and the production cost. Capital investment estimate is an important figure related to the amount of money that needs to be allocated in order to start the venture. Production cost estimate is important to both management and marketing groups. A preliminary economic evaluation can be completed only after the development of a provisional process flowsheet that involves at least the main processing steps. At this stage most of the unit operations assigned to the specific processing tasks are provisional and not necessarily proven as the best available technology for that purpose (this is mainly the case for downstream separation stages rather than the fermentation stage). There are several textbooks available that describe in detail the methodologies used for preliminary techno-economic evaluation of engineering projects (*e.g.* Peters *et al.*[283]).

Most of the techno-economic studies available in the open literature refer to the production of ethanol as fuel or commodity chemical. As described earlier, ethanol is nowadays a renewable fuel and basic chemical for the bio-economy era. The recent update of the NREL technical report on process design and economics for the biochemical conversion of lignocellulosic

biomass to ethanol is arguably the most complete and reliable document in the field.[284] This report (along with previous issues) has been used as a prototype in both industrial and academic studies while many of its cost elements and assumptions have been adopted by most studies reviewed in what follows. The NREL report concludes that the minimum ethanol selling price (MESP) is \$2.15 gal^{-1} (\$0.57 L^{-1} or \$0.72 kg^{-1}), 35% of which (\$0.25 kg^{-1}) is the feedstock contribution and 16% is the cost of enzymes used in the hydrolysis of the lignocellulosic feedstock. It should be mentioned that the current (May 2013) price of ethanol as a commodity chemical is approximately \$2.8 gal^{-1} and that large variations have occurred in the past (the price of ethanol was, for instance, \$1.2 gal^{-1} on May 2005 and \$5 gal^{-1} only a year later). The total capital investment is estimated to be M\$422. It is important to note that in the NREL study the feedstock used is corn stover with a price of \$59 t^{-1} of dry material and a yield to ethanol of 79 gal t^{-1} of dry material (0.236 g ethanol g^{-1} dry feedstock). Macrelli *et al.*[285] estimated the cost of 2nd generation ethanol production at \$0.97 L^{-1} and it was suggested that it can be reduced to \$0.78 L^{-1} in the future.

Apart from bio-based production of ethanol, the bio-based commercial production of lactic acid is also nowadays a reality. Although agro-industrial by-product and waste streams (*e.g.*, C5 and C6 sugar-based hydrolysates, whey) can be used in principle, at least on lab- or pilot scale, supplementation with expensive nutrient sources (*e.g.*, yeast extract) can increase significantly raw material costs. Fermentative production of lactic acid suffers also from the high cost of recovering lactic acid from fermentation broths. Tejayadi and Cheryan[286] and González *et al.*[287] studied the production of lactic acid from ultrafiltered whey and reported a production cost of \$0.98 kg^{-1} and \$1.25 kg^{-1} lactic acid, respectively.

Since the announcement by the DuPont Tate and Lyle Bio Products Company, LLC of the first commercial shipments of Bio-PDO™ from its \$100 $\times$ 10^6 facility at Loudon, Tennessee in 2006, there is an increasing interest in fermentative production of PDO. An earlier joint publication by DuPont and Genencor International[288] revealed that, using a metabolically engineered microorganism and glucose as substrate, a PDO concentration of 135 g L^{-1}, a glucose to PDO conversion yield of 0.51 g g^{-1} and a productivity of 0.35 g L^{-1} h^{-1} could be achieved. However, limited information has been made available in the open literature on the economics of large-scale production of PDO. Zeng and Biebl[49] estimated that the production cost of PDO from glucose is around \$1.3 kg^{-1} PDO, while Apostolakou *et al.*[289] estimated that the production cost is \$1.4 kg^{-1} PDO when glycerol is used as raw material (annual capacity of 15 300 t PDO). It is important to note that, building on the success story of biotechnological PDO production, Genomatica and DuPont Tate & Lyle Bio Products Company, LLC announced in February 2013 the first successful commercial-scale production of BDO.

Following successful pilot runs, in December 2009, Myriant was awarded a \$50 million grant from the U.S. Department of Energy for its bio-succinic acid plant in Lake Providence, Louisiana. In June 2012, Myriant became the first renewable chemicals company to receive a loan guarantee from the United States Department of Agriculture (USDA). The plant will produce annually 13 600 t (30 million pounds per year) of bio-succinic acid from grain sorghum and other commercially available sugars. Orjuela *et al.*[290] performed a detailed economic analysis of a succinic-acid production process with a novel recovery strategy and estimated that the minimum production cost (maximum production capacity, maximum titer and no byproduct formation) is \$1.85 kg^{-1} bio-succinic acid. Vlysidis *et al.*[291] estimated that the fermentative production of succinic-acid using crude glycerol, from a relatively small (<10 kt y^{-1}) biodiesel production plant, as carbon source can improve significantly bio-diesel economics.

Van Wegen *et al.*[292] reported a detailed techno-economic study on bio-technological production of PHB concluding that for an annual production of 4300 t PHA the production cost is \$6.08 kg^{-1} PHA and the total fixed capital investment is M\$28.6 (\$49.5 L^{-1} of installed fermentation capacity). Choi and Lee[293] reported that the unitary cost for an annual production of 100 000 t PHB can vary from \$2.6 kg^{-1} to \$8.32 kg^{-1}. The total fixed capital investment was estimated to be M\$396 (\$59.9 L^{-1} of installed fermentation capacity).

The most important characteristics of a bio-based process that determine, to a large extend, the overall economic performance are: (1) final product concentration (expressed in kg of desired product per m^3 fermentation broth), (2) productivity (expressed in kg of desired product per h and per m^3 fermentation broth), and (3) yield (kg of desired product per kg of substrate).[294] The final product concentration should exceed 50 kg m^{-3}, productivity should exceed 2.5 kg h^{-1} m^{-3} and yield must be at least 80% of the theoretical value for considering the potential of successful commercialization of a bio-technological process. These limits should be treated as general figures of merit for the production of any commodity chemical with a selling price in the range of \$0.3–5 per kg. An approximate support for these arguments can be based on an estimation of the fixed capital investment (FCI) which exhibits a strong correlation with the installed fermentation capacity as shown in Fig. 20. Data for the generation of this

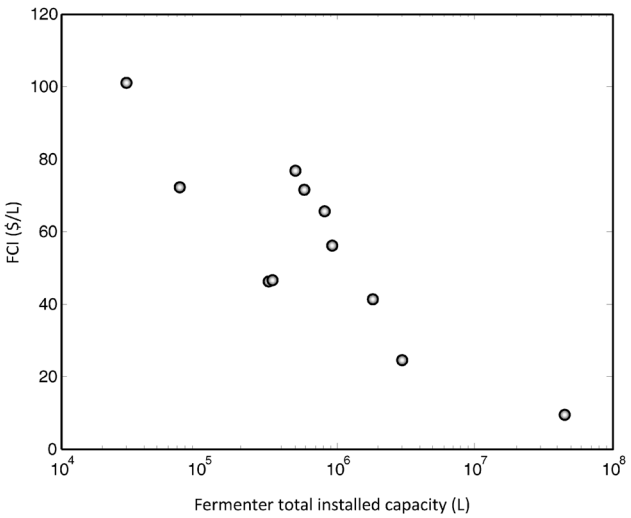

Fig. 20 FCI (expressed per L of installed fermentation capacity) as a function of fermentation capacity.

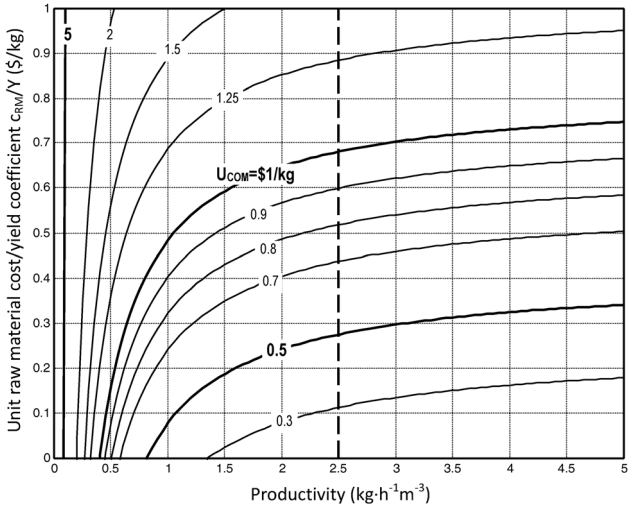

Fig. 21 Minimum achievable unit cost of manufacture (U_{COM}) as a function of productivity, unit cost of raw material (c_{RM}) and yield coefficient (Y).

figure have been extracted from the papers reviewed above and from work in our research group.[271,295] The data point that corresponds to the largest fermentation capacity corresponds to the ethanol production studied by the NREL.[284]

Using the corresponding minimum FCI (per L of installed fermentation capacity, approximately \$10 L^{-1}), we can develop an optimistic estimation of the cost of manufacture (COM) (Fig. 21), expressed in \$ kg^{-1} product, using a quick estimation method presented in Peters *et al.*[283] From Fig. 21, it can be concluded that the target of achieving a unit production cost of less than \$1 kg^{-1} can only be achieved for productivities larger than 0.5 kg h^{-1} m^{-3}. Reasonably competitive costs can only be achieved for productivities higher than 2.5–3.0 kg h^{-1} m^{-3}.

5. Case studies

5.1 Succinic acid production from wheat milling by-products

In 2012, the UK flour millers processed 5.2 × 10^6 t of wheat and generated 10^6 t of by-products, mainly consisted of wheat middlings and bran.[296] Dorado *et al.*[297] converted wheat milling by-product streams into a fermentation feedstock for succinic acid production. Wheat bran was used as substrate in solid state fermentations of *Aspergillus awamori* and *Aspergillus oryzae* that can produce significant quantities of amylolytic and proteolytic enzymes. Remaining solids from both solid state fermentations were mixed with wheat middlings and simultaneous starch and protein hydrolysis together with fungal autolysis led to the production of significant glucose (100 g L^{-1}) and free amino nitrogen (300 mg L^{-1}) concentrations. The utilisation of hydrolysates from wheat milling by-products as the sole source of nutrients in fermentations of *Actinobacillus succinogenes* resulted in the production of 50.6 g L^{-1} succinic acid, demonstrating also a promising productivity of 1.04 g L^{-1} h^{-1} and conversion yield of 0.73 g g^{-1}.[297] Based on the experimental results from this study and the annual production of wheat milling by-products in the UK, a total succinic acid production of around 80 000–120 000 t (assuming a modest 10–15% starch content in wheat milling by-products) could be produced on an annual basis in the UK.

5.2 Biorefinery development for integrated biodiesel and succinic acid production

As discussed in Section 2.1.6, succinic acid production from glycerol is favourable compared to other by-products due to the production/consumption of electrons produced in the glycerol–succinic acid metabolic pathway.[82] For this reason, in many studies, the glycerol to succinic acid conversion yields are higher than 0.80 g g^{-1} (Table 21). If the expected glycerol production by 2021 (3 × 10^6 t) from biodiesel plants is utilised for succinic acid production with a relatively modest yield of 0.8 g g^{-1} and accounting 8.4% losses during the downstream process,[291] then 2.2 × 10^6 t of succinic acid can be produced which is more than 40 times higher than the current global succinic acid production. Succinic acid productivities can be improved when continuous systems are implemented,[298] while higher final succinic acid concentrations can be achieved when yeast strains are employed (*e.g.*, genetically modified *Yarrowia lypolytica*).[299]

Vlysidis *et al.*[291] carried out a techno-economic analysis of an integrated biodiesel plant that co-produces succinic acid from crude glycerol together with biodiesel (Fig. 22). The incorporation of a succinic acid production facility improves the profits of the biodiesel biorefinery by 60% when an interest rate of 7% and a plant's lifespan of 20 years are applied.[291] The economics of such a plant are affected by a number of critical parameters, most important being the succinic acid price and the operational parameters of succinic acid production. The sensitivity of the succinic acid price should be taken into consideration as prices are expected to be drastically decreased to \$1–3 kg^{-1} due to the increase in succinic acid production. Crucial operational parameters were found to be the glycerol concentration that enters the bioreactor and the incubation time of the fermentation.[291,302] In Fig. 22, the parameter that

Table 21 Succinic acid production from glycerol by various microorganisms

Microorganism	System	Yield (g g^{-1})	Productivity (g L^{-1} h^{-1})	Final conc. (g L^{-1})	Ref.
Anaerobiospirillum succiniciproducens	F-B	1.60	0.16	19.0	300
Anaerobiospirillum succiniciproducens	C	1.37	2.10	15.5	298
Actinobacillus succinogenes 130Z	B	0.80	0.27	29.3	83
Escherichia coli (gen. mod.)	B	1.03	0.08	12.1	301
Y. lipolytica (gen. mod.)	B	0.36	0.27	45.5	299

B: batch, F-B: fed-batch, C: continuous.

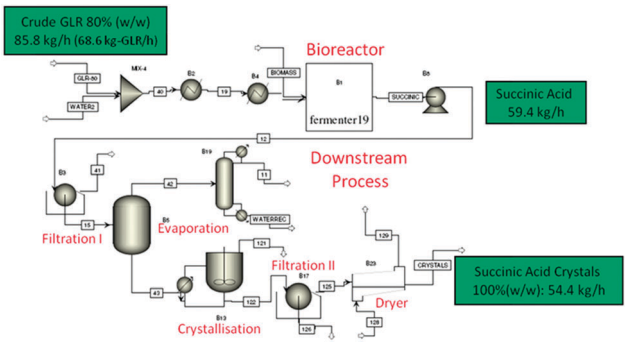

Fig. 22 Succinic acid production and purification from crude glycerol.[291]

controls the glycerol concentration is the water stream that dilutes the crude glycerol to levels where the fermentation can start. The succinic acid is produced with a yield of 0.86 g g^{-1} and it undergoes a number of purification steps, including filtration, evaporation, crystallisation, and drying, in order to be recovered as white pure crystals.

Improvement of the succinic acid fermentation is possible both by setting up more sophisticated systems like fed-batch, repeated batch or continuous processes and by generating genetically modified microbial strains. Moreover, like petroleum refineries, future biorefineries are complex facilities that should apply optimum designs for heat exchange networks in order to minimise their total energy consumption. Kastritis *et al.*[303] showed that there are elevated opportunities for heat integration in a biorefinery facility that produces biodiesel and succinic acid. The authors have designed a more sustainable and cost-efficient plant as they optimised the heat exchange network of the overall plant by applying heat integration techniques like pinch analysis. They managed to decrease by 46.4% the operational cost of the heat exchange network and by 17.2% the total annual cost of the plant demonstrating the significance of improved designs.[303]

5.3 Succinic acid and PHB production from bakery waste

Based on the large quantities of food waste generated at Hong Kong on a daily basis, The City University of Hong Kong has collaborated with the retailer 'Starbucks Hong Kong' in order to evaluate the potential to produce succinic acid and PHB from bakery waste, mainly pastries and cakes.[192] Fungal solid state fermentation was employed to produce hydrolytic enzymes that were subsequently employed through simultaneous hydrolysis and fungal autolysis for the production of nutrient-rich media. Both cake and pastry hydrolysates were found to be rich in glucose (35.6 and 54.2 g L^{-1}) and free amino nitrogen (685.5 and 758.5 g L^{-1}), whereas the protein hydrolysis yields were 23.2 and 22.5%, respectively. These cake and pastry hydrolysates, together with magnesium carbonate (10 g L^{-1}) were subsequently used as feedstock in *Actinobacillus succinogenes* fermentation, which resulted in succinic acid concentrations of 24.8 g L^{-1} and 31.7 g L^{-1}, respectively. A cation-exchange resin-based process (*via* vacuum distillation and crystallisation) was subsequently used to recover the succinic acid crystals from fermentation broth with a crystal purity of 96–97.7%.

Lam *et al.*[304] carried out preliminary costing of fermentative succinic acid production from bakery waste. With complete mass and energy balances of the biorefinery process simulated using SuperPro Designer, the total capital investment (US$1 118 000) and the total production cost (US$230 750 y^{-1}) were estimated. The total revenue from sales of 29 t succinic acid y^{-1} and 270 t solid biomass y^{-1} and the received waste treatment charge was US$374 000 per y. The return on investment of the production was 12.8%. The breakeven of the capital investment was 7.2 years and the internal rate of return was 15.3%. Therefore, the succinic acid production from bakery waste can be economically feasible. Variations of product prices and operating labour costs were the major uncertainties to the profitability. It was worth noting that the production plant reached shutdown point when the bakery waste feed was less than 0.26 t day^{-1}. This work demonstrates that the biorefinery process using bakery wastes as raw material is feasible.

5.4 Biodiesel industry by-products

Research at the Agricultural University of Athens focuses on biorefinery development using the by-product streams from an oilseed-based biodiesel plant. Research started with the enzymatic conversion of sunflower meal into a nutrient-rich supplement that was mixed with glycerol for the production of more than 25 g L^{-1} PHB.[244] However, using the whole sunflower meal for the production of fermentation nutrient supplements does not take advantage of the full potential of the meal, which contains significant quantities of antioxidants and protein.

Optimisation of sunflower meal component utilisation (Fig. 23) led to an advanced sunflower-based biorefinery producing a protein isolate of high purity, an antioxidant-rich fraction and PHB or P(3HB-*co*-3HV). A simple sedimentation–flotation process was initially used to fractionate the sunflower meal *via* suspension in water.[305] This process led to the production of three fractions: a protein-rich fraction, a lignocellulose-rich fraction and a liquid fraction. The protein- and lignocellulosic-rich fractions were used for the extraction of antioxidants (mainly chlorogenic acid). The remaining protein-rich fraction was used for the production of a protein isolate (~97% pure protein) *via* treatment with acid and alkaline solutions. This co-product could be utilized for the production of biopolymers, amino acid and edible films. It could be also enzymatically hydrolysed to enhance its applications.

After the extraction of value-added co-products, remaining streams were used for the production of fermentation meda for PHB production. The lignocellulosic fractions were employed as solid substrate in solid state fermentation with a fungal strain of *Aspergillus oryzae* for the production of enzyme consortia. Fermented solids were used as an enzyme-rich source for hydrolysis of macromolecules contained in remaining sunflower streams. The liquid fraction generated during fractionation of sunflower meal was employed as process water in enzymatic hydrolysis. This nutrient-rich medium was mixed with glycerol and was used as the sole fermentation medium leading to the production of more than 40 g L^{-1} PHB. Production of P(3HB-*co*-3HV) was also

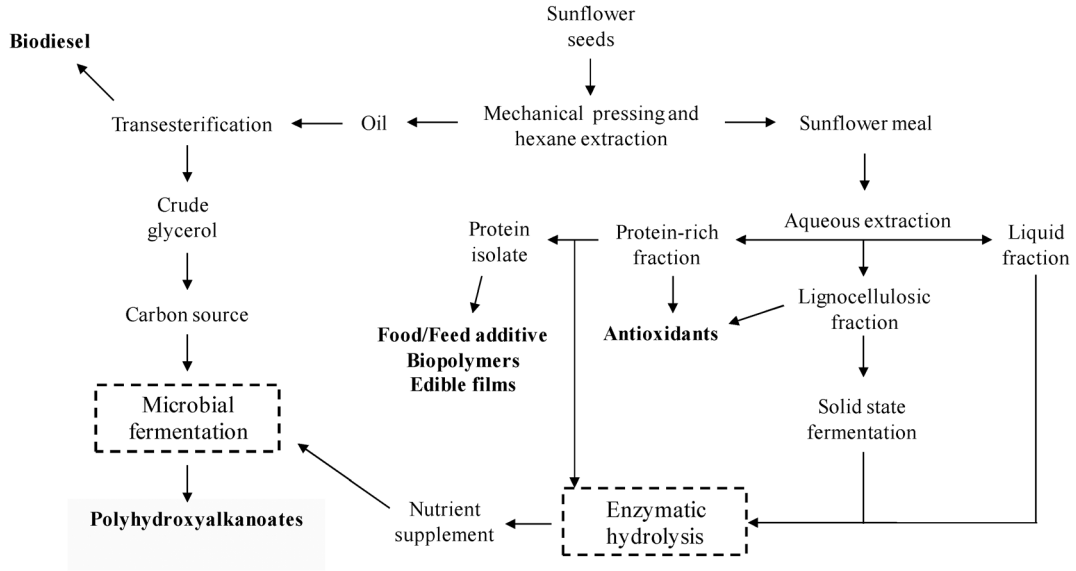

Fig. 23 Advanced sunflower-based biorefinery concept.

accomplished by supplementing the fermentation medium with commercial levulinic acid. In future biorefineries, levulinic acid could be produced from cellulose.

6. Conclusions and outlook

The aim of this contribution is to raise the awareness of all stakeholders on the potential and opportunities of utilizing waste and by-product streams from major industrial activities for the production of platform chemicals and biopolymers as a mean to achieve the illusive target of sustainability. It focuses on quantifying the potential contributions of advanced waste valorisation practises to our society, which sees the development of white biotechnology as a key strategic objective. The intention is to facilitate the step changes necessary in order to move towards a bio-based economy, where production and use of materials and energy are taking place in a responsible and environmentally friendly manner. The achievements of literature-cited research in producing platform chemicals and biopolymers using principles of white biotechnology are reviewed, and short as well as medium term research objectives are identified, when possible. The important role of the idea of integrated biorefinery development and its implications are discussed extensively and case-specific examples are analysed. The need for step changes in the way that industrial activities and industrial infrastructures are conceived is identified with the ultimate goal to improve bioprocess economics, which is a key driver for achieving commercialization. The most important change is arguably the adoption of the ideas relevant to the biorefinery concept where industry identifies waste and by-product streams as potential sources of wealth, and strives for their complete recycling and utilisation rather than for their mere rejection as undesirable or unwanted. Significant technological challenges need to be met before such changes are adopted by industry to satisfy current and future societal needs.

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