

# Chapter 11

## Microbial Technologies for Biorefineries: Current Research and Future Applications



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**Abstract** Conventional resources becoming limited due to the increase in population and energy demand. This rise in energy demand has increased consumer prices and pressure on the environment. This prompted researchers to take care of sustainable energy resources. In this case, biomass is only environmentally friendly renewable resource which is used for the production of chemicals and fuels. A system similar to a petroleum refinery is required to produce fuels and useful chemicals from biomass and is known as a biorefinery. Biorefineries have been subdivided into various categories on the basis of technology and biomass used. In this chapter, types of biorefineries and microbes which are used for the production of valuable products are discussed.

### 11.1 Introduction

International Energy Agency (IEA) Bioenergy Task 42 has defined biorefinery as the sustainable processing of biomass into a variety of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat) (de Jong and Jungmeier 2015). The National Renewable Energy Laboratory (NREL) defined biorefinery as a facility that facilitates conversion of biomass into fuels, power, and chemicals. A biorefinery can utilize all types of biomass and producing agricultural by-products (wheat bran, rapeseed meal, straw, corn stover, bagasse), waste from the food industry (including kitchen and household waste), grains/cereals (wheat, maize, corn, soybean), starch and sugars, aquatic biomass (algae and seaweeds), as well as wood and lignocellulosic materials. A biorefinery is not a completely new concept.

According to Berntsson et al., biorefinery promotes industrial trades, economic, and environmental sustainability. Biorefineries are found helpful in generating added-value products, bio-based products, and bioenergy utilizing sustainable biomass (de Jong and Jungmeier 2015). As per the increasing energy demand nowadays, interest

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229

of scientists is increasing in renewable and sustainable biotechnological processes for energy, biofuels, and chemicals. Use of microorganisms in chemical industries is to derive the same product; using biological materials is an alternative sustainable and economical approach. It is estimated that by 2025, 15% of chemical products will be bioformulated (Vijayendran 2010). Thus, the development of biorefineries is an alternative to diesel and petroleum-based products. Biorefineries can be defined as processing of biomass (mainly lignocelluloses) into marketable and commercial products (food, feed, material, and chemicals) and energy (fuels, power, and heat) mediated by physical, chemical, or biological materials (IEA 2010).

The biorefinery concept is eye-catching because it facilitates production of high added-value products at lesser price and reducing waste disposal and maintaining ecological harmony. Few biorefineries have established, for instance, the pulp- and paper-based biorefinery, Borregaard, in Norway (Borregaard 2014), but attempts are required to establish such biorefineries in several other countries as well. Microorganisms are the basis of biorefineries and backbone of industrial bioprocesses; they either produce desired chemical or produce intermediate required for the process. Most of the industries in world utilize the potential of microorganisms for the production of food additives, medicines, antibiotics, enzymes, bioethanol, biodiesel, and other chemicals. Lignocellulosic biomass is the most abundant biomass on earth obtained as agricultural by-product and renewable source of sugars, and is an advisable feedstock for the production of biodiesel, biogas, biohydrogen, and chemical products through the biorefinery processes (Menon and Rao 2012). In biorefinery processes, lignocellulosic biomass is firstly pre-treated, and then cellulosic and hemicellulosic are decomposed into simple sugars mediated by enzymes (Rastegari et al. 2019a). Microbes metabolize and ferment these simple sugars producing chemical products such as alcohols, fatty acids, organic acids, and amino acids. Bioethanol is a more preferred alternative over conventional petroleum-based transport fuels. However, complex structure of lignocellulosic biomass is a challenge in its bioconversion than simple starch and sugar materials (Mussatto et al. 2010; Yadav et al. 2020). Cellulose, hemicellulose, and lignin are building blocks of lignocellulosic biomass.

Biorefineries have led new opportunities to the industrial application of microorganisms. Potential of unexplored or new microbe for desired product can be checked. New substrates may be added, and along with these industrial processes can be optimized to achieve maximum conversion processes. In addition, we highlight and exemplify general strategies to develop microorganisms that are able to produce fuels and chemicals from renewable feedstocks. All types of biomass from forestry, aquaculture, agriculture, organic and forest residues, and aquatic biomass (algae and seaweeds) are converted into valuable products of humankind. Many of the industries converting sugar, starch, pulp, and paper industries are considered as biorefineries. There are many differences between refineries and biorefineries (Table 11.1).

**Table 11.1** Comparison of refineries and biorefineries regarding feedstocks, building block composition, processes, and chemical intermediates produced at commercial scale

Sources	Refinery	Biorefinery
Feedstock	Feedstock relatively homogeneous	Feedstock heterogeneous regarding bulk components e.g., carbohydrates, lignin, proteins, oils, extractives, and/or ash Most of the starting material present in polymeric form (cellulose, starch, proteins, lignin)
	Low in oxygen content	High in oxygen content
	The weight of the product (mole/mole) generally increases with processing	The weight of the product (mole/mole) generally decreases with processing. It is important to perceive the functionality in the starting material
	Sometimes high in sulfur	Sometimes high in inorganics, especially silica
Building block composition	Main building blocks: Ethylene, propylene, methane, benzene, toluene, xylene isomers	Main building blocks: Glucose, xylose, fatty acids (e.g., oleic, stearic, sebacic)
(Bio)chemical processes	Introduction of heteroatoms (O, N, S)	Removal of oxygen
	Relative homogeneous processes to arrive at building blocks: Steam cracking,	Relative heterogeneous processes to arrive building blocks
Chemical intermediates produced at commercial scale	Many	Few but increasing (e.g., ethanol, furfural, biodiesel, mono-ethanol glycol, lactic acid, succinic acid)

## 11.2 Classification of Biorefineries

Biorefineries have been classified in different categories on the basis of different criteria (de Jong and Jungmeier 2015). On the basis of technologies used, biorefineries are divided into conventional and advanced biorefineries: first-, second-, and third-generation biorefineries. On the basis of raw material used, biorefineries are divided into whole crop biorefineries, oleochemical biorefineries, lignocellulosic feedstock biorefineries, green biorefineries, and marine biorefineries. On the basis of conversion process used, biorefineries are divided into thermochemical biorefineries, biochemical biorefineries, and two-platform concept biorefineries. On the basis of intermediate produced, biorefineries are syngas platform biorefineries and sugar platform biorefineries. On the basis of availability of biomass, biorefineries

have been classified into six types (Lange 2017). Yellow biorefinery utilize straw, corn stover, and wood. Green biorefinery utilizes fresh green biomass, grass for protein-rich feed. Blue biorefineries use fish by-catch/cut-offs, fish discards and innards, mussels as biomass, brown seaweed, red and green algae, and invertebrates such as sea cucumber. Red biorefinery utilizes slaughterhouse waste. White biorefinery uses agro-industry-side streams.

### 11.3 Microbial Fermentation Processes for the Development of Biorefineries

Due to large consumption of fuels and foods, sustainable way to produce new foods and fuels from agro-residues is required. Sustainable production is an effective technology utilizing raw materials, agro-waste to produce new, commercial, and valuable products. Solid-state fermentation is an alternative and long term used approach for the production of biotechnology-based commercial products. Fermentation technology of microbes has been used in East for the manufacture of fermented foods and for manufacture of mold-ripened cheese in West. In fermentation technology, microbes are allowed to grow on solid material with low moisture content. Fermentation is an economical, large-scale process of bioconversion and biodegradation process. With the aid of this technology food, enzymes, chemicals, cosmetics, and pharmaceutical compounds have been produced (Kour et al. 2019a; Kumar et al. 2019). This fermentation technology is driving attention of researchers widely nowadays. Various alternative terms are currently being used as synonyms of solid-state fermentation likewise solid-state fermentation, surface cultivation, surface culture, solid-state digestion, and solid-state fermentation.

Botella et al. (2009) used a new term “particulate bioprocessing”, in order to define solid-state fermentation. Particulate bioprocessing defines growth of microorganism in moist condition in a particulate solid medium. Amore and Faraco (2012) used the term consolidated bioprocessing (CBP) defining fungi as alternative microbe for the degradation of lignocellulosic materials. Cellulose degrading fungi produce saccharolytic enzymes for the digestion of lignocellulose and converting sugars to ethanol. These technologies reduce the cost of production of ethanol and show that the fungi have all the pathways required for conversion of lignocellulose to bioethanol. Viniegra-González (1997) defined solid-state fermentation as a process where microbes grow on the surface of solid material without the addition of nutrients. Pandey et al. (2000) defined solid-state fermentation, a technology, where microbes are grown on moist solid support, either on inert carriers or on insoluble substrates that can also be used as carbon and energy source.

Rahardjo et al. (2006) defined solid-state fermentation as the growth of microorganisms on moistened solid substrate with enough moisture is to maintain microbial growth and metabolism. Adopting the technology of solid-state fermentation, microbes have been used in biorefineries for conversion of sugar containing polymers

such as cellulose and hemicellulose in commercial products. Biofuels, bioethanol, biomethanol, biogas, pharmaceutical products, and biodegradable products have been produced using microbes (Koutinas et al. 2007). Webb et al. proposed a model for wheat-based biorefining strategy in economical way using microbial fermentation (Fig. 11.1).

## 11.4 Genetic Improvement of Microorganisms for Development of Biorefinery Products

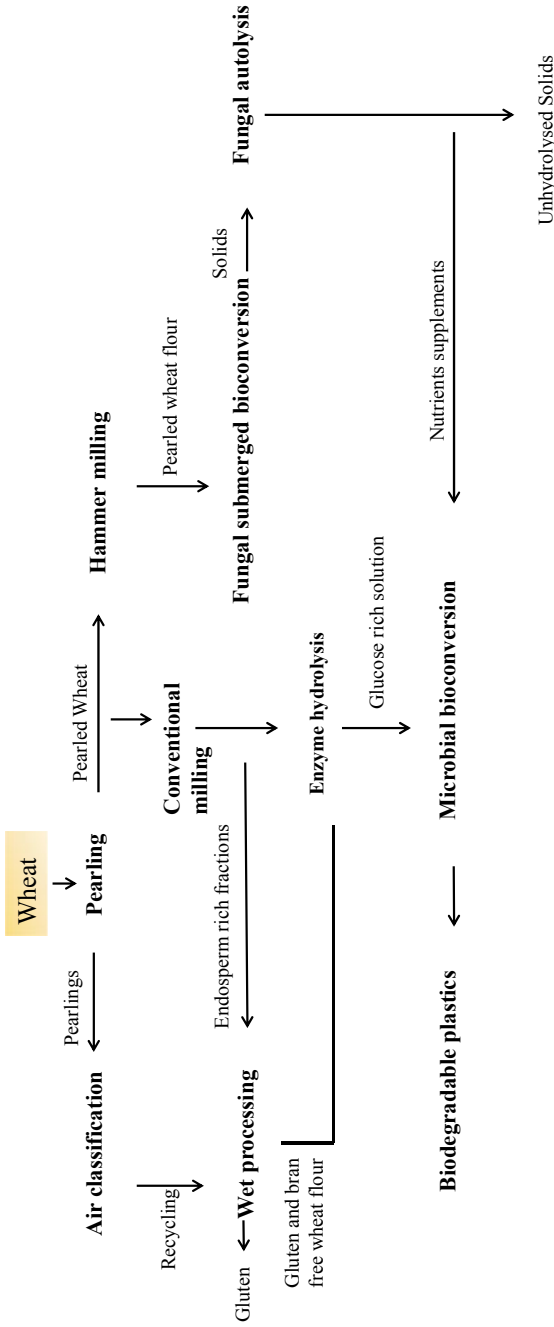
Microbial strains are required which can result in high yield and productivity of compounds tolerating several stresses (Rastegari et al. 2019b, c). For the same, microbes are genetically modified. *S. cerevisiae* has been used in bio-industries since last 30 years, each year with an improved version. Different strategies have been adopted for this genetic engineering likewise (i) driving carbon flux, (ii) increase tolerance to toxic compounds, (iii) increase of substrate uptake range, and (iv) generation of new products (Fig. 11.2).

### 11.4.1 Driving Carbon Flux

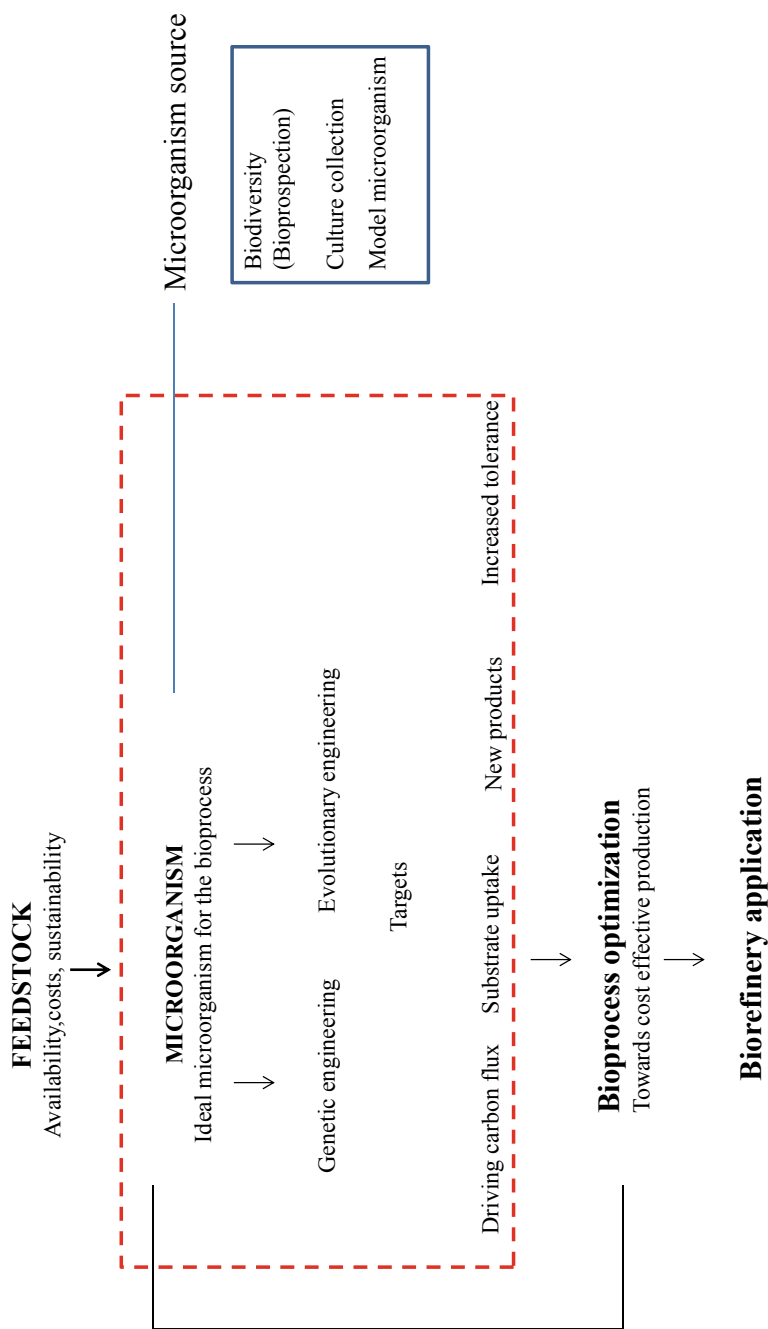
Naturally, microbes have capability to produce desired chemical compounds, and they are optimized for maximal growth. But the production of bioactive compounds is hindered due to expense of carbon, energy, and by-product formation. Thus, modifications in microorganisms which lead to higher production are driving carbon flux. Microbes of different groups such as bacteria, fungi, and yeast have been genetically modified to enhance production of biofuel and desired compounds. Microbial strains which are able to produce 90% m/m of desired chemical compound are available (Table 11.2). There are many steps where microbes have been modified such as modification in microbial metabolism by overexpression or knockout of enzymes (Jiang et al. 2009; Mojzita et al. 2010), modification in transcription and change in redox reactions (Alper and Stephanopoulos 2007; Almeida et al. 2009; Nissen et al. 2000). For instance, *S. cerevisiae* is modified to produce ethanol from sugars present in lignocellulosic biomass (Hahn-Hägerdal et al. 2007).

### 11.4.2 Increased Tolerance to the Substrate

Low tolerance to end product also hampers product formation by microbes. Fermentation medium also causes a harsh environment for the microorganism. In case of unavailability of tolerant strains, genetic engineering approaches have been used to



**Fig. 11.1** Schematic diagram of microbial fermentations proposed in a possible biorefinery utilizing wheat for the production of poly-hydroxyl butyrate and succinic acid



**Fig. 11.2** Main steps for the development of a new bioprocess integrated to a biorefinery

**Table 11.2** Microbial bioresources and biofuel production

Organism	Product	Main substrate	Yield*	Productivity	Concentration	Outcomes	Main genetic modifications	References
<i>Driving carbon flux toward the desired pathway</i>								
<i>E. coli</i> SY4	Ethanol	Glycerol	0.42 g g <sup>-1</sup>	0.15 g L <sup>-1</sup> h <sup>-1</sup>	7.8 g L <sup>-1</sup>	Yield improved 69-fold. Engineered strains efficiently utilized glycerol in a minimal medium without rich supplements	Deletion of genes to minimize the synthesis of by-products	Durbin et al. (2009)
<i>E. coli</i> LA02Δdld	Lactic acid	Glycerol	0.80 g g <sup>-1</sup>	1.25 g g <sup>-1</sup> h <sup>-1</sup>	32 g L <sup>-1</sup>	Low-value glycerol streams to a higher value product like D-lactate. Yield improved sevenfold	Overexpression of pathways involved in the conversion of glycerol to lactic acid and blocking those leading to the synthesis of competing by-products	Mazumdar et al. (2010)
<i>E. coli</i>	Acetate	Glucose	0.456 g g <sup>-1</sup>	1.38 g g <sup>-1</sup> h <sup>-1</sup>	53 g L <sup>-1</sup>	Reduction of the fermentation by products concentration by 1, 25 (succinate) to 33 fold (lactate). Yield improved over sevenfold	Deletion of genes involved in the succinate formation as fermentation product	Causey et al. (2003)
<i>Y. lipolytica</i>	Succinic acid	Glycerol	0.45 g g <sup>-1</sup>	Not determined	45 g L <sup>-1</sup>	Succinic acid production yield increased over 20 fold	Deletion in the gene coding one of succinate dehydrogenase subunits	Blankschien et al. (2010)

(continued)



Table 11.2 (continued)

Organism	Product	Main substrate	Yield*	Productivity	Concentration	Outcomes	Main genetic modifications	References
Y-3314 Mannheimia succiniciproducens	Succinic Acid	Glucose	0.76 g g <sup>-1</sup>	1.8 g g <sup>-1</sup> h <sup>-1</sup>	52.4 g L <sup>-1</sup>	Nearly complete elimination of fermentation by-products, (acetic, formic, and lactic acids) and carbon recovery increased to 58–77% by fed-batch culture	Disruption of genes responsible for by-product formation (ldhA, pfIB, pta, and ackA)	Lee et al. (2006)
<i>Increasing of tolerance to toxic compounds</i>								
<i>C. acetobutylicum</i>	Butanol	Glucose	Not determined	Not determined		Increased tolerance and extended metabolism response to butanol stress	Overexpression of spo0A, responsible for the transcription of solvent formation genes	Alsaker et al. (2004)
<i>C. acetobutylicum</i>	Butanol	Glucose	70.8%	Not determined	13.6 g L <sup>-1</sup>	Reduction of acetone production from 2.83 g L <sup>-1</sup> to 0.21 g L <sup>-1</sup> and enhanced butanol yield from 57 to 70.8%	Disruption of the acetoacetate decarboxylase gene (ade) avoiding acetone production and optimization of medium	Jiang et al. (2009)
<i>S. cerevisiae</i>	Ethanol	Glucose plus HMF (inhibitor)	0.43 g g <sup>-1</sup>	0.61 g g <sup>-1</sup> h <sup>-1</sup>	Not determined	Four times higher specific uptake rate of HMF and 20% higher specific ethanol productivity	Overexpression of alcohol dehydrogenases ADH6 or ADH1-mutated	Almeida et al. (2008)

(continued)

Table 11.2 (continued)

Organism	Product	Main substrate	Yield*	Productivity	Concentration	Outcomes	Main genetic modifications	References
<i>S. cerevisiae</i>	Ethanol	Spruce hydrolysate	Not determined	$0.39 \text{ g g}^{-1} \text{ h}^{-1}$	Not determined	HMF conversion rate and ethanol productivity for the engineered strains four to five times and 25% higher than for the control strain	Overexpression of alcohol dehydrogenases ADH6 or ADH1-mutated	Almeida et al. (2008)
<i>E. coli</i> XW068(pLOI4319)	Lactate	Xylose plus HMF	85% of the theoretical maximum	Not determined	Not determined	Furfural tolerance increased by 50%. Minimal growth and lactate production occurred after 120 h for the control strain	Overexpression of NADH-dependent propanediol oxidoreductase (FucO)	Wang et al. (2011)
<i>Increasing substrate uptake range</i>								
<i>E. coli</i>	Ethanol	Xylose	$0.48 \text{ g g}^{-1}$	$2.00 \text{ g g}^{-1} \text{ h}^{-1}$	$43 \text{ g L}^{-1}$	Rapid co-fermentation due to reduced repression of xylose metabolism by glucose, and 60% less time required for fermentation of 5-sugar mix to ethanol	Deletion of methylglyoxal synthase gene (mgsA), involved in sugar metabolism	Yomano et al. (2009)

(continued)

Table 11.2 (continued)

Organism	Product	Main substrate	Yield*	Productivity	Concentration	Outcomes	Main genetic modifications	References
<i>Lactobacillus plantarum</i>	Lactic Acid	Corn starch	0.89 g g <sup>-1</sup>	4.51 g g <sup>-1</sup> h <sup>-1</sup>	86 g L <sup>-1</sup>	First direct and efficient fermentation of optically pure D-lactic acid from raw corn starch reported	Deletion of L-lactate dehydrogenase gene (ldhL1) and expression of <i>Streptococcus bovis</i> 148 $\alpha$ -amylase (AmyA)	Okano et al. (2009)
<i>S. cerevisiae</i>	Ethanol	Xylose	0.43 g g <sup>-1</sup>	0.02 g g <sup>-1</sup> h <sup>-1</sup>	7.3 g L <sup>-1</sup>	Higher ethanol yields than XR/XDH carrying strains	Overexpression of <i>Piromyces</i> sp. xylose isomerase (XI)	Kuyper et al. (2003)
<i>S. cerevisiae</i>	Ethanol	Xylose	0.33 g g <sup>-1</sup>	0.04 g g <sup>-1</sup> h <sup>-1</sup>	13.3 g L <sup>-1</sup>	Higher specific ethanol productivity and final ethanol concentration than XI carrying strains	Overexpression of xylose reductase (XR) and xylitol dehydrogenase (XDH) enzymes from <i>Scheffersomyces stipitis</i>	Karhuma et al. (2007)
<i>E. coli</i>	Butanol	Glucose	6.1%	0.02 g g <sup>-1</sup> h <sup>-1</sup>	1.2 g L <sup>-1</sup>	Anaerobic production of butanol by a microorganism expressing genes from a strict aerobic organism	Expression of <i>C. acetobutylicum</i> butanol pathway synthetic genes in <i>E. coli</i>	Inui et al. (2008)

(continued)

Table 11.2 (continued)

Organism	Product	Main substrate	Yield*	Productivity	Concentration	Outcomes	Main genetic modifications	References
<i>Generation of new products</i>								
<i>E. coli</i>	Fatty acid ethyl esters (FAEEs)	Glucose	7%	Not determined	30.7 g L <sup>-1</sup>	Tailored fatty ester (biodiesel) production	Heterologous expression of a "FAEE pathway" engineered in <i>E. coli</i>	Steen et al. (2010)
<i>S. cerevisiae</i>	Butanol	Galactose	Not determined	Not determined	2.5 mg L <sup>-1</sup>	First demonstration of n-butanol production in <i>S. cerevisiae</i>	N-butanol biosynthetic pathway engineered in <i>S. cerevisiae</i>	Steen et al. (2008)
<i>E. coli</i> K12	1,3-propanediol	Glycerol	90.2%	2.61 g g <sup>-1</sup> h <sup>-1</sup>	104.4 g L <sup>-1</sup>	Substantially high yield and productivity efficiency of 1,3-PD with glycerol as the sole source of carbon	Heterologous overexpression of genes from natural producers of 1,3-PDO	Tang et al. (2009a, b)

improve strain response for toxic and end product. Strains have been improved to produce biofuels from lignocellulosic hydrolysate. Lignocellulose is composed of cellulose, hemicellulose, and lignin (Hahn-Hägerdal et al. 2007). Prior to fermentation, this hydrolysate is allowed for pretreatment to reduce its recalcitrance. Later, it is allowed for hydrolysis where sugar monomers have been formed from cellulose and hemicellulose. These sugar monomers form biofuels. During this pretreatment and hydrolysis, many toxic compounds are produced which inhibit microbial processes, microbial metabolism, and microbial growth as well. Compounds like furaldehyde, organic acids (acetic, levulinic, and furoic), and phenolic derivatives are found in lignocellulose. These compounds inhibit microbial growth, cause lowering in product yield, and reduce cellular viability (Almeida et al. 2007, 2011). Metabolic engineering and genetic engineering have been applied to make these strains tolerant. *S. passalidarum*, *S. cerevisiae*, and *P. stipites* have been evolutionary engineered to ferment lignocellulose more than the native strains (Heer and Sauer 2008; Hughes et al. 2012; Liu et al. 2004; Kour et al. 2019b). Yeast tolerance to lignocellulose has been improved by genetic engineering (Almeida et al. 2011) (Table 11.2). Genes having resistance to inhibitors are transferred in microbial strain for providing tolerance to end product.

### 11.4.3 Increase of Substrate Uptake Range

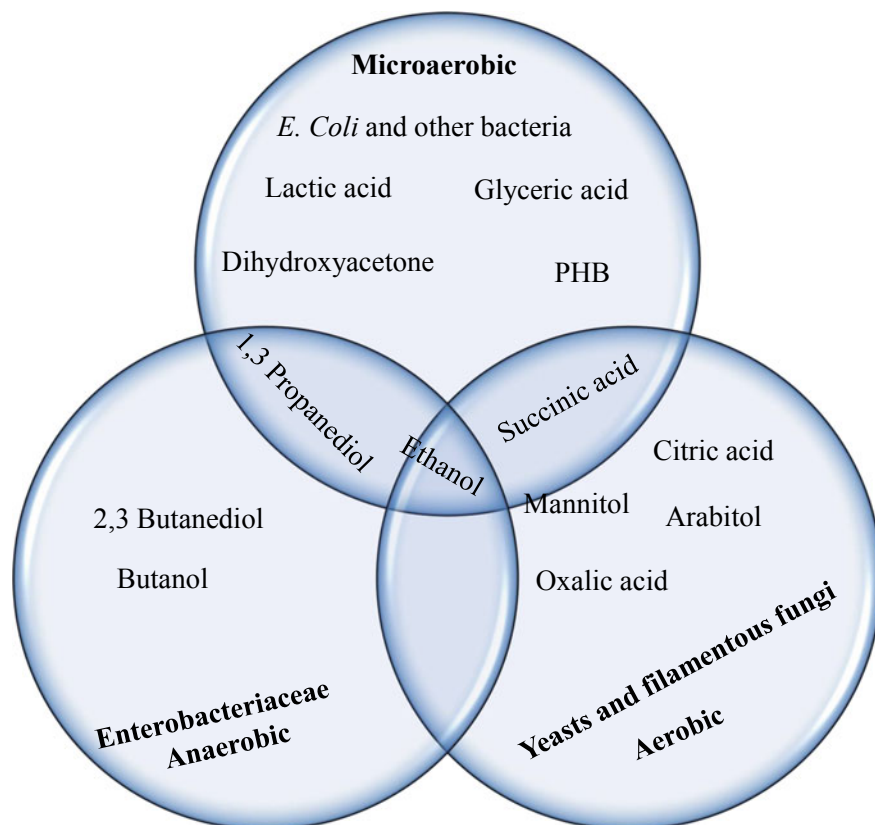
Genetic engineering of microbes has been done to increase substrate and its better utilization in product formation. Utilization of lignocellulosic biomass requires xylose utilization. Xylose is the second most abundant pentose sugar present in sugarcane bagasse (30%) (Ferreira-Leitão et al. 2010). Naturally, *S. cerevisiae* does not utilize pentose sugars; it is genetically modified to use this pentose sugar (Table 11.2).

### 11.4.4 New Products

Genetically modified microorganisms are able to produce compounds that are not possible by natural pathways. For this, enzymes and pathways from one organism have been transferred in an organism of choice. Nowadays, many new compounds have been reported by microbes rather than bioethanol which increase economy and can be produced in lesser time (Table 11.2). Acids produced from this lignocellulose serve as precursors of plastics (Werpy et al. 2004). *Acetobacter*, *Aerobacter*, *Pseudomonas*, *Gluconobacter*, and *Erwinia* produce a five-carbon acid xylonic acid, derived from xylose. Obviously, wild-type bacteria are able to produce this xylonic acid; however, this yield was very low. *E. coli*, *S. cerevisiae*, *Kluyveromyces lactis*, and *Pichia kudriavzevii* have been produced by genetic recombination to enhance yield of this xylonic acid (Toivari et al. 2010; Nygård et al. 2011; Liu et al. 2012).

## 11.5 Microbial Technologies for Biodiesel-Based Biorefineries

Production of biofuels from renewable feedstocks is demanded in the period of crisis of energy where petrol fuels are becoming limited and expensive (Rastegari et al. 2020; Yadav et al. 2019). Production of biofuels is a costly process, and various residues are produced; however, this cost can be reduced if residues can be converted into valuable coproducts (Zhang 2011; Yazdani and Gonzalez 2007). Biodiesel is an alternative biofuel obtained by the transesterification of fat and vegetable oils and reduces net greenhouse effect (O'Connor 2011). Many plants such as sunflower, soybean, rape, and palm oils are used to produce biodiesel. In Brazil, soybean oil was the source of 80% of biodiesel in 2010. Pies and glycerol are produced as residues in the production of biodiesel. Pies are used as animal feed or fertilizers, whereas glycerol is used as crude sample in biorefineries and many valuable products are formed (Fig. 11.3).



**Fig. 11.3** List of chemicals produced by microbes by the fermentation of glycerol

Many microbes such as *Klebsiella*, *Enterobacter*, *Clostridium*, Yeasts, and filamentous fungi are used for the production of organic acids, polyols, 1,3-propanediol, 2,3-butanediol, butanol, and ethanol (Yadav et al. 2017). 1,3-propanediol (1,3-PDO) can be produced by *Klebsiella* spp. and *Clostridium* spp. from glycerol (Celinska 2010). *K. pneumoniae* G31 also produces 2,3-Butanediol (BDO) from the fermentation of glycerol (Petrov and Petrova 2009). This BDO can be used in the preparation of synthetic rubber, plastics, and as a precursor of pharmaceutical drugs and medicine (Syu 2001; Ji et al. 2011). Ethanol is a widely used fuel and solvent in industries, produced from lignocellulose by yeasts. However, there are many reports where glycerol also acts as a source of ethanol (Liu et al. 2007; Petrov and Petrova 2009). *E. coli* can convert glycerol to ethanol aerobically and anaerobically (Dharmadi et al. 2006; Durnin et al. 2009). *Hansenula polymorpha*, a methylotrophic yeast, possesses potential to produce ethanol from glycerol (Hong et al. 2010). Genes encoding for pyruvate decarboxylase and aldehyde dehydrogenase II, from *Zymomonas mobilis*, are transferred into *H. polymorpha*, and increase in ethanol production was found (Hong et al. 2010). Butanol is an alternative fuel which is used in the manufacturing of plastics, paints, resin formulation, and lacquers (Harvey and Meylemans 2011). *C. pasteurianum* has been found to produce butanol from glycerol (Taconi et al. 2009). Apart from these, glycerol has been used to produce mannitol, arabitol, erythritol, succinic acid, lactic acid, oxalic acid, citric acid, and glyceric acid (Table 11.3).

## 11.6 Conclusion

Plant cell wall is composed of cellulose and lignin, which are very complex and poorly understood. Utilization of this for bioenergy needs more understanding and research inputs. In biorefineries, a consortium of microbes is used, where microbe–microbe interaction takes place. Attention should be paid toward population dynamics, interrelationship between species for scale-up of a process. It is possible to optimize microbial processes with the aid of computer simulations. Application of biotechnological aspects such as CRISPR/Cas, genome shuffling, transcription, and translational machinery in microbes can make them more potent for biorefineries

**Table 11.3** Chemicals produced at high yield and/or high concentration by microbial fermentation of glycerol

Product	Organism	Fermentation mode	Oxygen availability	Yield (product/glycerol)	Productivity	Product concentration	References
1,3-Propanediol	<i>K. pneumoniae</i> DSM 2026	Fed-batch	Microaerobic	0.52 mol/mol	1.57 g/L/h	59.50 g/L	Chen et al. (2003)
	<i>K. pneumoniae</i> LDH 526	Fed-batch	Aerobic	0.52 mol/mol	2.13 g/L/h	102.1 g/L	Xu et al. (2009)
	<i>C. butyricum</i> F2b	Batch	Anaerobic	0.53 g/g	1.05 g/L/ha	47.1 g/L	Papanikolaou et al. (2008)
2,3-Butanediol	<i>E. coli</i> K12	Fed-batch	Anaerobic	90.2%	2.61 g/L/h	104.4 g/L	Tang et al. (2009b)
	<i>K. pneumoniae</i>	Fed-batch 1 m <sup>3</sup>	Anaerobic	61 mol/mol	2.2 g/L/h	75 g/L	Liu et al. (2010)
	<i>K. pneumoniae</i> G31	Fed-batch	Microaerobic	0.36 mol/mol	0.18 g/L/h	49.2 g/L	Petrov and Petrova (2009)
Ethanol	<i>K. pneumoniae</i> G31	Fed-batch	Aerobic	0.39 g/g	0.47 g/L/h	70.0 g/L	Petrov and Petrova (2009)
	<i>E. coli</i> SY 4	Batch	Microaerobic	85%	0.15 g/L/h	7.8 g/L	Durbin et al. (2009)
Butanol	<i>C. pasteurianum</i>	Batch	Anaerobic	0.36 g/g	Not determined	1.8 g/La	Taconi et al. (2009)
	<i>G. oxydans</i> ZJB09112	Fed-batch	Aerobic	88.7%	Not determined	161.9 g/L	Hu et al. (2010)
Glyceric acid	<i>G. fraterii</i> NBRC103465	Fed-batch	Aerobic	0.76 g/g	0.81 g/L/ha	136.5 g/Lc	Habe et al. (2009)
	<i>A. tropicalis</i> NBRC16470	Fed-batch	Aerobic	0.46 g/g	0.71 g/L/ha	101.8 g/Ld	Habe et al. (2009)

(continued)



Table 11.3 (continued)

Product	Organism	Fermentation mode	Oxygen availability	Yield (product/glycerol)	Productivity	Product concentration	References
Lactic acid	<i>E. coli</i> AC-521	Fed-batch	Aerobic	0.9 mol/mol	0.49 g/g/ha	85.8 g/L	Hong et al. (2009)
Succinic acid	<i>E. coli</i> LA02Δdld	Batch	Microaerobic	0.83 g/g	1.25 g/g/h	32 g/L	Mazumdar et al. (2010)
	engineered <i>E. coli</i>	Batch	Microaerobic	0.69 g/g	~4 g/g/h	14 g/L	Blankschien et al. (2010)
Citric acid	<i>Y. lipolytica</i> Y-3314	Batch	Oxygen limited	0.45 g/g	Not determined	45 g/L	Yuzbashev et al. (2010)
	<i>Y. lipolytica</i>	Repeated batch	Aerobic	0.77 g/g	0.85 g/L/h	124.2 g/L	Rymowicz et al. (2010)
Oxalic acid	<i>A. niger</i>	Batch	Aerobic	0.62 g/g	Not determined	21 g/L	Andre et al. (2010)
Mannitol	<i>C. magnoliae</i>	Batch	Aerobic	0.51 g/g	0.53 g/L/h	51 g/L	Khan et al. (2009)
Erythritol	<i>Y. lipolytica</i> Wratisslavia K1	Fed-batch	Aerobic	0.56 g/g	1.0 g/L/h	170 g/L	Rymowicz et al. (2009)
Arabitol	<i>D. hansenii</i> SBP1	Batch	Aerobic	0.50 g/g	0.12 g/L/h	14 g/L	Koganti et al. (2011)
PHB	<i>E. coli</i> Arc2	Fed-batch	Microaerobic		0.18 g/L/h	10.81 g/L	Nikel et al. (2008)
	<i>Z. denitrificans</i> MW1	Fed-batch	Aerobic	0.25 g/g	1.09 g/L/h	54.3 g/L	Ibrahim and Steinbuechel (2009)

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