Microbes in Production of Commodity Chemicals

7

Ethanol, Acrylamide, Citric Acid, Adipic Acid, 1, 2-Propanediol and Penicillin

7.1 Introduction

Commodity chemicals are inexpensive, have larger demands and are produced and sold in bulk. They generally are intermediates involved in the syntheses of high end products (Table 7.1). Initially the chemical industry was dependent on nonrenewable resources for virtually all commodity chemicals. The cost of the feedstocks for commodity chemicals is directly associated with the cost of the petroleum and hence represents 50–75 % of the manufacturing cost of the commodity chemicals. However, considering the enhanced cost of the petroleum and natural gas resources as well as their possible exhaustion in the future due to continuous industrial demand, newer alternatives are being explored. One of the major technologies being explored by the industries in the USA, Europe and Japan is conversion of biomass into commodity chemicals using microbial interventions. Biomass generally comprises of crop and forest product wastes and municipal and agricultural wastes. Technologically it is possible to produce all the commodity chemicals from biomass feedstocks like starch and cellulose.

Microbes offer to be the best manipulative systems which could be exploited for customised synthesis of commodity chemicals, thereby decreasing the reliance on petroleum for production of chemical feedstocks. Microbes which could be exploited in this process could be natural isolates as well as genetically/metabolically engineered to produce the desired product. The commodity chemicals which are being produced from biomass include ethanol, acetone, citric acid, propanoic acid, fumaric acid, butanol and 2,3-butanediol.

7.2 Commercial Production of Ethanol

Previously ethanol was being made from ethylene derived from petroleum sources. Presently the ethanol/alcohol is being produced by fermentation through the conversion of biomass. Ethanol is a renewable energy which is being produced by fermentation of sugars and is being used as a blending agent up to 15 %v/v in petroleum in many countries of the world. Ethanol-blended petroleum for automobiles can significantly reduce the use of petroleum as well as bring down the emission of greenhouse gases. Brazil is one of the largest producers of motor grade fuel ethanol (MGFE).

The common fermentation substrates for the production of ethanol are corn starch, molasses, sugarcane juice, cassava starch and other fermentable carbohydrates. There are a variety of microbes which convert these substrates into ethanol (Table 7.2). Corn starch is generally used as a raw material for the commercial production of motor grade fuel ethanol. The broad process of production of motor grade fuel ethanol comprises grinding, cooking, fermentation, distillation and dehydration (Fig. 7.1).

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General assumption of ethanol production is based on the amount of the fermentable sugar available for the process. It has been estimated that approximately 18–23 kg of ethanol is produced from 45 kg of fermentable sugar, i.e. glucose. For starchy material, the yield is about the same, i.e. between 40 and 50 % based on the dry weight of the carbohydrate. Commonly the substrates used for ethanol production are directly fermentable or starchy materials which could be easily hydrolysed to fermentable sugars. Recently technologies are being developed to use the cellulosic as well as lignocelluloses biomass by pretreating them so that they are easily hydrolysed for use as a fermentable substrate.

Table 7.1 Important commodity chemicals and their uses

Commodity chemicals	Major uses
Ethanol	Detergent, solubiliser, cosmetics, solvent, fuel
Acetic anhydride	Cellulose esters
Adipic acid	Nylon
Cyclohexane	Nylon, caprolactam
Isopropanol	Acetone, solvents
Propylene oxide	Propylene glycol, urethanes
Butadiene	Rubber
Acrylonitrile	Polymers

During the commercial production of MGFE, corn is generally ground by dry milling process into proper consistency for efficient conversion of corn starch into ethanol. The purpose basically is to prepare the grain for efficient and rapid

Table 7.2 Ethanol-producing microorganisms

Class	Organism name	Carbon source
Yeasts	*Saccharomyces cerevisiae*	Glucose
	Schizosaccharomyces pombe	Xylulose
	Kluyveromyces lactis	Xylulose
Fungi	*Pachysolen tannophilus*	Glucose, xylose
	Mucor indicus	Glucose
Bacteria	*Zymomonas mobilis*	Glucose
	Thermobacteroides saccharolyticum	Glucose, xylose
	Thermoanaerobacter ethanolicus	Glucose, xylose
	Clostridium thermohydrosulfuricum	Glucose, xylose

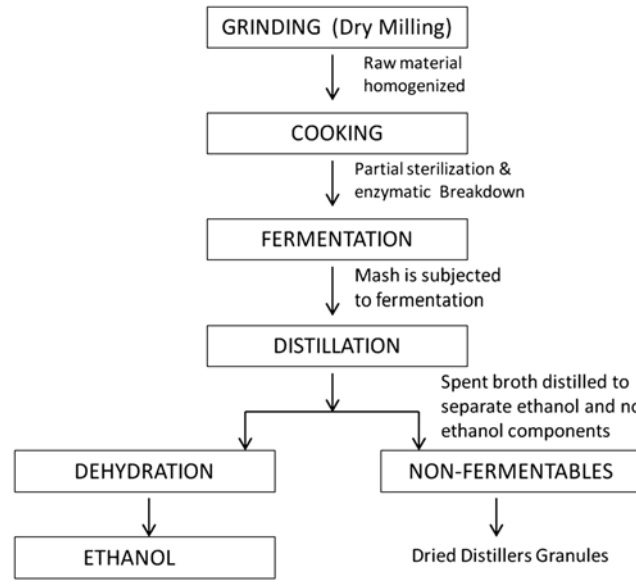


Fig. 7.1 Broad processes for the production of motor grade fuel ethanol

introduction of water and enzymes to achieve a homogenous mixture which could be easily pumped.

To have 25 % solids in the fermentation substrate, 1.149 kg of ground corn is mixed with 2.85 l of water at 60 °C and stirred continuously in a homogeniser held at a temperature ranging between 80 and 90 °C for 4–8 h and then mixed with two different enzymes. Approximately 3.3 ml of α-amylase (145,000 amylase units/ml) per kg of ground corn is mixed to hydrolyse the starch (i.e. amylase) and reduce the viscosity. This process is referred to as liquefaction and also prevents starch retrogradation. The slurry is then autoclaved at 121 °C for 20 min. Subsequently the sterilised slurry is held at 85 °C for 1 h and then mixed with 6.7 ml of amylase (saccharification step) which is just before fermentation. This process is known as saccharification and results in the formation of mash, the final fermentable substrate. Mash is cooled at room temperature and the water lost is made up with sterile water. Antibiotic lactoside is added 5 μg/ml to prevent bacterial contamination.

The pH value for fermentation is between 4.5 and 5.5 which are typically encountered in fuel ethanol plants. In the fermentation medium, urea is added at 0.016 % of the weight of mash as a nitrogen source. The fermentation was carried out at a temperature of 30 °C. Yeast growth and ethanol formation are generally inhibited by solutions having high osmotic pressure and accumulating high concentrations of ethanol. In batch fermentation for MGFE production, the inoculum size is six to eight million cells per ml or 0.24 kg of yeast solids per kilolitre. A five- to tenfold multiplication is generally expected during the batch process which approximately gives a yield of 1.2–2.2 kg of yeast solids per kg of mash. *Saccharomyces cerevisiae* requires biotin for enhancing the rate of fermentation. The total batch fermentation time ranges between 48 and 72 h. The alcohol produced during the fermentation process ranges between 6 and 8 % by volume. Twenty-five kilograms of corn on fermentation usually yields 8.5–10 l of ethanol and about 7.5 kg of distillers dried grains in a batch fermentation process.

The production of MGFE from the fermented beers is similar to those found in beverage spirits industry. Previously the dehydration step was achieved by azeotropic distillation, but currently molecular sieve dehydration utilising integrated pressure swing adsorption (PSA) technology is being used which is an energy-efficient process when compared to combined distillation and dehydration. Molecular sieves are manufactured from materials such as potassium aluminosilicates and are hard, granular, spherical or cylindrical extrudates. Their grading is done according to the nominal diameter of myriad internal pores that provide access to the interstitial free volume found in the microcrystalline structure. The grade used for ethanol dehydration is type 3Å, which refers to average diameter of the interstitial passageways is 3 Angstroms (Å). As the water molecule has a diameter of less than 3 Å and the ethanol molecule has an average diameter of more than 3 Å, the water molecules are retained in the interstitial spaces and the ethanol molecules move out. Thus, ethanol is also recovered from the azeotropic concentrations. The molecular sieving process generally occurs in the liquid or vapour phase. This dehydration step produces a final product that is nominally 100 % ethanol (200 proof, ethanol). After the centrifugation, the non-fermentable solids, i.e. unfermented grains, are concentrated thin stillage with 10–12 % moisture. These are known as Dried Distillers Granules Soluble (DDGS). DDGS has been used in poultry diets and in livestock diets for the improvement in the yield of milk and biomass.

7.3 Industrial Production of Acrylamide

Acrylamide ($CH_2{=}CHCONH_2$) is a commodity chemical which is used as a starting material for different polymeric substances like polyacrylamide and similar polymers which find applications as thickening, binding, strengthening and flocculating agent in industrial applications. The current annual global demand for acrylamide is ~250,000 metric tonnes. Conventionally acrylamide was produced using acrylonitrile as a

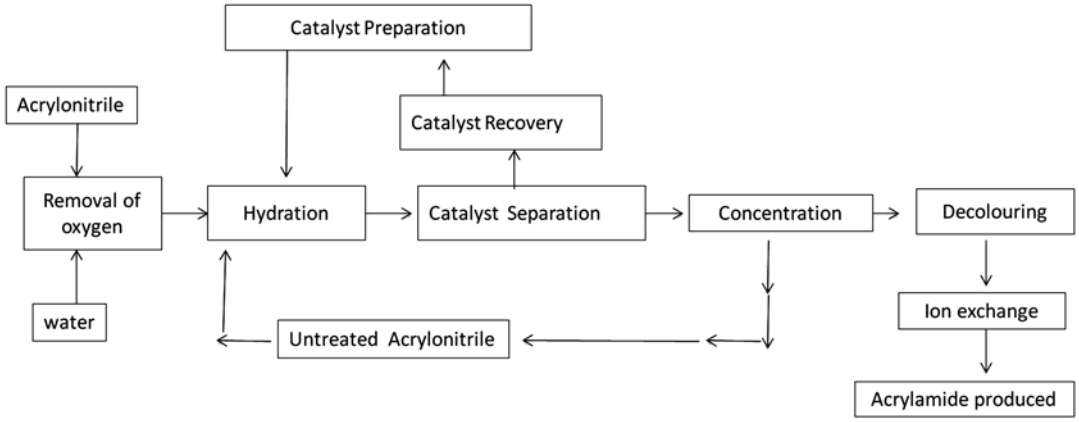


Fig 7.2 Conventional (chemical) process of acrylamide production

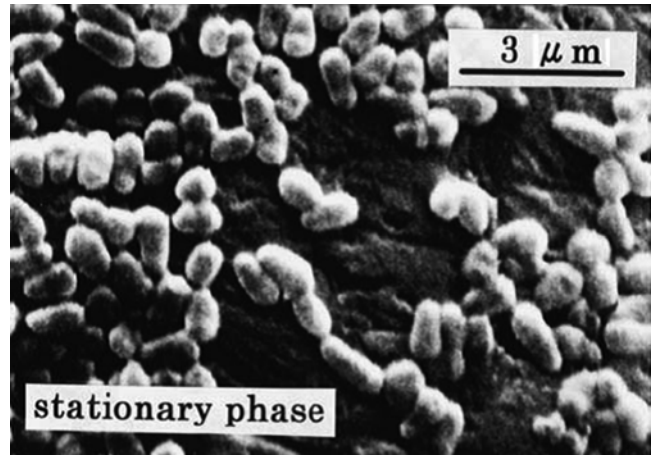


Fig. 7.3 Electron micrograph of *Rhodococcus* species N-774 (Taken from Dia-Nitrix Co., Ltd website)

starting material by passing it over a Raney copper catalyst at about 100 °C (Fig. 7.2). However, the major drawback of the process is the complex nature of the preparative procedure for the catalyst, difficulties in regenerating the used catalyst and problems associated with separating impurities from acrylamide. The impurities are ethylene cyanohydrin, β-hydroxypropionamide and nitrilotrispropinamide which may interfere with the chain reactions. The Nitto Chemical Company in Japan started the commercial acrylamide production using an enzymatic process which was jointly developed by researchers at Kyoto University and Nitto Chemical Industry (now Mitsubishi Rayon). The process was based on the biocatalyst nitrile hydratase which was discovered during studies on degradation of nitrile compounds by microorganisms and catalyses the hydration reaction of nitrile to amide (Asano et al. 1980).

During the extensive screening process, some microorganisms under resting conditions were able to accumulate acrylamide when incubated with acrylonitrile. *Rhodococcus* sp. N-774 was the first strain that was put into commercial use to produce acrylamide from acrylonitrile (Fig. 7.3). The nitrile hydratase enzyme is produced in this strain without an inducer. The resting cells of *Rhodococcus* sp. N-774 and *Pseudomonas chlororaphis* B23 were incubated with acrylonitrile provided that acrylonitrile was added gradually to avoid inhibition of nitrile hydratase activity, thereby accumulating approx. 400 g of acrylamide per litre at 10 °C. Ninety-nine percent conversion

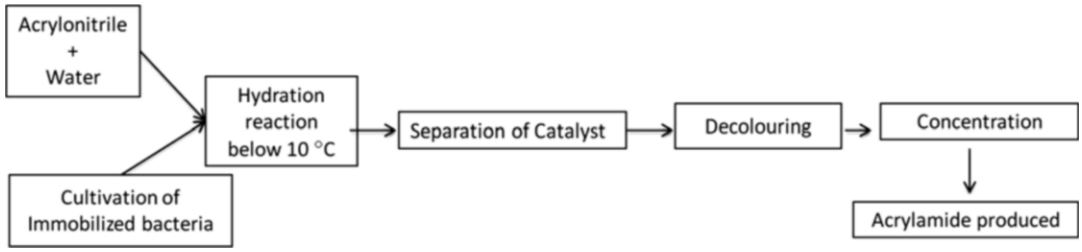


Fig. 7.4 Process outline for microbial production of acrylamide (Catalyst here refers to the immobilised bacteria producing nitrile hydratase)

Table 7.3 Performance of microorganisms producing nitrile hydratase for acrylamide production

	Reaction conditions			Reaction performance		
	Temp (°C)	pH	Acrylonitrile conc. (%)	Conversion of acrylonitrile (%)	Selectivity of acrylamide (%)	Acrylamide concentration at outlet of reactor (%)
Rhodococcus sp. N-774	0–5	7.5–8.5	1.5–2.0	99.9	99.9	20
Pseudomonas chlororaphis B23	0–5	7.5–8.5	1.5–2.0	99.97	99.98	30
Rhodococcus rhodochrous J1	0–15	6.5–8.0	1.5–2.0	99.97	99.98	50

of acrylonitrile into acrylamide was achieved without the formation of impurities (Fig. 7.4). *Rhodococcus rhodochrous* J1 strain produced cobalt containing nitrile hydratase which could produce 700 g/l acrylamide in the reaction solution. Mitsubishi Rayon's acrylamide production using bacterial nitrile hydratase produces approximately 30,000 tpa, consumes less energy and steers clear of heavy metal problems in the wastewater (Table 7.3).

7.4 Industrial Production of Citric Acid

Citric acid is a naturally occurring organic acid which is found in citrus fruits, pears and pineapples. This tricarboxylic acid is a critical intermediate of metabolism in plants and animals as it is the first product formed in the aerobic respiration, i.e. citric acid cycle or Krebs cycle. Carl Wilhelm Scheele isolated and crystallised citric acid from lemon juice in 1784. Citric acid is commodity chemical and is available in anhydrous or monohydrate forms. The commercial success of citric acid is attributed to its use in industry. Approximately 70 % of the citric acid produced is used in food and beverage industry with carbonated products having a market size of approximately 50 %. The pharmaceuticals consume approximately 12–14 %, and the remaining 16–18 % is used for other industrial applications like metal cleaning and detergent markets, in preparation of blue print paper, in passivation of stainless steel, etc. The annual global production of citric acid is to the tune of over 1.4 million tonnes.

The citric acid is currently being produced by fermentation. Microorganisms that can produce citric acid were first observed by C. Whemer (1893). He found that the mould *Penicillium glaucum* could accumulate significant quantities of citric acid when grown on sugar solution. It was James N. Currie (1917) who found that an isolate of *Aspergillus niger* produced better yields of citric acid and is still considered the organism of choice for industrial production of citric acid (Table 7.4). Currie joined Pfizer and in 1923, Pfizer started the commercial production of citric acid. Currently the major producers of

Table 7.4 Microorganisms producing citric acid

Fungi	*Aspergillus niger*
	A. aculeatus
	A. carbonarius
	A. awamori
	A. foetidus
	A. fonsecaeus
	A. phoenicis
	Penicillium janthinellum
Yeasts	*Candida tropicalis*
	C. oleophila
	C. guilliermondii
	C. citroformans
	Hansenula anomala
	Yarrowia lipolytica
Bacteria	*Arthrobacter paraffinens*
	Bacillus licheniformis
	Corynebacterium ssp.
	Brevibacterium flavum
	Bacillus subtilis

citric acid are Archer Daniels Midland (ADM), USA; Cargill, USA; Tate & Lyle, UK; DSM, Netherlands; Jungbunzlauer, Switzerland; Gadot Biochemical Industries, Israel; and Anhui BBCA Biochemical Co. Ltd., China.

The basic fermentation processes used in industry for production of citric acid by *Aspergillus niger* are (1) surface fermentation, (2) submerged fermentation and (3) solid substrate or the Koji fermentation.

7.4.1 Citric Acid Production by Surface Fermentation

This was the very first method adopted for the industrial manufacture of citric acid. The process is still used in small and medium scale industries as the installation is cost-effective and the process operations are cost- and energy effective. The raw material used for production of citric acid comprise of molasses, hydrolyzed corn starch or other inexpensive sugary solutions. Dextrose or beet molasses is generally preferred raw material wherein it is diluted to 15–20 % with dilute sulphuric acid and the pH is adjusted between 5.5 and 6.5. Inorganic nitrogen is provided by ammonium sulphate, ammonium nitrate, sodium nitrate, potassium nitrate and urea. Phosphorus is the third major constituent of the fermentation medium, and its concentration ranges between 0.1 and 2 % based on the type of strain used for carrying out the fermentation process.

Shallow trays made of high purity aluminium or stainless steel with a capacity of 200–1,000 l are used for the surface fermentation process. These trays are stacked in a rack under aseptic conditions. Media is pumped through the trays aseptically and then spore inoculation is carried out into the liquid medium or via air. Aeration is important for the process of fermentation as well as removal of heat from the aseptic system. The heat generation during the fermentation is at the rate of 1 kJ/h/m^3, but the surface and the medium temperature is maintained in the range of 28–30 °C throughout the fermentation by air circulation in the fermentation chamber. Air flow at initial fermentation stage is low but increases after 12 h, and when the growth is maximal, it is supplied at a rate of 10 $m^{3.}$ The air is humidified between 40 and 60 % to prevent the loss of moisture from the surface of the medium and is passed through bacteriological filter prior to entering the fermentation chamber. The fermentation duration is between 8 and 15 days. After the fermentation process, the tray contents are separated into crude fermentation fluid and mycelial mats are washed to remove the impregnated citric acid. The productivity of citric acid is 1 kg per sq. m/day.

7.4.2 Submerged Fermentation for Citric Acid Production

In industrialised countries, the submerged process is the choice method since it is less labour intensive, uses less space compared to surface fermentation and gives higher production rate. Submerged process is generally carried out in stirred tank fermentation though air lift fermenters with higher aspect ratio are also being used industrially. The reactors are designed using high stainless steel grade due to low pH being developed during the fermentation process.

At times, two-stage fermentation process is adopted in which sufficient inoculum is developed by using the growth medium in the first stage and

in the second stage the whole biomass is transferred to the production medium. However, the temperature for inoculum production and that for citric acid production is the same at 30 °C. Low aeration rates are generally used since oxygenation is toxic hence low aeration rate of 0.1 vvm is generally used at the beginning of the fermentation process which is slowly enhanced to 1 vvm as the growth proceeds. Fed-batch processes are generally preferred over continuous process for citric acid production by submerged fermentation. The raw material generally used is molasses amended with nitrogen source like urea, peptone, ammonium nitrate and potassium dihydrogen phosphate as phosphate source. The duration of the fermentation is 3–5 days and the fermentation mother liquor is drained off, mycelium washed from which citric acid is extracted.

7.4.3 Solid–Substrate Fermentation for Citric Acid Production

Solid state fermentation process or Koji process was first developed in Japan. The process is characterised by the development of organism with low water activity environment on insoluble material which served as nutrient as well as solid support. A variety of agro-industrial wastes have been used for citric acid production. These include wheat bran, rice bran, coffee husk and pineapple waste husk. Solid substrate fermentation is carried out in trays or horizontal drum bioreactor.

Solid state fermentation is carried out in trays (0.0045 m^3) where the moist and pre-inoculated substrate (10^6–10^7 spores/g of dry substrate) was distributed in order to have thickness of 6 cm (0.45 Kg of dry substrate). The trays are placed in a room with controlled temperature of 28 °C and humidity of about 97 %. Fermentation is carried out for 120 h.

In horizontal drum bioreactor (Fig. 7.5), 2 kg of substrate with initial moisture of 60 % and is placed inside the drum. The drum is made using steel 360 with 32 cm diameter and 30 cm length (internal volume of 0.024 m^3) which consisted of shovel coupled to a motor axle which is rotated with a controlled speed. The HD reactor is rotated three to four times a day. After 20 h of fermentation, saturated air is passed continually into the drum in order to control substrate temperature and moisture. The air flow is maintained at 5 L/min. Fermentation is carried out for 144 h. *A. niger* is the common organism which is generally employed for solid substrate fermentation.

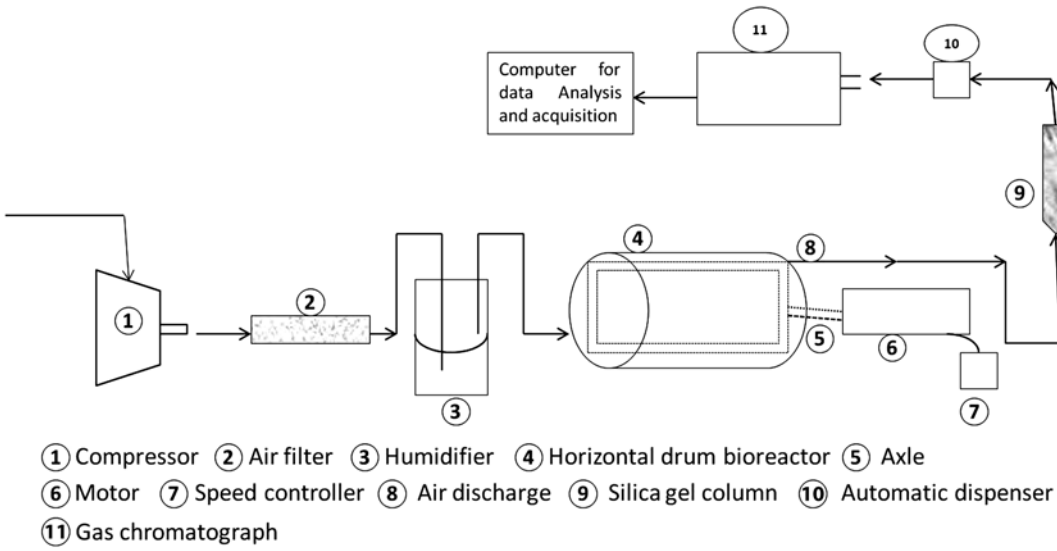


Fig. 7.5 Solid state fermentation for citric acid production using horizontal drum bioreactor

7.4.4 Recovery of Citric Acid

The process of citric acid recovery is same for surface and submerged fermentation wherein the mother liquor containing citric acid is mixed with mycelial washes so as to recover the impregnated citric acid. Broadly the three procedures adopted are (1) precipitation, (2) extraction and (3) adsorption. Precipitation is the first method which is a conventional process of mixing the filtered mother liquor with calcium oxide (hydrated lime) in the ratio of 2:1 at a temperature of 50 °C for 20 min to achieve 100 % precipitation of citric acid. The citric acid is converted into tricalcium citrate tetrahydrate. The precipitated calcium citrate is filtered off and then washed several times with deionised water. The precipitate is finally recovered by filtration and then treated with sulphuric acid which results in the formation of calcium sulphate (gypsum) which can be filtered off and the mother liquor containing citric acid is obtained. Further this mother liquor of citric acid is treated with activated charcoal and passed through cation and anion exchangers. Finally, the liquor is concentrated under vacuum crystallisers between 20 and 25 °C wherein the citric acid crystallises as citric acid monohydrate. Anhydrous citric acid is obtained when the crystallisation temperature is higher than 36.5 °C. The second process of recovery of citric acid is solvent extraction method which involves the use of tridecylamine or triisononylamine with water insoluble ester, ketone or alcohol. The third process which has been recommended by the US Food and Drug Administration uses a mixture of n-octyl alcohol, synthetic isoparaffin petroleum hydrocarbons and tridodecylamine used for the recovery of citric acid from fermented liquors.

7.5 Microbial Production of Adipic Acid

Adipic acid is a dibasic acid which was essentially used as a chemical feedstock for the production of Nylon 6, 6. Apart from its role in the production of nylon, today adipic acid sports a position of commodity chemical due to its versatile use in adhesives, coatings, hydraulic fluids, flue gas desulfurisation scrubber additive, cleaning additive, soil conditioners, glass protection agents, polymer/plasticiser additives, leather tanning, personal care emollients and chemical intermediates. A green method has been developed for the synthesis of the dibasic acid, adipic acid. In this process the dextrose is converted into cis, *cis*-muconic acid which is subsequently by catalytic hydrogenation leads to the formation of adipic acid (Fig. 7.6). This process of synthesis is not known and has been engineered in *E. coli* to develop an ecofriendly process so as to reduce the greenhouse effects due to the production of nitrous acid. More recently, Verdezyne Inc. has engineered a yeast for the commercial production of adipic acid. It has set up a fermentation plant for adipic acid production at Carlsbad, California, in 2011.

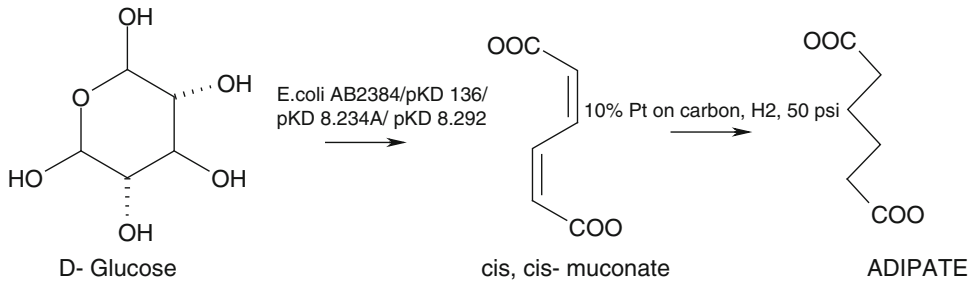


Fig. 7.6 Microbial production of adipic acid from d-glucose via cis, *cis*-muconate

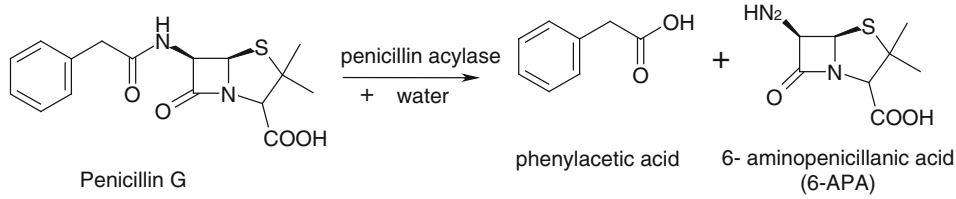
7.6 Microbial Production of 1, 2-Propanediol

1,2-Propanediol (PDO) is major commodity chemical which is used to carry out polycondensations to produce biodegradable plastics and polymer resins. Apart from this, PDO is also being used for the production of non-ionic detergents, as anti-freezing agent, de-icing agent in cosmetics and liquid detergents. It is also used as a feed additive in dog food after being recognised as GRAS by USFDA. The global consumption of 1, 2-propanediol is over 1.5 million metric tonnes, and the biggest producers are Dow and Lyondell. Microbial production of 1, 2-propanediol was first reported from *Clostridium thermobutyricum* (Enebo 1954). The other microorganisms which metabolised sugars, viz. fucose, rhamnose, glucose, xylose to produce 1, 2-propanediol, are *Salmonella typhimurium*, *Klebsiella pneumonia*, *Bacteroides ruminicola*, *Clostridium sphenoides*, *Clostridium thermosaccharolyticum* and *Thermoanaerobacterium thermosaccharolyticum* HG-8. *Thermoanaerobacterium thermosaccharolyticum* exhibits a unique feature of producing enantiomerically pure (R)-1, 2-propanediol and hence been explored for developing a fermentative mass production process.

Attempts are also being carried out to develop recombinant microbial strains for cost-effective production of 1, 2-propanediol from renewable resources. One of the strategies involves development of a recombinant bug which could convert glucose to glycerol and glycerol to 1, 2-propanediol and subsequently optimise the fermentation process. Recently, Cargill and Ashland Inc. have started a joint venture for production of propylene glycol from glycerol coming out of biodiesel plant industry. Cargill has announced the process wherein carbohydrates would be converted into 1, 2-propanediol by *E. coli* or *T. thermosaccharolyticum*.

7.7 Penicillin as a Commodity Chemical

Penicillin was discovered as an antibiotic and marked the golden era of antibiotic drug discovery and development. However, since the late 1990s, it is sporting a status of a commodity chemical for the production of semi-synthetic penicillins. The key factors which led penicillin to become a commodity chemical were (1) addition of precursors in the fermentation medium to enhance the yield, (2) development of high penicillin-yielding strains by strain improvement programmes (see Chap. 10) and (3) development of appropriate industrial equipments for the production as well as isolation of penicillin in bulk quantities. Another important reason for penicillin being converted into a commodity chemical was antibiotic resistance encountered by the pathogenic bacteria and hence newer versions of more potent antibiotics were required which could overcome antibiotic resistance. The discovery of the enzyme penicillin acylase helped in isolation of the β-lactam structure known as 6-aminopenicillanic acid (6-APA) which could be used as a template for development of semi-synthetic penicillins with better antimicrobial activity against drug-resistant bacteria as well as broader spectrum of activity against pathogenic microbes (please refer to Chap. 8). This process of enzymatic splitting of penicillin has been referred to as biosplitting (Fig. 7.7). The global production of penicillins is approximately



Penicillin G phenylacetic acid 6- aminopenicillanic acid (6-APA)

Fig. 7.7 Biosplitting of penicillin G to 6-APA by penicillin acylase

75,000–85,000 tonnes annually. Majority of the penicillin being produced is used for the development of semi-synthetic penicillins, and a very limited amount is directly used as an antibiotic.

7.7.1 Production of Penicillin

Currently the penicillin is produced by high-yielding strains of *Penicillium chrysogenum* which have been developed using classical mutagenesis and recombinant DNA technology. Commercially the production is carried out in a Fed-batch reactor having a tank volume of 20,000–60,000 gal. The carbon source is glucose which is provided as molasses, while the nitrogen source is corn steep liquor. The pH of the fermentation medium is maintained between 6.4 and 6.8, and the temperature during the fermentation process is maintained throughout at 25 °C. Membrane filters are used for providing air during the fermentation process. Thirty percent dissolved oxygen is critical for the production of penicillin. Phenoxyacetic acid and phenylacetic acid are used as precursors for production of penicillin V and penicillin G, respectively. There are three-staged seed fermenters for the production of the inoculum and the fermentation time is 120–200 h for penicillin production. The production of penicillin is monitored by HPLC during the fermentation process. The production of penicillin, i.e. titer achieved in the above process, ranges between 40 and 60 g/l.

7.7.2 Recovery and Purification of Penicillin

Whole broth is filtered through a membrane filter which separates the insolubles from the aqueous liquor. The mycelium is rewashed with distilled water to recover entrapped penicillin in the mycelial cake due to capillary force. The aqueous liquor/mother liquor obtained is adjusted to a pH range of 2–2.5, and then penicillin is extracted in butyl acetate. The butyl acetate fraction is re-extracted with a buffer of pH 6.0 to yield penicillin-rich buffer solution. This penicillin-rich solution is re-extracted with butyl acetate to yield a solvent solution consisting of a high potency of penicillin. This is further concentrated using multiple back extractions using buffer and solvent at different pH using countercurrent contractors, leading to a considerable penicillin concentration in early stages of recovery.

Subsequently, the pigment and broth impurities are removed by activated charcoal. The penicillin is re-crystallised by addition of potassium acetate and isolated as crystalline potassium salt. Additional carbon treatment and solvent washes ensure highly purified penicillin V.

7.8 Summary

Microbes are playing an important role in development of greener industrial processes for the production of commodity chemicals. Thus, reliance of petrochemical for harnessing them is progressively reducing with parallel development of microbially catalysed processes, thereby saving energy and environment. Production of ethanol, acrylamide and adipic acid through microbial process clearly indicates the shift in the paradigm.

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