Chapter 8
Date Palm Waste: An Efficient Source for Production of Glucose and Lactic Acid

Muhammad Tauseef Azam and Asif Ahmad

Abstract Plant based by-products are naturally available in large quantities and they can be exploited as cheap and feasible substrate for their biological transformation into valuable products. Date palm is a good example from plant source having a great value for its by-products owing to presence of cellulosic material that can be converted into valuable products like glucose and lactic acid as an option to reduce environmental pollution. Production of glucose from cellulosic date palm waste can be achieved with the help of cellulose enzyme from selective microorganisms. Similarly, date palm cellulosic material can also be converted into lactic acid with the help of lactic acid bacteria through fermentation process. Conditions may be optimized for the production of glucose and lactic acid during fermentation process. Lactic acid production is decreased if the substrate concentration is high initially in the fermentation experiment while maximum production is achieved by increasing the enzyme concentration in the experiment. The desirable yield of glucose can be achieved at 50 °C and pH of 5.0. Adopting a two-step hydrolysis process can increase the glucose production by 94.88% in 24 h process. Lactic acid yield can be achieved maximum at temperature 40–45 °C and pH 6. These results are promising and these suggest that yield of sugar and lactic acid from date palm waste is practical and it may be employed as a best practice to minimize the environmental pollution by using date palm cellulosic by-products as an inexpensive source. This chapter envisage the suitability of date palm waste as inexpensive cellulosic source for obtaining commercially valuable products i.e. glucose and lactic acid.

Keywords Date palm · Biological waste · Cellulosic waste · Cellulase enzyme · Glucose · Lactic acid · Lactic acid bacteria · Enzymatic fermentation

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M. Naushad, E. Lichtfouse (eds.), *Sustainable Agriculture Reviews 34*,
Sustainable Agriculture Reviews 34, https://doi.org/10.1007/978-3-030-11345-2_8

8.1 Introduction

Date palm (*Phoneix dactylifera*) trees are considered as one of the oldest tree of the world. These are ancient cultivated plants and their history goes back to 10,000 years ago (Al-Shahib and Marshall 2003). It is produced as principal agriculture product in North Africa and Middle East region. Saudi Arabia and Egypt are the major countries which are famous for their date production. It is cultivated across the globe and it is estimated that about 3% of the world's cultivable area is occupied by date palm. From this cultivable area, global marketable date production has reached about 8 million metric tons (mmt) tons per year (Zamzam et al. 2018). Date plant is mainly cultivated for its nutritional value however, its medicinal properties is also available in scientific literature. These health properties are attributed to presence of bioactive compounds including antioxidants and phenolics which can be exploited in curing diseases like cancer, diabetes, microbial infections etc. (Khalid et al. 2017a). On nutrition side, date fruit is rich in carbohydrate (80%), protein (2–6%) and fiber (6.4–11.5%) while fats (0.2–0.4%) exists in minor amounts. Besides these date palm also contains traces of important minerals and vitamin (Al-Farsi* and Lee 2008). The presence of these nutrients in variable amounts to maintain the health are also supported by other scientists (Al-Shahib and Marshall 2003; Khalid et al. 2017b).

Date is mainly cultivated for its fruit, however, it also provides large amount of valuable products that are useful for many applications of daily life. It also provides raw material for many industrial products as well (Agoudjil et al. 2011). Date palm fronds are very famous agricultural by-products that have been used as feed stuff in animal diet (Chandrasekaran and Bahkali 2013). Date palm waste products includes tree trunk, leaves (midrib, leaflets, spines, sheath), stems, fruit stalk, spikelets, spath, fronds, seeds, date pits and coir etc. (Chao and Krueger 2007). Date pit mass is about 10–15% of the total mass of the date fruit. Date pit contains about 10% of crude oil and cellulose (Hamada et al. 2002). Date palm waste products could be utilized for the production of different valuable products like glucose and lactic acid.

Dates can be used for fermentation process to produce different biochemical products like alcohol, organic acids and glucose etc. (Naik et al. 2010). The conversion of cellulosic waste into useful products remained at priority in the last few decades. Conversion of cellulosic date palm waste into glucose and lactic acid is important for environment as such agricultural wastes utilize a lot of land resources and when the process of decomposition starts, air in the surrounding area stinks which is responsible for the dissemination of microbes. In this way such agricultural wastes brings a lot of pressure to the environment. However, this conversion of agricultural waste into useful products like energy, proteins and chemicals remained costly due to cost of cellulosic material, and cellulosic enzymes where technical problems also creates hindrance in cellulosic saccharification (Goyal et al. 2008).

Date palm contains huge amount of cellulose i.e. 45.3%, hemicellulose i.e. 29.12% and lignin amount is around 25.82%. Fermentation technology is the one which is used to produce valuable products from waste materials. Lignocellulosics are not easily saccharified by enzyme like cellulases to produce sugar until it is carried through different processes like physical, chemical and mechanical

pretreatments which remove undesired inhibitory complexes like lignin complex. Such complexes reduce the crystallinity whereas polymerization process of cellulose molecules helps the exposure of substrate for action of enzyme by increasing the surface area (Agbor et al. 2011).

Lactic acid is a very useable organic acid which is produced by the action of lactic acid producing bacteria through the process of fermentation. Wide range of application for lactic acid has been reported in the literature for various industries like pharmaceutical, leather, biodegradable plastic and food industry (Vijayakumar et al. 2008). Lactic acid is employed as pH regulator, acidulant and flavor enhancer in these industries. Lactic acid keep higher amount of chemical reactivity because of carboxylic and hydroxyl group so it is involved in variety of conversions into useful chemical components (Rogers et al. 2006). In recent decades, lactic acid fermentation drew higher attention due to increased demand for new biomaterial such as biodegradables and biocompatible polylactic acid (PLA) (Lim et al. 2008). PLA is used for making bioplastics which has wide applications. There are many economic and efficiency problems in lactic acid fermentation but scientist are focusing to discover new and effective nutritional resources using progressive fermentation techniques so they can achieve high substrate conversion along with high productivity. Use of cheap agricultural waste materials such as date palm cellulosic waste for the yield of important products like lactic acid through process of fermentation is attractive (de Oliveira et al. 2018).

There are various reports available relating to production of glucose and lactic acid from date palm and other agricultural waste products. This study represents the review of production of glucose and lactic acid from date palm by fermentation technology. Suitability of date palm as a cellulosic waste for the production of valuable products is also part of discussion.

8.2 Glucose

Glucose is a monosaccharide (simple sugar). Its chemical formula is $C_6H_{12}O_6$ and it contains aldehyde group. Commercial production of glucose involves the hydrolysis, enzymatic hydrolysis of complex carbohydrates or fermentation of biological wastes from different sources i.e. sugarcane bagasse, rice, wheat, corn husk (Raman 2010). Glucose has wide industrial applications. It is used for the synthesis of many other useful products e.g. vitamin C, citric acid, ethanol, amino acids, sorbitol, gluconic acid etc. (Pandey 2003).

8.2.1 Raw Material for the Production of Glucose

For the production of glucose in large quantities, it is important that raw material or substrate used must be cheap and easily available. Substrate must be clean with less

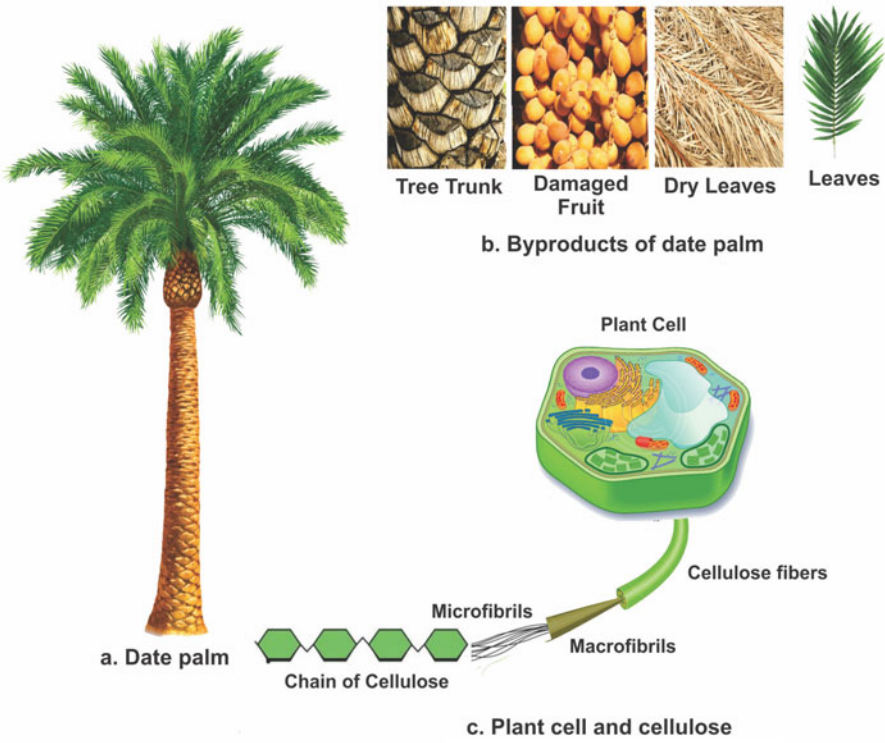

Fig. 8.1 Date palm and its cellulosic by-products with depiction of cellulose fibers

contaminant and it should give large production rate/yield without requirement of extra processing and in this way it reduces the cost of production. Availability is also important; substrate must be available all the year easily (Kimura et al. 2016). Date palm waste material provides the desired starting material for glucose production. During regular date palm collection activity a lot of material is produced which cannot be utilized by man as a food but this waste material can be used as part of feed for animals or it can be used as substrate for the production of useful products as it contains cellulose (Nancib et al. 2015). The details of such date palm waste is shown in Fig. 8.1.

8.2.2 Microorganisms Involved in the Production of Glucose

Cellulases are the main enzymes which are used as a source for the production of glucose from cellulosic material. These enzymes may be isolated from bacterial strain *Geobacillus stearothermophilus* Y-1. Separate saccharification and fermentation of lignocellulosic biomass is also recommended because of its much higher

temperature and substrate range (Himmel et al. 2010). Achieving complete cellulose hydrolysis is a difficult process, however, it can be achieved by utilizing three types of cellulases, i.e. glucosidase, exoglucanases and endoglucanases. Glucosidase can cleave glucose units from cello-oligosaccharides; exoglucanases has high efficiency to cleave cellobiosyl units from the end of cellulose whereas endoglucanases can cleave internal glucosidic bonds (Scully et al. 2015).

8.2.3 Enzymatic Hydrolysis of Date Palm Waste

Date fruits are collected and cleaned manually and thoroughly to remove dust or any other unnecessary substance present on it. Date pulp is removed usually and date seeds are separated manually by splitting the fruit. Regular tap water is added into the date fruit in a way that two parts of water is mixed with one part (by weight) of fruit. Heat (80 °C) is then applied to the mixture for 02 h with continuous stirring by a rod. Cellulosic material from date fruit is removed by the process of centrifugation at the rotor speed of 20,000 revolutions per minute (rpm) for 10 min. After completion of the process of centrifugation, supernatant is obtained which is important as it serves as carbon source in the experiment.

Hydrolysis of cellulosic substrate is required and it is performed at appropriate conditions. Culture growth for fermentation process is carried out by initial inoculation of bacterial cultures from primary agar plates into a 50 mL tube containing 5 mL of medium nutrient broth and incubation at 50 °C with continuous shaking at 200 rpm. This culture stock is enough for preparation of media (250 mL) in a flask containing 50 mL Bushnell Haas medium (BHM) media. Recipe of production medium (BHM) includes: $CaCl_2$ (0.02 g/L), KH_2PO_4 (1 g/L), $FeCl_3{\cdot}6H_2O$ (0.05 g/L), K_2HPO_4 (1 g/L), Tween 80 (0.2%) $MgSO_4{\cdot}7H_2O$ (0.2 g/L) and yeast extract (1.0 g/L). As source of carbon, leaves of date palm pre-treated with 2.0% alkaline solution can be used (Yadav et al. 2011).

For the fermentation process, the optimized conditions for production of cellulose enzyme can be achieved at pH 7.0 and mild temperature i.e. 45 °C with shaking at shaker incubator at 200 rpm to avoid clump formation in media. Centrifugation process helps in removal of insoluble cells and materials, when centrifugation is performed for 10 min at 10,000 rpm. The supernatant is now free of cells and it could be used as the enzyme source (Alrumman 2016).

For enzymatic hydrolysis, experimental conditions could be optimized and then date palm cellulosic waste (2%) can be mixed with cellulase enzyme (optimized units) in a 100-mL Erlenmeyer flask containing buffer (acetate buffer. pH 5.0). For inhibition of microbial growth in the media, sodium azide can be added. To carry out the enzymatic hydrolysis successfully, flasks can be incubated at 50 °C for 24 h at shaker incubator with continuous shaking at 200 rpm. During hydrolysis, complex sugars would be broken down into simple ones and after successful saccharification procedure, centrifugation is again performed at 400 rpm at 30 °C to remove the unhydrolyzed substrate. Hydrolyzed product should be well mixed with sodium

acetate (0.167%) and is supplemented with yeast extract (6%), $FeSO_4.7H_20$ (0.1%),), $NaPO_3$ (0.167%), $MnSO_4.H_2O$ (0.005), $MgSo_4.7H_20$ (0.1%) made to final volume 50 ml in a 100 ml flask. To prevent acidification, $CaCO_3$ is added because acidification will alter the pH of the medium that in turn affects the efficiency of whole process (Nancib et al. 2015).

8.3 Lactic Acid

The first organic acid which is discovered by a Swedish Chemist Carl Wilhem Scheele in 1780 by fermentation process in sour milk was lactic acid. Its chemical formula is $C_3H_6O_3$ and it is also known as 2-hydroxypropionic acid (Datta et al. 1995). History of lactic acid discovery and production is described in Table 8.1. It is an essential metabolic product and is available as metabolic intermediate in all living organisms. Naturally, it is also available in numerous fruits and vegetables and can also be produced in lab and industrial conditions through microbial fermentation. Lactic acid has different physical and chemical characteristics which are presented in Table 8.2. Lactic acid is produced commercially by the process of fermentation in many countries since its production is started in the United States in 1881 (Wang et al. 2015).

8.3.1 Potential Applications of Lactic Acid

Lactic acid is an important naturally occurring organic compound which has tremendous uses in our daily life. There are four main and important fields in which lactic acid is used i.e. cosmetics, food, chemical industry and pharmaceutics. The potential fields and products where lactic acid has tremendous applications are represented in Fig. 8.2.

Table 8.1 The history of lactic acid production

Sr #	Developments	Year	References
1	Discovered by Scheele	1780	Mohanty et al. (2016)
2	First industrial production	1881	Martinez et al. (2017)
3	First usage in leather and textile processes	1894	Saxena (2015)
4	Used for armoured tank coolant and glycerol substitute	1939-1945	Harding and Harrison (2016)
5	Synthetic production of lactic acid to make stearoyl-2-lactylates	Early 1960's	Carocho et al. (2015)

Table 8.2 Physical and chemical properties of lactic acid

Sr #	Property	Characteristics	References
1	Optical activity	Exists as L(+), D(−) and racemic mixture	Chafran et al. (2016)
2	Crystallization	Forms colourless monoclinic crystals when highly pure	Salas-Papayanopolos et al. (2017)
3	Color	None or yellowish	Carrasco et al. (2010)
4	Odour	None	Carrasco et al. (2010)
5	Consistency	Syrupy liquid	Carrasco et al. (2010)
6	Solubility/miscibility/ hygroscopicity	Soluble in all proportions with water; miscible with water, alcohol, glycerol, furfural; insoluble in chloroform, carbon disulphide; hygroscopic	Salas-Papayanopolos et al. (2017)
7	Volatility	Low	Salas-Papayanopolos et al. (2017)
8	Self-esterification	In solutions of >20%, forming a cyclic dimer or a linear polymer	Södergård and Stolt (2002)
9	Reactivity	Versatile, e.g. as organic acid or organic alcohol	Wasewar et al. (2004)
10	Physical	Formula weight: 90.1; melting point 18 °C, boiling point 122 °C at 15 mm Hg; density 1.2; specific gravity 1.248	Södergård and Stolt (2002)

Cosmetic industry is the main industry which utilizes lactic acid in a lot of formulations. It is an important ingredient in moisturizers for its water retaining properties and it can help in pH regulation and microbial growth inhibition. Lactic acid is a natural product and it is considered safer for its use in cosmetic industry. It has some additional properties like it can be used as skin lightening agent as it inhibits formation of tyrosinase (John et al. 2007).

Lactic acid is considered safe and it is approved by US FDA for its use as additive to food. Now lactic acid is widely used as flavoring agent, pH regulator, mineral fortification agent and inhibitor of microbial growth in food items. It is used in poultry and meat industry to improve the shelf life of products and to control the growth of pathogenic microbes. Lactic acid is used as flavoring agent on salads, pickles, backed food items, beverages etc. due to its acidulant properties. Lactic acid is also used in confectionaries for its ability to control pH and to add flavor in candies etc. (John et al. 2007).

There are a lot of chemical processes which involves use of lactic acid. Lactic acid and its derivatives are part of many chemical products. In chemical industry, lactic

Fig. 8.2 Potential applications of lactic acid

acid represents itself as an excellent solvent. It is safe as it is natural product and it has great solubility property so it is used as cleaning agent to remove polymer and resin. Lactic acid is used for the manufacturing process of different herbicides and pharmaceutical products (Martinez et al. 2013). It has excellent descaling properties so it is used for the production of cleaning products like toilet and bathroom cleaners. It is utilized as pH regulator, neutralizer, antimicrobial product and metal complexing agent. Product of lactic acid e.g. Ethyl lactate is produced naturally and it does not have any toxicological effects so it is used in the preparation of many anti-acne formulations (John et al. 2007; Datta and Henry 2006). Lactic acid is comprised of very reactive functional groups i.e. carboxylic group and a hydroxyl group. These functional groups are involved in variety of chemical conversions to produce different chemicals e.g. propylene oxide, acetaldehyde, acrylic acid and propanoic acid etc. Production of biodegradable plastic i.e. polylactic acid (PLA) is achieved because of lactic acid which is used for food packaging and for making of trash bags, containers and short shelf-life trays (John et al. 2007).

In the pharmaceutics, lactic acid has wide applications. Lactic acid is employed in production of n different varieties of important medical preparations including surgical items, tablets, artificial body parts etc. It is used in preparation of intravenous solutions (I.V.) which serves as electrolyte to replenish all the nutrients in human body e.g. Hartmann's solutions or Lactated Ringer's solution. Lactic acid is also used in dialysis solution for dialysis process (John et al. 2007).

Table 8.3 Quantitative comparison of production of lactic acid from different substrates

Sr.no.	Substrates	Production of lactic acid in g/L	References
Expensive substrates			
1.	Barley	162.00	Linko and Javanainen (1996)
2.	Wood	108.00	Moldes et al. (2001)
3.	Beet molasses	90.00	Kotzamanidis et al. (2002)
Cheap alternative substrates			
4.	Cellulose	24.00	Yáñez et al. (2003)
5.	Corn cob	24.00	Miura et al. (2004)
6.	Waste paper	23.10	Yáñez et al. (2005)

8.3.2 Raw Material for the Production of Lactic Acid

There are lots of reported investigations which attempt to search for the feasible and cheap cellulosic raw materials for the economical lactic acid production. Any cellulosic waste of date palm can be used as a substrate for fermentation process (Nancib et al. 2015). Recent reports regarding the use of different raw materials for the lactic acid production and their quantitative comparison is listed in Table 8.3.

Use of cellulosic waste as raw material/substrate is preferred for being utilized for the lactic acid production as it is feasible, cheap and available round the year. It mainly contains xylan, arabinan, galactan and lignin. There are several published reports regarding production of lactic acid by using different raw materials which contain cellulose. It is reported that pure cellulose can be utilized through the processes of enzymatic hydrolysis and fermentation for lactic acid yield (Venkatesh 1997; Yáñez et al. 2003). There are reports on the use of corn stover, waste paper, wheat bran, wood, wheat straw, alfalfa fiber and corn cob for the reasonable and economic lactic acid production. It is investigated that yield of lactic acid is enhanced by using cellulase and pectinase enzymes synergistically (Sreenath et al. 2001). Different lactic acid bacteria e.g. *L. pentosus* and *L. brevis* can be co-cultured for the lactic acid production. They were reportedly used for complete hydrolysis of substrate i.e. wheat straw into lactic acid (Garde et al. 2002). During pretreatment process of substrate, different compounds were produced e.g. furfural, 5-hydroxymethyl furfural, and acetic acid. These compounds have inhibitory action during fermentation process and they hinder hydrolysis of lignocellulosic material. To decrease inhibitory action of these compounds several methods are studied including physical and chemical treatment of hydrolysate. It is possible to minimize the inhibition of fermentation process if lactic acid bacteria are adapted directly on substrate based medium i.e. wood hydrolysate (Wee et al. 2004).

Commonly agricultural and industrial waste products e.g. whey and molasses are exploited to get valuable products such as lactic acid. Whey is very important as it contains fats, protein, minerals and lactose. Whey is obtained from dairy source. If whey substrate is supplemented with additional nitrogen source e.g. yeast extract, it will be completely hydrolyzed. It makes the production of lactic acid viable economically as it utilizes all the nutrients completely (Kulozik and Wilde

1999; Schepers et al. 2002). Whey protein hydrvolysate can be added into whey medium and hydrolyzed into lactic acid (Amrane and Prigent 1998; Kulozik and Wilde 1999).

During production process of sugar, molasses is produced which is a waste product and contains large quantities of sucrose. There are several reports available regarding the use of molasses in the process of lactic acid production (Shukla et al. 2004). For efficient fermentation process there is a requirement for supplementation of fermentation media with extra nutrients. Media can be supplemented by using yeast extract but it is expensive and it increases the production cost. Corn steep liquor can be used as alternatives of yeast extract in the media. Corn steep liquor is obtained from the corn. Most of the nitrogenous compounds which are part of corn steep liquor relies on the steeping process and it contains proteins, amino acids and peptides.

Media supplementation is advantageous and it is studied that wheat bran or rice bran contains fermentable sugars and their use brings lots of nutritious value to media (Wee et al. 2005). Other media supplementations are also reported to increase nutritious value of media including use of ram horn waste and vinification lees (Bustos et al. 2004). Electrodialyzed fermentation water contains nutrients which can be employed by gram positive lactic acid bacteria to produce lactic acid by enzymatic fermentation. There is published data regarding the use of electrodialyzed fermentation water which demonstrate that it enhances the efficiency of fermentation water (Wee et al. 2005).

8.3.3 Pretreatment of the Date Palm Waste

The cellulosic waste of date palm e.g. plant leaves, leaves stalks and fibers of date palm can be used as a cellulosic substrate. These waste date palm plant materials can be ground and pretreated by two methods. (1) alkaline pretreatment and (2) acid-steam pre-treatment. Alkaline pretreatment method involves treatment of substrate with 2 N NaOH at room temperature for at least 48 h while during acid steam pretreatment process substrate has reaction with H_2SO_4 (1%) at high temperature (120 °C) for 100 min. After pretreatment procedures, the plant waste must be cleaned. Tap water can be used to thoroughly wash the treated substrate. By using water, substrate gets neutralized. Substrate is then dried at 70 °C in hot oven. Dried material will then thoroughly ground for further processing by using Wiley Mill (Model 2 Thomas Co., USA). Grinding process will generate particle size $\leq$1 mm, which is preferable. Smaller sized particles have more surface area that increases its availability for enzyme action. Substrate may be comprised of major contents i.e. cellulose, hemicellulose and lignin (Bozoglu and Ray 2013).

8.3.4 Micro-organisms Used for the Production of Lactic Acid

Commonly used microorganisms for the lactic acid production from cellulosic waste material are known as lactic acid bacteria. Lactic acid bacteria are mainly gram positive bacteria which can produce lactic acid by the process of fermentation into the fermentation medium when they are cultured on substrate which contain cellulose or hemicellulose (Wee et al. 2006). On the basis of mechanism of action utilized to achieve the final product i.e. lactic acid, lactic acid bacteria are of two different types i.e. homofermentative and heterofermentative. Those bacteria which have ability to convert glucose into lactic acid exclusively are known as homofermentative lactic acid bacteria while the heterofermentative lactic acid bacteria are group of those bacteria which can produce lactic acid as well as some other products such as CO_2 and alcohol (ethanol) (Fig. 8.3) (Wee et al. 2006).

Only the homofermentative lactic acid bacteria belonging to genus *Lactobacillus* are preferably employed for the lactic acid production commercially because of their higher conversion, yield and rate of metabolism (Table 8.4).

There are reports for the use of batch culture of *L. helveticus* and *L. rhamnosus* to get lactic acid. Substrates used in the experiment were concentrated cheese whey and lactose (Berry et al. 1999; Schepers et al. 2002). There are investigations regarding the use of different substrates for the conversion into lactic acid by using *L. bulgaricus*. Kinetic models were also established for the lactic acid production (Burgos-Rubio et al. 2000). Investigations were conducted on studying the effects of variation of temperature and use of nitrogen from different sources on lactic acid production by *L. casei* strain (Hujanen et al. 2001; Roukas and Kotzekidou 1998). Investigations were made to study the kinetics of lactic acid production from lactose by using *L. plantarum* and *L. amylophilus* (Fu and Mathews 1999; Altaf et al. 2005). Lactic acid can also be produced by using *L. pentosus* from vine-trimming wastes (Bustos et al. 2004).

Investigations are made and it is obvious that some other strains other than the *lactobacilli* from lactobacillus genus can also be employed for lactic acid production e.g. *lactococci*. There are reports on the lactic acid production by cassava starch by using *Lactococcus lactis* with *Aspergillus awamori* together and attempts are made to establish the model of kinetics of lactic acid production by using strain *Lactococcus lactis* from substrate of whole wheat flour (Åkerberg et al. 1998).

However, after reviewing a lot of investigation reports, it is obvious that among the genus *Lactobacillus* the most commonly used species is *L. delbrueckii* which can be employed for lactic acid yield. It is reported for its use in many reports related to enzymatic fermentation and lactic acid production (Kotzamanidis et al. 2002; Alrumman 2016). Another species, a homofermentative lactic acid producer i.e. *L. casei* subsp. *rhamnosus* can also be employed for fermentation process. Bacterial stock cultures can be preserved in *Lactobacilli* MRS (Chang et al. 1999)

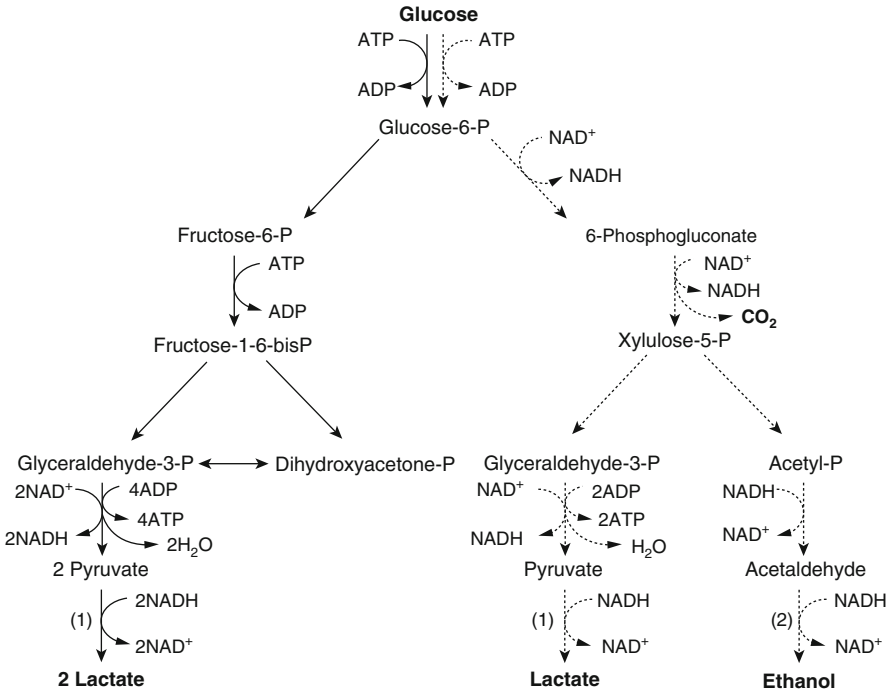

Fig. 8.3 Lactic acid synthesis via different metabolic pathways by using enzyme (*1*) lactate dehydrogenase (*2*) alcohol dehydrogenase. (Courtesy Wee et al. 2006)

Table 8.4 Biotechnological production of lactic acid by using different starting materials

Sr. #	Raw material	Organism	References
01	Cassava	*Lactobacillus amylovorus*	Xiaodong et al. (1997)
02	Molasses	*Lactobacillus delbrueckii*	Kotzamanidis et al. (2002)
03	Potato	*Lactobacillus amylovorus*	Yun et al. (2004)
04	Rice	*Lactobacillus sp.*	Yun et al. (2004)
05	Rye	*Lactobacillus paracasei*	Richter and Berthold (1998)
06	Barley	*Lactobacillus casei*	Linko and Javanainen (1996)
07	Cellulose	*Lactobacillus coryniformis*	Yáñez et al. (2003)
08	Wheat	*Lactobacillus lactis*	Hofvendahl and Hahn-Hägerdal (1997)
09	Waste paper	*Lactobacillus coryniformis*	Yáñez et al. (2005)
10	Wood	*Lactobacillus delbrueckii*	Moldes et al. (2001)
11	Whey	*Lactobacillus casei*	Büyükkileci and Harsa (2004)

media in −20 °C refrigerator with glycerol 25% (v/v) (Nancib et al. 2015). *L. rhamnosus* and *L. acidophilus* also have very good fermenting efficiency for the lactic acid yield from date cellulose as a substrate. However, certain modification are required in setting conditions in order to get the desired results (Alrumman 2016).

8.3.5 Inoculum Preparation

Bacterial culture preparation is an important step in the fermentation experiments. Bacterial culture can be prepared by transferring (1 mL) of the glycerol stock solution to the Erlenmeyer flask which already contains 100 mL MRS medium in liquid form. In order to achieve the exponential growth of bacteria, flask can be incubated for 12 h at 38 °C. For the process of fermentation, Fermenter contains production medium which can be inoculated with a portion of starter culture. Usually, 10% starter culture or inoculum in MRS medium is required for fermentation process (Bozoglu and Ray 2013).

8.3.6 Fermentation Conditions

Fermentation conditions for both batch and Fed –batch cultures are different. For batch culture, stirred tank fermenter is required which has a fermentation capacity of 1 L. For fermentation, 5 N NH_4OH solution is added in the medium which maintains the pH at 6. Cultures are usually incubated with continuous shaking at speed of 200 rpm at 38 °C.

For Fed-batch culture, stirred tank fermenter (LKB, Bromma, Sweden) is required which has fermentation capacity of 10 L. Fed-batch can be started with the addition of feeding medium. Feeding medium has date palm fruit juice (glucose 100 g/L) as main constituent which should be added continuously into fermenter at different feeding rates i.e. (18, 22, 33, 75 and 150 mL/h) (Nancib et al. 2015). Batch Fermentation can be done in 24 h after inoculation.

8.3.7 Fermentation Process in Lactic Acid Production

Lactic acid is produced from the cellulosic date palm waste by fermentation process in which substrate is biodegraded with the help of enzymes obtained from microorganisms into products i.e. lactic acid and ethanol. Lactic acid is produced from glucose with the help of process of glycolysis and it is available either in levorotatory (L) configuration or dextrorotatory configuration (D) (Fig. 8.4) (Martinez et al. 2013).

Fermentation process is completed in different stages that are carried out in bio reactor. Firstly, samples are prepared for fermentation process (Fig. 8.5).

Fermentation processes is employed to get valuable products e.g. lactic acid and it is of many types e.g. Batch, fed-batch, repeated batch and continuous fermentation process. Advantage of using batch and fed batch culture is that it gives higher lactic acid concentrations while other kind of fermentation processes e.g. continuous cultures gives higher productivity and fermentation processes can be continued for the longer time in this case (Oh et al. 2003).

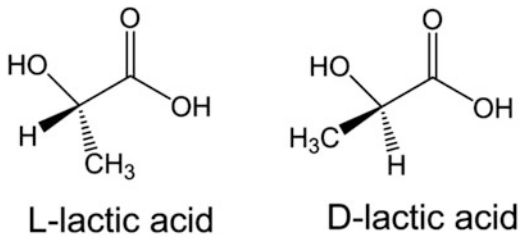

Fig. 8.4 Enantiomers of lactic acid

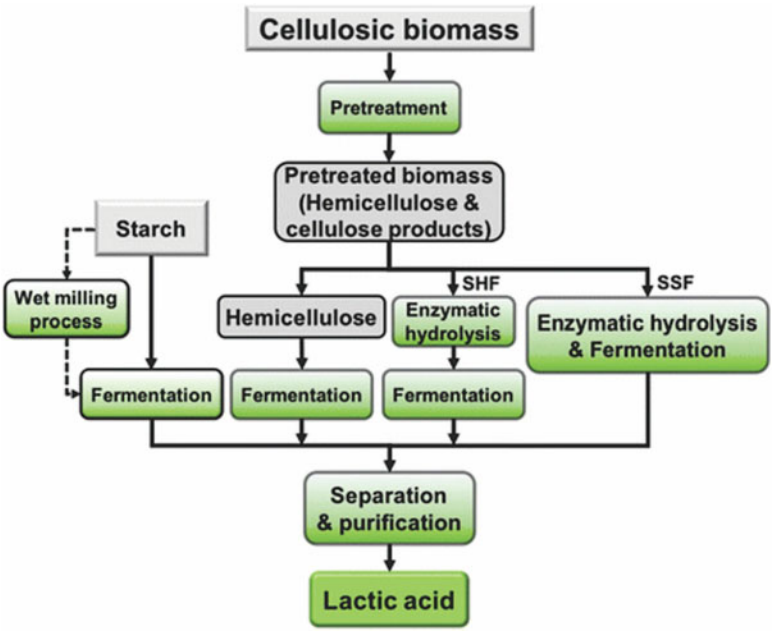

Fig. 8.5 Flow diagram for production of lactic acid

Two types of fermentation is mostly used for fermentation of date palm. One is batch fermentation whereas other one is fed batch fermentation.

8.3.7.1 Batch Fermentation

Batch fermentation is an excellent method that is used for commercial production of lactic acid at industrial level. However, it is observed that during batch fermentation, productivity and yield of lactic acid is decreased because of some issues. Presence of larger amount of initial substrate concentration cause inhibition of lactic acid production during batch fermentation process (Hujanen et al. 2001).

8.3.7.2 Fed Batch Culture

Another type of fermentation process is fed-batch culture. This type of culture is constantly provided with substrate quantity and in addition it must have some mechanism for the transfer of broth of fermentation. Fed batch fermenter has some advantages over the batch fermenter that it is more efficient and productivity of biomass is higher in this case as it is continuous and constantly provided with the substrate. It is beneficial as final production of desired product i.e. lactic acid in this case is enhanced as nutrients are provided continuously in the process (Ding and Tan 2006). (Roukas and Kotzekidou 1998; Nancib et al. 2015). Data regarding the use of fed batch process to get lactic acid biotechnologically is scanty.

High productivity of lactic acid can be achieved by using repeated batch and continuous fermentation processes. It was reported that lactic acid is produced at a rate of 6.4 g/L by using repeated batch fermentation system with very low production cost as compared to traditional fermentation processes as only 26% yeast extract is used in such experiments (Oh et al. 2003). Lactic acid production was also attempted by cell retention in cell recycle culture system using *L. rhamnosus.* Lactic acid was obtained 92 g/L in cell recycle bioreactor successfully.

8.3.7.3 Kinetics

The study of kinetics is important to design and control biological reactors. It is extremely important to obtain maximum yield. The Hanson and Tsao in 1972 studied about kinetics of batch and continuous fermentation on a glucose-yeast extract medium at controlled pH levels. They give a detail idea about maintenance of pH during fermentation process and suggested that any change in pH affects the fermentative process (Eş et al. 2018). Stieber and Gerhard in 1979 succeeded in developing a mathematical model for the continuous process that also carry dialysis for the production of ammonium lactate from deproteinized whey (Hofvendahl and Hahn–Hägerdal 2000). In structured models for fermentations the compositions of biomass remain constant during the processes which are responsible to limits their applicability to experiments with different operating conditions. It is suggested by Nielsen in 1991 that a theoretical study of lactic acid fermentation that was based on simple structured model of *Streptococcus cremoris*, in which the cell physiology is taken into consideration (Dien et al. 2003). These kinds of models and their modifications suggest better characterization of lactic acid fermentation in different situations such as non-growth conditions. These models can be modified into suitable way depending on other parameters of fermenting process and conditions. Nielsen also claimed that their model described experimental observations as *S. cremoris* carbohydrate adaptation and substrate preference on mixtures of galactose, glucose and lactose (Dien et al. 2003). These models suggest idea about setting the optimal condition for desired process.

8.3.8 Fermentation Equipment and Technology

The target of producing lactic acid by active microbial organisms usually follows basic established technology. The utilization of right technology and right microorganism is extremely important in carrying out effective fermentation.

In conventional fermentation methods the use of free microbial cells in batch or continuous fermenters is good choice but they usually require the separation of cells from the medium at the end of each process. Whereas, on the other hand, immobilization of cells on solid supports or their entrapment in a gel matrix can introduce diffusional resistances but it requires additional expenses which are associated with the immobilization step (Kailasapathy 2013).

Product inhibition in lactic acid fermentation processes is a serious problem and its effect on lactic acid productivity has been reported by various authors (Carroll and Somerville 2009). It has been studied that due to product inhibition, the benefits of continuous fermentation are not fully achieved in lactic acid processes. In case, if substrate concentrations in the reaction is very low then specific growth rate of the microorganism can be more dependent on the concentration of the product as compare to substrate (Keller and Gerhardt 1975). So, processes that remove the product from the fermentation medium could easily improve the efficiency of the system. In such cases, the dialysis culture system was utilized which can remove lactic acid from the medium and restore the efficiency of the system (Friedman and Gaden 1970). The system maintained a low lactate concentration after the log phase which causes the increase in specific microbial growth rates and lactic acid production. The study also claims that these results inveterate the product inhibition effect in lactic acid fermentation. It was suggested that relative to non-dialysis continuous or batch processes, the dialysis continuous fermentation endorsed the use of more concentrated substrate that causes increase in the efficiency of substrate conversion into product i.e. lactic acid (Friedman and Gaden 1970). Monitoring of process variables is very important in a fermentation process. A semi-on-line monitoring system is required to study the lactic acid fermentation process which can successfully measure the Glucose, lactic acid, protein, and optical density in a computer based controlled fermenter (Nielsen et al. 1990). The authors reported that the response of this system was very fast and reliable and that it can be used for the study of mathematical fermentation models that may help in designing appropriate protocols (Spann et al. 2018).

Lactic acid produced in fermentation processes needs to be recovered or extracted and then purified. However, few fermentation problems make its difficult. For example, to control the pH of the lactic acid fermentation, many chemicals such as ammonia, calcium carbonate, or sodium hydroxide are added into the medium. But, calcium carbonate causes precipitation of calcium lactate, which in turn hinders the production of a polymer-grade material from lactic acid. Whereas the continuous removal of lactic acid from the fermentation medium can be a solution to this problem, by avoiding the lowering of the pH of the broth. A system which is

known as reactive liquid-liquid extraction (RLLE) that uses amines can achieve this target (San-Martín et al. 1992). Extractive fermentation process can be used to get high yield of lactic acid by *Lactobacillus delbrueckii* utilising a tertiary amine (Alamine 336) and oleyl alcohol, at acidic pH was also studied by (Berry et al. 1999). In general the processes used for the recovery of lactic acid or lactate salts from the fermentation medium are a significant part of the total cost of the process (Nampoothiri et al. 2010).

8.4 Factors Affecting Yield of Glucose and Lactic Acid

8.4.1 Effect of Pretreatments on Wastes of Date Palm on Yield of Glucose and Lactic Acid

It is experimentally proved that if date palm cellulosic waste material is pretreated that will give rise to increased production of glucose as compared to starting material which is non-treated. It is because of activity of cellulose enzyme which is more active on pretreated substrate date palm waste as compared to non-treated substrate material. The low production of glucose may be due to the presence of high content of lignin in non-treated substrate which makes it difficult for the large enzymatic protein molecules to act on the structure of plant cell wall which is complex and packed. So, pre-treatment of the substrate which contains lignocellulose is necessary for activity of enzyme effectively. Pretreatment with alkaline solution lowers down the hardness and crystalline nature of cellulose in plant leaves as compared with leaf bases and it also increases the surface area available for the activity of cellulose enzyme. The maximum glucose production i.e. 19.57% (4.37 mg/mL) is achieved in date palm leaves then in leaf bases, 15.66% (3.47 mg/mL) respectively (Nancib et al. 2015). The alkaline pre-treatment process is very important factor that affects the production of valuable products because it can dissolve the complicated complexes of lignin and hemicelluloses and swelling of the cellulose (Hofvendahl and Hahn–Hägerdal 2000). Fermentation process requires efficient conversion of cellulosic content into lactic acid with high yield.

It is reported that lactic acid yield is also enhanced in case of pretreatment of cellulosic substrate. Pretreatment results in effective hydrolysis of substrate which leads to efficient and increased productivity. Pretreatment with alkali dissolves lignin and hemicellulose which are present in natural agricultural wastes. In this way surface contact with enzymes are increased which results in better yield of lactic acid. It is investigated that pretreatment of vinasse with alkali or microwave method results in enhancement of lactic acid production i.e. 71% (Wang et al. 2010). There are reports that hydrothermal pretreatment of substrate with addition of $CaCO_3$ prior to fermentation increases the enzyme action which leads to increased production of glucose and lactic acid (Bretón-Toral et al. 2017).

8.4.2 *Effect of Substrate Concentration on Yield of Glucose and Lactic Acid*

Rate of enzymatic reaction also depends on the substrate concentration. The increase in substrate concentration in the fermentation reaction can decreases sugar yield, which may be caused because of poor stirring in the reaction. Enzyme may be inhibited by by-products of the reaction and it decreased synergistic action between cellulases enzymes (Idris and Suzana 2006).

Initial high substrate concentration could result in low productivity of glucose and lactic acid. In case of fed-batch process it is reported that lactic acid production is enhanced if low substrate concentration is maintained (Michelz Beitel et al. 2017).

8.4.3 *Effect of Enzyme Concentration on Yield of Glucose and Lactic Acid*

Enzyme concentration is important in determining the rate of an enzymatic reaction. For the production of glucose, enzyme concentration should be 10–80 FPU/g of palm date waste substrates. It is investigated that production of glucose from date palm waste is also dependent on the enzyme-substrate ratio. Maximum sugar production i.e. 71.03% and 31.57 mg/mL is achieved at 30 FPU/g substrate However, no effect on increase in glucose production is seen with increasing the enzyme concentration. Cellulase enzyme also affects the process of sugar production by its role in the transglycosylation reactions (Hofvendahl and Hahn–Hägerdal 2000).

It is investigated that addition of increased concentration of enzymes i.e. cellulose and pectinase can result in enhanced productivity and yield of lactic acid from alfalfa but it also increases the cost of production as well. Increased concentration of enzymes results in increased release of sugar for lactic acid production (Sreenath et al. 2001).

8.4.4 *Effect of pH on Yield of Glucose and Lactic Acid*

For the maximum activity of an enzyme, optimum pH for a chemical reaction is necessary. Production of sugar from date palm waste substrate is maximum achieved at pH 5.0. The pH conditions of a chemical reaction affects significantly on the enzymatic hydrolysis carried out by cellulase enzyme. For the action of enzyme, at optimum pH value, enzyme-substrate complex is formed for hydrolysis reaction. The effect of pH on hydrolysis as well as on adsorption is same and that occurs at around acidic pH 4.8 (Hofvendahl and Hahn–Hägerdal 2000).

During fermentation process, pH maintenance is very important. At the start of fermentation process, pH is maintained but due to production of acid in the process,

pH is decreased. To control pH of the process, base titration method is used usually. Various studies have been carried out to check the effect of pH on the lactic acid yield. It is reported that maximum lactic acid production is achieved between pH 5–7 (Göksungur and Güvenç 1997). Lactic acid production yield was 79% at pH 6 which was optimum. When the pH is increased from 6 to 6.5, lactic acid production is decreased to 31.25%. Lactic acid production is also decreased to 16,55% when pH is lowered till 5.5 (Pailin 2010).

8.4.5 Effect of Temperature on Yield of Glucose and Lactic Acid

Optimum temperature is necessary for the maximum activity of any enzyme. If there is any increase or decrease in the temperature (which is ideal for activity of any enzyme) it may change and reduce the production of valuable products. For the production of glucose, complete enzymatic hydrolysis is achieved at 50 °C with glucose production of 71.23%. If temperature is increased from 50 to 55 °C, it results in decrease of glucose production i.e. 66.48% from 71.23% (Nancib et al. 2015).

Yield of lactic acid from cellulosic date palm waste is also affected by temperature which is one of the most important environmental factor. Investigations have been made to study the effect of temperature on the lactic acid yield and it is found that optimum temperature for the lactic acid production is 41–45 °C (Hofvendahl and Hahn–Hägerdal 2000). Lactic acid bacteria are mesophilic and their growth is optimum between 20 and 45 °C. Maximum lactic acid yield (79.8%) is reported at 40 °C (Pailin 2010). It is also observed that lactic acid productivity is decreases (35.30%) with a increase in temperature from 45 °C (Göksungur and Güvenç 1997).

8.5 Conclusion

The bioconversion of cellulosic wastes into valuable bio-products by fermentation processes is an important process that converts useless waste into very important products e.g. glucose and lactic acid. Development in the production of lactic acid from date palm is linked to the present general trends in biotechnology and its main factors include availability of date palm waste and progress in related scientific and technological areas. Date palm cellulosic waste represents an excellent starting material for its use in fermentation process for the production of glucose and lactic acid as it is cheap and it is easily available round the year. Production of glucose and lactic acid are significantly influenced by the substrate concentration, enzyme concentration, temperature and pH etc. Maximum glucose production is achieved at substrate concentration (4%) and enzyme concentration (30 FPU/g). Lactic acid production is affected by initial higher concentrations of substrate. Optimum temperature and pH for the production of glucose is 50 °C and 5 respectively, while

Lactic acid bacteria produce lactic acid at optimum condition of temperature 40 °C and pH 6. Cellulosic waste material i.e. date palm waste is an excellent alternative for the expensive raw material for industrial production of glucose and lactic acid through fermentation process. Such results are attractive and these suggest that date palm cellulosic material is an excellent and cheap source for glucose and lactic acid production enzymatically.

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