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Single-Step Bioconversion of Unhydrolyzed Cassava Starch in the Production of Bioethanol and Its Value-Added Products

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1. Introduction

The global economic recession that began in 2008 and continued into 2009 had a profound impact on world income (as measured by GDP) and energy use. Since then the price of the energy supply by conventional crude oil and natural gas production has been fluctuating for years which has resulted in the need to explore for other alternative energy sources. One of the fastest-growing alternative energy sources is bioethanol, a renewable energy which can reduce imported oil and refined gasoline, thus creates energy security and varies energy portfolio. Global biofuel demand is projected to grow 133% by 2020 (Kosmala, 2010). However, the biofuel supply is estimated deficit by more than 32 billion liters over the same period and the deficit is worse for ethanol than biodiesel. Ethanol may serve socially desirable goals but its production cost is still remained as an issue. Extensive research has been carried out to obtain low cost raw material, efficient fermentative enzyme and organism, and optimum operating conditions for fermentation process. In addition to that, researchers have been trying to capitalize certain features of the plant equipment and facilities to increase the throughput of ethanol and other high value by products as well as to apply suitable biorefinery for the product recovery. At the same time, effort has been made to reduce utilities costs in water usage, cooling or heating, and also consumables usage via minimizing the effluent production.

Aimed to provide an alternative means for ethanol production, this book chapter introduces a single-step or direct bioconversion production in a single reactor using starch fermenting or co-culture microbes. This process not only eliminates the use of enzymes to reduce the production cost but also yield added value by-products via co-culture of strains. Before further elaboration on this single-step fermentation, we will visit the conventional process, the substrate preparation and microbe used. By this way a clear picture of the differences between conventional process and the proposed single-step fermentation with the advantages and disadvantages of both processes will be discussed.

1.1 Conventional process of starch fermentation

Traditionally, production of ethanol from starch comprises of three general separate processes namely; liquefaction using *α*-amylase enzyme, which reduces the viscosity of the starch and fragments the starch into regularly sized chains, followed by saccharification whereby the starch is converted into sugar using glucoamylase enzyme. Each of the process operated at different temperature and pH optima with respect to the maximum enzyme reaction rate. The final process involved the fermentation of sugar into ethanol using yeast. The simplified flow of the process can be summarized as in Figure 1.



Fig. 1. Conventional Starch Fermentation.

1.2 Substrate and the preparation

In this chapter, starch as carbon source will be primarily discussed in the application for single-step or direct bioconversion. Starch is a polysaccharide and the most abundant class of organic material found in nature. Sources of starch that are normally used in the production of ethanol are derived from seeds or cereals such as corn, wheat, sorghum, barley, soy and oat. Other sources of starch can be from tuber or roots such as potato, yam or cassava. By using starch as substrate for bioethanol production has distinct advantages in terms of its economical pretreatment and transportation compared to other types of biomass. For example cassava or tapioca tuber that has received an enormous attention in the production of biofuel in particular bioethanol in East Asia region such as China, Thailand, Malaysia and Indonesia (Dai et al., 2005; Hu et al., 2004; Nguyen et al., 2006). Cassava is a perennial woody shrub, ranks second to sugarcane and is better than both maize and sorghum as an efficient carbohydrate producer under optimal growing conditions. It is also the most efficient producer under suboptimal conditions of uncertain rainfall, infertile soil and limited input encountered in the tropic (Fregene and Puonti-Kaerlas, 2002).

Before undergo conventional or traditional fermentation, starch regardless of its sources required to be hydrolyzed. Two types of hydrolysis usually applied are mineral acid hydrolysis and enzymatic hydrolysis. The mineral acid or acid-base involved in the hydrolysis can be of diluted or concentrated form. The dilute acid process at 1-5% concentration is conducted under high temperature and pressure and has fast reaction time in the range of seconds or minutes. The concentrated acid process on the other hand uses relatively mild temperatures and the reaction times are typically much longer as compared to those in the dilute acid hydrolysis. The biggest advantage of dilute acid processes is their fast reaction rate, which facilitates continuous processing for hydrolysis of both starch and cellulosic materials. Their prime disadvantage is the low sugar yield and this has opened up a new challenge to increase glucose yields higher than 70% (especially in cellulosic material) in an economically viable industrial process while maintaining high hydrolysis rate and minimizing glucose decomposition (Xiang et al., 2004; McConnell, 2008). The concentrated acid hydrolysis offers high sugar recovery efficiency, up to 90% of both hemicelluloses and cellulose sugars. Its drawback such as highly corrosive and volatility can be compensated by low temperatures and pressures employed allowed the use of relatively low cost materials such as fiberglass tanks and piping. Without acid recovery, large quantities of lime must be

used to neutralize the acid in the sugar solution. This neutralization forms large quantities of calcium sulfate, which requires disposal and creates additional expense. In addition to that, this type of hydrolysis has resulted in the production of unnatural compounds that have adverse effect on yeast fermentation (Tamalampudi et al., 2009).

Enzymatic hydrolysis of starch required at least two types of enzymes. This is due to that the starch or amylum comprises of two major components, namely amylose, a mainly linear polysaccharide consisting of α-1,4-linked ɒ-glucopyranose units and the highly branched amylopectin fraction that consists of α-1,4 and α-1,6-linked ɒ-glucopyranose units (Knox et al., 2004). Depending on type of plants, starch typically contains 20 to 25% amylose (van der Maarel et al., 2002) and 75 to 80% amylopectin (Knox et al., 2004). These two type linkage, α-1,4 and α-1,6-linked required an efficient starch hydrolysis agent or enzyme that can fraction α-1,4 and promote α-1,6 debranching activity. Since starch contains amylose and amylopectin, single or mono-culture cells are usually added during fermentation stage where starch has already been hydrolyzed to reducing sugar by hydrolysis agent such as acid-base or microbial enzymes in pretreatment and saccharification steps. The microbial enzyme of α -amylase cleaves α-1,4 bonds in amylose and amylopectin which leads to a reduction in viscosity of gelatinized starch in the liquefaction process. The process is the hydration of starch by heating the starch in aqueous suspension to give α-amylase an access to hydrolyze the starch. Dextrin and small amount of glucose and maltose are the end products. Exoamylases such as glucoamylase is then added during saccharafication which hydrolyses 1,4 and 1,6-alpha linkages in liquefied starch (van der Maarel et al., 2002). Enzyme has an advantage over acid-based hydrolysis. Amylolytic enzymes hydrolysis work at milder condition with the temperature lower 110°C (Cardona et al., 2010). However, enzyme is expensive especially cellulosic enzyme where it was reported the most expensive route accounted for approximately 22%-40% of total total cost (Wooley et al., 1999; Yang and Wyman, 200; Rakshit, 2006). Furthermore, fermentation of high concentration of starch to obtain high yield of ethanol is unfeasible due to reducing sugar inhibition on enzyme. This was shown in the work of Kolusheva and Marinova (2007) where the high reducing sugar produced from hydrolysis of high concentration not only inhibited the enzyme activity but also the fermenting yeast.

1.3 Microbes

Many investigators offer direct fermentation of starch using amylolytic microorganisms as an alternative to the conventional multistage that employs commercial amylases for liquefaction and saccharification, and followed by yeast fermentation. By using the amylolytic microorganism, ethanol production cost can be reduced via recycling some of the microorganism back to fermentors, thereby maintaining a high cell density, which facilitates rapid conversion of sugar into ethanol. However, there are very few types of amylolytic yeasts that are capable of efficiently hydrolyzing starch. Recombinant microbes and mix of amylolytic microorganism with glucose fermenting yeast in co-culture fermentation can resolve this setback. To further minimize contaminations and process handling cost, a single step or direct bioconversion of cassava or tapioca starch to bioethanol in one reactor (i.e. simultaneously saccharification and fermentation) in place of separate multistage processes will be focused upon in this chapter.

2. Single-step bioconversion

The idea of single-step bioconversion is to integrate all processes such as liquefaction, saccharafication and fermentation in one step and in one bioreactor. This alternative process

will reduce contamination and the operation cost resulted from multistage processes of ethanol production. This also will reduce energy consumption of the overall process. The one-step bioconversion can be done by using recombinant clone or by co-culture or consortium of microorganisms that able to degrade or digest starch into intermediate product such as oligosaccharides and reducing sugar by starch fermenting microorganism(s). Then, the fermentation followed by fermenting the intermediate products into ethanol by microbe in the mixture. This process not only eliminates the use of enzymes to reduce the production cost but also yield added value by-products via co-culture of microbes. Besides, it also has a distinctive advantage as far as biorefinery is concerned. Unlike enzymes which normally required purification before recycled and added into the process, microbial growth can replace cells that have been removed. Even if cell separation and recycle are required, the processes are simpler compared to the more complex and sophisticated enzyme separation and purification process such as enzyme membrane reactor (Iorio et al., 1993) using ultrafiltration, extraction in aqueous two-phase system (ATPS) of water-soluble polymers and salts and/or two different water soluble polymers (Minami and Kilikian, 1998; Bezerra *et al.*, 2006) and selective precipitation (Rao et al., 2007).

2.1 Carbon source

The cost of raw material is important and cannot be overlooked since it governs the total cost which represents more than 60% of total ethanol production cost (Ogbonna et al., 2001). Using cassava (*Manihot esculenta*) or tapioca starch as substrate in bioethanol production will reduce the production cost since cassava plants are abundant, cheap and can easily be planted. It is a good alternative at low production cost. It is a preferred substrate for bioethanol production especially in situation where water availability is limited. It tolerates drought and yields on relatively low fertility soil where the cultivation of other crops would be uneconomical especially on idle lands. Furthermore, the starch has a lower gelatinization temperature and offers a higher solubility for amylases in comparison to corn starch (Sanchez and Cardona, 2008).

Cassava is one of the richest fermentable substances and most popular choice of substrates for bioethanol production in the Asian region. The fresh roots of cassava contain 30% starch and 5% sugars while the dried roots contain about 80% fermentable substances. Its roots can yield up to an average 30-36 t/ha. Several other varieties of its non edible tubers maybe selected based on the cyanide content which can be categorized as sweet, bitter, non-bitter and very bitter cassava contains 40-130 ppm, 30-180 ppm, 80-412 ppm and 280-490 ppm, respectively (Food Safety Network, 2005). Since fresh cassava tubers cannot be kept long, it needs to be processed immediately or produced ethanol from dried root. Alternatively, its roots can be milled and dried to form pallet or flour. This will prolong its storage time and save storage spaces. Cassava tuber also can be kept in soil after maturating for several months unharvest without deteriorating. Besides the tuber, cassava waste also can be utilized for ethanol production due to its high content of cellulose, hemicelluloses and starch respectively at 24.99%, 6.67% and 30-50% (w/w) (Ferreira-Leitão et al., 2010).

One of the advantages of using starch such as cassava is that most of the plants can be intercropped with other plants such as cover crops (legume plant) or tree crops (such as cocoa plant and palm oil plant) which can simultaneously grow together (Aweto and Obe, 1993; Polthanee et al., 2007). Polthanee et al. (2007) discussed four possible ways of intercropping practice. They are i) mixed inter-cropping, simultaneous growing of two or more crop species; ii) row-intercropping where simultaneous growing of two or more crop

species in a well-defined row arrangement in an irregular arrangement; iii) strip inter-cropping, simultaneous growing of two or more crop species in strips wide enough to allow independent cultivation but, at the same time, sufficiently narrow to induce crop interactions and iv) Relay inter-cropping, planting one or more crops within an established crop in a way that the final stage of the first crop coincides with the initial development of the other crops. This will improve the land productivity and better land usage without the need to explore new land which might lead to deforestation. Figures 2 and 3 show the row-



Fig. 2. Soyabean in four-year-old oil palm (Ismail et al., 2009)



Fig. 3. Cassava intercrop with oil palm (Ismail et al., 2009)

intercropping of immature oil palm plantation intercrop with soyabean and cassava, respectively by Malaysian Palm Oil Berhad (MPOB), Malaysia.

2.2 Preparation method

The first step in the pre-separation process of starchy root or cassava tuber is to remove the adherent soil from roots by washing in order to prevent any problem later caused by the soil and sand. The process is followed by disintegration of cell structure to break down the size mechanically (i.e. milling) or thermally (i.e. boiling or steaming) or by combination of both processes. Slurry will be produced from the disintegration process which contains a mixture of pulp (cell walls), fruit juice and starch. This slurry can be cooked directly to gelatinized starch. When it is required, it can also be separated to produce flour by exploiting the difference in density using hydrocyclone and/or centrifuge separators as presented in Table 1.

Component	Density g/ml
Starch	1.55
Cell walls (fibers)	1.05
Water	1.00
Soil, sand	above 2

Table 1. Density of root components, water and soil (International Starch Institute, 2010).

For direct fermentation from starch to ethanol, there are two techniques normally employed in preparing starch medium which are non-cooking and low-temperature cooking fermentation. In non-cooking technique no heating is required however an aseptic chemical or method may be required to avoid contamination. Since it is uncooked, some aeration or agitation may also be required to avoid sedimentation of the starch particle. In low-cooking temperature fermentation, the medium is either semi or completely gelatinized first prior to inoculation of fermenting microorganism. Gelatinized starch forms a very viscous and complex fermentation media. It contains nutrients that required by microorganisms to grow and to produce different fermentation products. During fermentation, various physical, biochemical and physical reactions take place in the media. The nature and composition of the fermentation media will also affect the efficiency of the fermentation process. Many difficulties in designing and managing biological processes are due to the rheologically complicated behavior of fermentation media. Due to that, a pseudoplastic of a non-Newtonian behavior of starch solution is essential for cooked or gelatinized starch. This pseudoplastic property of gelatinized starch is important because it has suspending properties at low shear rates and its viscosity becomes sufficiently low when it is processed at higher rates of shear. Any fermentation medium which does not apply any viscosity reduction agent such as enzyme, its viscous nature combined with non-Newtonian flow will affect the mass heat transfer, dissolved oxygen homogeneity, mixing intensity, cell growth rate and eventually, the product accumulation state. Thus, it is imperative to minimize the viscosity to eliminate these problems. Starch slurry or flour concentration, temperature, agitation speed and cooking/gelatinization time are the major factors affecting media preparation. Optimization study of these conditions is useful prior to single-step fermentation of consortium or co-culture microorganisms. Table 2 gives the gelatinization temperature for different sources of starch. This information is helpful to prepare cooked or gelatinized starch for direct bioconversion at low temperature cooking.

Starch	Gelatinization Temperature Range (°C)
Potato	59-68[a,b,c]
Cassava/ tapioca	58.5-70[a,c]
Corn	62-80[a,b]
Paddy, rice and brown rice	58-79[a]
Sorghum	71-80[a]
Waxy corn	63-72[b]
Wheat	52-85[a,d]

[a]Turhan and Sağol (2004), [b] Whistler and Daniel (2006), [c]Tulyathan et al. (2006), [d] Sağol et al. (2006)

Table 2. Starch gelatinization temperature range

2.3 Direct starch fermentation without enzyme

In the industry whereby ethanol is produced from starch, temperature around 140˚C-180˚C is applied to cook the starch during hydrolysis using α-amylase prior to liquefaction. This high-temperature completely sterilizes harmful microorganisms and increases the efficiency of saccharification for high ethanol yield (Shigechi et al, 2004a, b). Consequently, this resulted in high energy consumption and added cost to amylolitic enzymes used in the process which ultimately increased the overall production cost. Several methods have been developed to reduce the energy consumption by applying milder liquefaction and/or saccharafication temperatures (Kolusheva and Marinova, 2007; Majovic et al., 2006; Montesinos and Navarro, 2000; Paolucci-Jeanjean et al, 2000) and also by exercising non-cooking fermentation (Shigechi et al., 2004b; Zhang et al, 2010). However, these types of fermentation usually required longer process time and sometimes may demand for additional volume of enzyme to maintain same productivity. The cost of enzyme will upset the total process cost.

To overcome this shortcoming, an alternative method of direct fermentation from starch may be employed to reduce the cost of enzyme. However, there are relatively few fermentation microorganisms that are capable of converting starch directly to ethanol since they do not produce starch-decomposing enzymes. One of the attempts to resolve this problem is by constructing recombinant microbes to coproduce α-amylase and glucoamylase with incorporating low temperature cooking of starch prior to fermentation by many research teams as shown in Table 3.

Several investigators reported that direct fermentation of starch using amylolytic microorganism offers a better alternative to the conventional multistage using commercial amylases for liquefaction and saccharification followed by fermentation with yeast (Abouzied and Reddy, 1986; Verma et al., 2000; Knox et al., 2004). By using this amylolytic microorganism in direct fermentation, the ethanol production cost can be reduced via recycling some of the microorganism back to fermentors, thereby maintaining a high cell density, which facilitates rapid conversion of substrate into ethanol. Furthermore by using cell exhibiting amylolytic activities, unlike using liquid enzyme that needs to be replenished or recycled unless if it is in immobilized system, the cell can multiply and reproduce with the enzymes. Fermentation using recombinant microbes, the starch medium can be prepared at low temperature cooking or uncook as a raw starch.

Another attempt of the direct fermentation without utilising any enzyme is by using co-culture microbes in the process. Instead of having enzyme separated and purified in different processes and subsequently to be used for hydrolysis in another separated process

Author	Transformed/ recombinant strain	Source of α-amylase	Source of glucoamylase	Type of starch	Starch concentration (g/L)	Max. ethanol concentration (g/L)
Altıntaş et al (2002)	*Saccharomyces cerevisiae*	*Bacillus subtilis*	*Aspergillus awamori*	Pure starch in 2.5 L fedbatch	40	29.7
Ülgen et al. (2002)	*Saccharomyces cerevisiae*	*Bacillus subtilis*	*Aspergillus awamori*	Starch	5- 80	47.5 (fed-batch culture) 15.6 (batch culture)
Knox et al. (2004)	*Saccharomyces cerevisiae*	*Lipomyces kononenkoae*	*Saccharomycopsis fibuligera*	Pure starch (Merck)	55	21
Shigechi et al. (2004a)	*Saccharomyces cerevisiae*	*Bacillus stearothermophilus*	*Rhizopus oryzae*	Corn starch cook at 80°C	50 90	18 30
Shigechi et al. (2004b)	*Saccharomyces cerevisiae*	*Streptococcus bovis*	*Rhizopus oryzae*	Raw corn starch in shake flask	200 g/L total sugar	61.8
Öner et al. (2005)	Respiration-Deficient Recombinant *S. cerevisiae*	*Bacillus subtilis*	*Aspergillus awamori*	Starch	5% starch + 0.4% (wt/vol) glucose	6.61
Khaw et al. (2007)	*S. cerevisiae* (non- and flocculent)	Not stated	Not stated	Raw corn starch	100	8
Kotaka et al. (2008)	*S. cerevisiae* (Sake yeast strain)	Not required	*Aspergillus oryzae Rhizopus oryzae*	Corn starch	50	18.5
He et al. (2009a)	*Zymomonas mobilis*	Not required	*Aspergillus awamori*	Raw Sweet potato	20.00 50.00	10.53 13.96

Table 3. Recombinant microbes for direct fermentation at low cooking temperature.

which contribute to higher expense, co-culture fermentation is worth to be considered as it might reduce the cost by omitting the unnecessary steps. While recombinant microorganism is constructed to provide the amylase activities, co-culture is simply selecting the microorganisms that naturally possess these amylase activities and combine them to work together to produce ethanol from starch.

Not many research works dedicated and related to co-culture fermentation for direct bioconversion of starch to ethanol. From just a few, same conclusions were drawn on the fermentation yield of the co-culture was better compared to mono-culture with improvement in the ethanol fermentation process. For instance study done by Verma et al. (2000), the co-culture fermentation of liquefied starch to ethanol can be carried out effectively with fermentation efficiency up to 93% compared to 78% and 85% when two-step bioconversion process using α-amylase and glucoamylase were applied to hydrolyze starch. Abuzied and Reddy (1986) observed that higher cell mass was produced in monoculture than in co-cultures which suggesting that substantially more carbon is used for cell production in monoculture, whereas in the co-culture most of the substrate carbon is utilized for ethanol production. Studies on co-culture microorganisms and systems are summarized in Table 4. The co-culture fermentation can either be simultaneous or subsequent mode for direct fermentation of low-temperature-cooking starch.

Strains for co-culture fermentation can also be obtained inexpensively from dry starter such as Ragi Tapai or Ragi Tape. This is similar to other oriental starter such as Ragi in Malaysia and Indonesia, Bubod in Philipine, Loog-pang in Thailand, Nurok in Korea, Koji in Japan, Banh Men in Vietnam, Chinese yeast in Taiwan and Hamei and Marcha in India. It is a dry-starter culture prepared from a mixture of rice flour and water or sugar cane juice/extract (Merican and Yeoh, 2004, Tamang et al., 2007). Clean rice flour is mixed with water or sugar cane juice to form thick paste. Sometime spices such as chilies, pepper, ginger and garlic which are assumed to carry desirable microorganism or may inhibit the development of undesirable microorganism are added to the paste (Basuki *et al*., 1996; Merican and Yeoh, 2004). Then the thick paste is shaped into hemispherical balls. Ragi from previous batch is inoculated either on thick paste before or after it is shaped into hemispherical balls. Hesseltine *et al.* (1988) reported that at least one yeast and one *Mucoraceous* mold (*Mucor, Rhizopus,* and *Amylomyces*) were present with one or two of cocci bacteria in every sample of the dry starter. Apart from the *Rhizopus sp.* which capable of producing lactic acid besides fermentable sugar and ethanol (Soccol *et al*., 1994), lactic acid bacteria are among the integral of the dry starter such as *Pediococcus pentosaceus, Lactobacillus curvatus, Lactobacillus plantarum* and *Lactobacillus brevis* (Sujaya et al., 2002; Tamang et al., 2007).

The traditional fermented food of tapai or tape' usually contains ethanol at concentration of 1.58% with high sugar content at concentration of 32.06%. Microaerophilic condition is required for the fermentation condition since fungi are unable to grow under anaerobic conditions and will result in unhydrolyzed starch. At lower temperature of 25˚C, higher alcohol content will be produced after 144 h whereas at temperature of 37˚C the tapai produces higher sugar content and becomes sweeter. (Merican and Yeoh, 2004). Tapai may contain up to 5% (v/v) of ethanol concentration (Basuki et al., 1996).

The benefit of using strains from dry starter such as ragi is that its application to produce fermented food such as tapai, is proven edible. Moreover, with addition of *S. cerevisiae* into the medium, the residue from ethanol recovery will contain yeast extract which can be processed as animal feed since it is edible and contain valuable nutrient that suitable for animal consumption as compared to fermentation using microbe such as *Escherichia coli.*

Direct fermentation has several advantages. First, to have multistage processes carried out in one reactor in which the glucose is produced during saccharification and simultaneously is fermented to ethanol can reduce contaminations and process handling cost. Second, direct fermentation reduces energy consumption. The starch medium can be prepared either at low-cooking temperature or by using the raw starch (uncooked starch). Even though some aseptic chemical or method may be required especially in raw starch fermentation, the cost incurred is still lower than the cost of energy consumption used in conventional fermentation.

Third, by applying direct fermentation, it is able to reduce inhibition of reducing sugar on fermenting yeast. In conventional fermentation, when starch is hydrolyzed using enzyme or mineral acid, certain amount of reducing sugar will be produced depending on the starch concentration. High level of reducing sugar in the fermentation medium (above 25% (w/v)) exerts osmotic pressure to the cells and limits their fermenting activity. This value may vary with different fermenting yeasts. However in direct fermentation, the osmotic pressure can be reduced by simultaneous converting starch to sugar and sugar to ethanol. This is particularly true in the recombinant clone which can co-express both the degrading enzymes. In the case of co-culture fermentation, the suitable inoculation time for the second microorganism needs to be determined. This is to avoid high yield of reducing sugar in

Author	1st microorganism	2nd microorganism	Co-culture System/ Fermentation procedure	Type of starch and concentration	Maximum ethanol concentration
Hyun and Zeikus (1985)	*Clostridium thermohydrosulfuricum*	*Clostridium thermosulfurogenes*	14 L microfermentor	5 % Starch with TYE medium (contains vitamin solution, ammonium chloride, magnesium chloride and trace mineral)	>120 mM
Abouzied and Reddy (1986)	*Aspergillus niger*	*Saccharomyces cerevisiae*	Simultaneous co-culture (500 mL shake flask)	Potato starch recovered from waste water of a potato chip manufacturing plant. (5% (w/v) starch)	5%(w/v)
Abouzied and Reddy (1987)	*Saccharomycopsis fibuligera*	*Saccharomyces cerevisiae*	Co-Culture fermentation (500 ml shake flask)	Similar to Abouzied and Ready (1986)	5%(w/v)
Reddy and Basappa (1996)	*Endomycopsis fibuligera* NRRL 76	*Zymomonas mobilis* ZM4	Shake flask	22.5% (w/v) cassava starch	10.5% (v/v)
Jeon et al. (2007)	*Aspergillus niger*	*Saccharomyces cerevisiae*	Separate fermentation in serial bioreactors (1.5 – 3.0 L).	Potato starch 55 g/L/day	22 g/L/day
He et al. (2009b)	*Paenibacillus sp.*	*Zymomonas Mobilis*	Simultaneously vs. subsequently co-cultured at 48 h of fermentation time. (100 mL shake flask)	50.0 g/L raw sweet potato starch (5% w/v starch)	6.6 g/L (120 h fermentation, pH 6.0) From subsequent co-culture
Yuwa-Amornpitak (2010)	*Rhizopus sp.*	*Saccharomyces cerevisiae*	Subsequently co-culture at 24, 48 and 72 h.	6%	14.36 g/L at 24 h subsequent co-culture

Table 4. The co-culture microorganisms in direct fermentations without enzyme addition.

medium before the second inoculation. When reducing sugar inhibition is avoided, fermentation of high starch concentration can be achieved for high ethanol yield and thus it reduces the water use. Subsequently this will reduce energy consumption in ethanol-water separation.

Direct fermentation is not limited to starch as it had been reported that different sugars from lignocellulosic hydrolysates such as mixture of glucose and pentose sugar for instance; xylose (Murray and Asther, 1984; Kordowska and Targonski, 2001; Qian et al., 2006) were fermented by glucose and pentose-fermenting microorganisms.

2.4 Ethanol by-products

During the fermentation process, several by-products are produced together with ethanol. In co-culture fermentation which involves different strains, different side-products besides ethanol are produced. The list of by-products and their applications in industry are listed in Table 5.

By-product name	Application
L-Lactic Acid (LA)	**Food and baverage** (acidulent, pH regulator, emulsifier, flavor enhancer & preservative), **cosmetics** (skin rejuvenating agent, moisturizer & exfoliant), **industrial** (degreasing agent, solvent & complexant), **pharmaceuticals** (sanitizer, drug delivery & administration, intermediate for optical active drug), **animal feed** (feed additive for farmed animals to reduce intestinal infection) (Hyflux ltd., 2008)
Polylactic acid (PLA)	**Food packaging** (disposable service ware, food containers & cartons), **medica**l (suture threads, bone fixation & drug delivery), **non-woven** (diapers, specialty wipes & geotextiles), **fiberfill** (mattresses, pillows & comforters), **woven fibers** (apparel, socks, decorative fabrics), **specialist applications** (automotive heads & door liners) (Hyflux ltd., 2008)
Acetic acid (ethanoic acid)	Vinegar, chemical reagent, industrial chemical, food industry (food additive code E260 as acidity regulator)
Acetoin	Food flavoring and fragrance
Carbon dioxide	Carbonated water, dry ice, fire extinguisher, photosynthesis
Glycerol	Cosmetic and toiletries, paint and varnishes, automotive, food and beverages, tobacco, pharmaceutical, paper and printing, leather and textile industries or as a feedstock for the production of various chemicals (Pagliaro and Rossi, 2010; Wang *et al*., 2001).

Table 5. Ethanol by-products and their applications.

The production of by-products somehow reduces the ethanol yield due to the competition from other metabolic conversions. The inhibition of lactic and acetic acids on yeast for ethanol production in corn mash was examined when both the acids synergistically reduced the rates of ethanol synthesis and the final quantities of ethanol produced by the yeast (Graves et al., 2007). The inhibitory effects of the acids were more apparent at elevated temperatures. So, a reduction in the formation of by-products is needed to achieve higher ethanol yield.

Alternatively, a fermentation process should not be only aimed for higher conversions of raw materials and ethanol productivity, but should rather take the advantage of the

byproducts released during the transformation of feed stocks and convert them into valuable co-products. To reduce the inhibition effect, in-situ separation can be applied to separate the valuable co-product from the process. In this way, economical and environmental criteria can be met. However, depending on the objective and the economic analysis of the particular ethanol plant, the by-products may either generate extra revenue for the plant or just an inhibition the conversion process.

Among the ethanol byproducts, glycerol and lactic acid are used extensively by industries and can increase the production profit. These fermentative products have attracted interest owing to their prospect environmental friendliness and of using renewable resources instead of petrochemical. These byproducts have broad applications which can generate lucrative profit for the processes i.e. lactic acid. The global market for lactic acid is predicted to reach 328.9 thousand tonnes by 2015 (Plastics Today, 2011). The world consumption of lactic acid is stimulated by its applications in key industries such as cosmetics, biodegradable plastics and food additives. Lactic acid is used as a pH balancer in shampoos and soaps, and other alpha hydroxy acid applications, was expected to elevate the consumption in the market. Polymer lactic acid (PLA) for biodegradable plastics has properties similar to petroleum derived plastic and was expected to increase the demand for environmental friendly packaging. Food additives will continue to be the largest application area for lactic acid globally, but biodegradable plastics represent the fastest growing end-use application.

Glycerol or glycerin is a simple alcohol produced by *S. cerevisiae* during glucose fermentation to ethanol to maintain the redox balance. The global market for glycerin is forecasted to reach 4.4 billion pounds by 2015 (PRWeb, 2010). The increased demand for glycerin was reported to originate from various end-use area such as oral care, personal care, pharmaceutical and food and beverage. In fact, there are over 1,500 end-uses for the chemical. In most products, glycerin is used in very small portions with exception in a few end-uses which require a significant amount of glycerin in their formulation. Glycerin is also used in several novel applications such as propylene glycol, syngas and epichlorohydrin and it is expected to improve the glycerin demand.

Glycerol also can be potentially used as fuel additives for diesel and biodiesel formulation that assist to a decreasing in particles, hydrocarbons, carbon monoxide and unregulated aldehydes emissions. It can also act as cold flow improvers and viscosity reducer for use in biodiesel and antiknock additives for gasoline (Rahmat et al., 2010). Since glycerol is also produced in the fermentation broth, it is attractive as an entrainer to reduce the use of fresh entrainer in extractive distillation of azeotrope mixture of ethanol-water system.

3. Conclusion

Cassava is an attractive alternative as the carbon substrate for ethanol production especially where water availability is limited as it can tolerate drought and yields on relatively low fertility soil. The conventional method for the ethanol production involves liquefaction, saccharification and fermentation steps which are time consuming and cost ineffective, in view of the use of enzymes. Therefore, direct fermentation with integrated steps that incorporating recombinant or co-culture strains in a single reactor offers a more convenient method for the production of ethanol and its high value by products. By co-culture fermentation, high starch concentration can be used to reduce water usage in fermentation and subsequently in ethanol-water separation system. Furthermore, the fermentation

medium can be prepared at lower temperature or raw starch can be used for direct fermentation to reduce the energy consumption. From the safety, economic and production process aspects, single-step bioconversion using co-culture microorganisms is a better alternative as far as production of ethanol and its by products from starch is concerned. The ethanol by-products such as lactic acid and glycerol can be value added co-products to generate extra revenue.

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Recent studies have shown strong evidence of human activity impact on the climate of the planet. Higher temperatures and intensification of extreme weather events such as hurricanes are among the consequences. This scenario opens up several possibilities for what is now called "green" or low carbon economy. We are talking about creating new businesses and industries geared to develop products and services with low consumption of natural resources and reduced greenhouse gases emission. Within this category of business, biofuels is a highlight and the central theme of this book. The first section presents some research results for first generation ethanol production from starch and sugar raw materials. Chapters in the second section present results on some efforts around the world to develop an efficient technology for producing second-generation ethanol from different types of lignocellulosic materials. While these production technologies are being developed, different uses for ethanol could also be studied. The chapter in the third section points to the use of hydrogen in fuel cells, where this hydrogen could be produced from ethanol.

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