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Fermentation of Sweet Sorghum into Added Value Biopolymer of Polyhydroxyalkanoates (PHAs)

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1. Introduction

The world today cannot deny the prospect of 'peak oil', higher prices and depletion of petrochemical feed stocks. At the same time, there is an environmental concern of the widely used synthesized plastic from petroleum industry because of its non- degradable nature. Plastics, solid wastes, and pollutants of all kinds not only accumulate as carbon footprint, but also pose a threat to the global warming issues. If they are disposed by open air burning. The United Nation's International Center for Science and High Technology (ICS) thus launched a program focusing on the development of degradable biopolymeric materials and plastic waste disposal in developing countries. Recycling, reuse, incineration, composting, and new technology for development of environmental friendly degradable plastics are making a highly efficient contribution to the mitigation of environmental problems. In addition, concerned researchers and the industrial sector have seen the importance of producing bio-based plastics and biopolymers from agricultural crops based on locally available biomass resources.

2. Polyhydroxyalkanoates (PHAs)

2.1 Definition

Polyhydroxyalkanoates (PHAs) or polyhydroxyalcanoic acid, the main kind of biodegradable and biocompatible biopolymer, are classified as linear polyesters. Typically, PHAs can be produced in nature by several microorganisms such as yeast, fungi and mostly by various bacterial strains. The bacteria could accumulate as intracellular carbon and energy storage under imbalanced growth conditions such as excess in carbon sources but limiting in nutrients of nitrogen, phosphorous and potassium etc (Yu et al., 2005). In addition, fermentation processes from renewable resources such as sugar, starch and even lipid based materials are also affected on the production of PHAs (Kaewkannetra et al, 2008).

2.2 Classification

Since 1925, PHAs were the first biomaterial, discovered by the French microbiologist, M. Lemoigne, accumulated as intracellular substance in a bacterial strain of *Bacillus*

megaterium (Lemoigne, 1926; Anderson & Dawes, 1990; Jacquel et al., 2008). Nowadays, biopolymers have been synthesised or are formed in nature during the growth curves of all microorganisms. Depending on the evolution of the synthesis process, different classifications of the different biopolymers have been proposed. In this case, they are classified into the following 4 categories. However, it should be noted that only 3 categories (from 2.2.1 to 2.2.3) are obtained from renewable resources and the remainder is obtained by chemical synthesis.

- Biopolymers derived from biomass such as from agro-resources (e.g., starch, lingo-cellulosic materials, protein and lipids)
- Biopolymers obtained by microbial production as the PHAs
- Biopolymers conventionally and chemically synthesised and the monomers are obtained from agro-resources, e.g., the poly-lactic acids or PLAs
- Biopolymers whose monomers and polymers are obtained conventionally by chemical synthesis such as aliphatic and aromatic hydrocarbon.

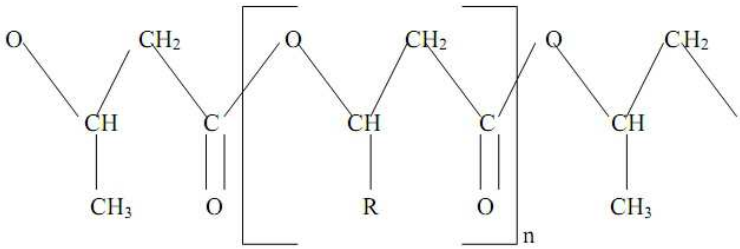
2.3 PHAs structures

PHAs are produced by the bacteria to store carbon and energy reserves (Keshavarz, Roy, 2010). Previous works stated that an intracellular accumulation of PHAs improves the survival of general bacteria under environmental stress conditions (Kadouri et al., 2005; Zhao et al., 2007). Various microorganisms are produced in different properties of biopolymer depending on the types of microorganisms and carbon sources used. More than 150 different monomers can be combined within this family to give materials with extremely different properties (Chen & Wu, 2005).

PHAs structure is composed of a monomer of 3-hydroxyalkanoic (HA) acid. The general formulae of the monomer unit is -[O-CH(R)-CH_2-CO]- as seen in Fig.1 (Lee, 1995). The (R)-3HA monomer units are all in the R configuration due to sterospecificity of polymerizing enzyme PHAs synthase. According to the size of the alkyl substituent (R), the mechanical properties of PHAs can typically be divided into three groups by number of carbon atoms in their side chain. Short chain length (*scl*) PHAs are composed of 3-5 carbon atoms, while medium chain length (*mcl*) PHAs consist of 6-15 carbon atoms and long chain length (*lcl*) ones comprise 15 and above carbon atoms. The structure of PHAs depends on supplying carbon sources and microbial types. The composition of PHAs depends on the microorganisms and nature of the carbon sources allowing the formulation of new polymers with different physicochemical properties, i.e., short or mid-chain and long-chain fatty acids.

The most common PHAs' forms found in microorganism cells are polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV). However, the majority of the published research on PHAs rather than PHB has concentrated on two bacterial strains, i.e., *Alcaligenes eutrophus* and *A. latus* (Slater, et al, 1988; Kim, et al, 1994; Yamane, et al, 1996, Shi, et al, 1997; Wang & Lee, 1997; Tsuge, et al, 1999; Yu, et al, 2005; Yezza, et al, 2007).

These monomers are biodegradeable and used for the production of bioplastics. PHAs produced from the process are usually composed of 100-30,000 monomers and exist in a short chain. Naturally, the properties of PHAs are similar to thermoplastics that are obtained from petrochemical industry such as polypropylene (PP) and polyethylene (PE) as shown in Table 1 (Evan and Sikdar, 1990).



n varies from 600 to 35000

R= hydrogen	Poly(3-hydroxypropionate)
R=methyl	Poly(3-Hydroxybutyrate)
R=ethyl	Poly(3-hydroxyvalerate)
R=propyl	Poly(3-hydroxyhexanoate)
R=pentyl	Poly(3-hydroxyoctanoate)
R=nonyl	Poly(3-hydroxydodecanoate)

Fig. 1. PHAs structure (Lee, 1995)

Property	PHB	Polypropylene
Melting point (T_m), ^oC	175	176
Crystallinity, %	80	70
Molecular weight, Daltons	5 x10^5	20 x10^5
Glass transition (T_g), ^oC	15	-10
Density, g/cm3	1.25	0.905
Tensile strength, Mpa	40	38

(Evan and Sikder, 1990)

Table 1. Properties of polyhydroxybutyrate (PHB) and polypropylene (PP)

They can be either thermoplastic or elastomeric materials with melting points ranging from 40 to 180°C and the percentage of crystallinity (up to 70-80) is similar (Blumm & Owen, 1995). Thus, they can tolerate organic solvents and even lipid and oil. The mechanical and biocompatibility of PHAs can also be easily changed by blending, forming, modifying the surface or combining PHAs with other polymers, enzymes and inorganic materials, making it possible for a wider range of applications such as bottles, bags and wrap films and even in pharmaceutical and medical areas such as drug coating and drug delivery (Steinbuchel & Fuchtenbusch, 1998, Jacquel, et al., 2008).

3. Sweet sorghum

Sweet sorghum (*Sorghum bicolor* L. Moench) is a 3 annual crop and a 4 carbons (C4) containing plant with high biomass productivity. As a high photosynthetic efficient crop, it does not only produce grains, but also yields large amount of sugars in the stems. Typically, it mainly consists of sucrose (up to 55% of dry weight biomass), fructose and

glucose which are ideal for preparing fermentation media (Kaewkannetra et al., 2008; Laopaiboon et al., 2009; Gao et al., 2010).

Fig. 2. Sweet sorghum (strain Khon Kean University 40 : KKU 40) from agricultural plantation area of Khon Kaen University, Khon Kaen, Thailand

In the age of petroleum crisis, sweet sorghum (see Fig. 2), especially grains and stems, has already proven advantages over other crops such as sugar cane, cassava, palm, in terms of residue or agricultural wastewater. These crops are feedstock for producing bio-fuels by squeezing the juice and then fermenting into bio-ethanol (Laopaiboon et al (2009), bio-hydrogen (Antonopoulou, et al., 2008). They have recently been used as carbon sources for algae during bio-diesel production (Gao et al., 2010) owing to their sustainability, processing efficiency and superior byproducts, such as bagasse, which serves as high-quality cattle feed. The crops can be cultivated under dry, non-arable land, or warm conditions and are inexpensive to grow. Thus, they are more typically grown for forage, silage, and sugar production than many other crops. The crops are also competitive on the cost aspect of ethanol production.

Presently, the cost of PHAs production is a main limiting factor for extensive production in an industrial scale. The carbon source contributes most significantly (up to 50%) to the overall cost in PHAs production. An attempt to produce PHAs by applying cheap carbon sources could reduce the total cost of the production. Previously, production of PHAs by using cheap carbon sources such as molasses, maple sap and cassava were studied (Grothe et al 1999; Yezza, et al., 2007; Koller et al., 2008). However, sweet sorghum had not been explored so far as a carbon source for PHAs production until in 2008 (Kaewkannetra, et al., 2008). Currently, it is planted in more than 90 countries around the world including Thailand, which is one of the agro-industrial countries in the Southeast Asia with plentiful cheap carbon sources. A number of crops such as cassava, sugar cane, corn, potato, can be extensively used as raw materials to produce degradable polymer and biomaterials via fermentation process by microorganisms. Sweet sorghum, among all the crops mentioned, is of high potentiality to help mitigate environment problems if it can be produced as biodegradable plastics.

3.1 Classification

Sorghum can be classified into 5 categories: grain, grass, broom, pop and sorgo or sweet sorghum. Typically, it is used for animal feed and as sweeteners, primarily in the form of sweet sorghum syrup similar to sugar cane. Recently researchers found rich sugar content in the stem of sweet sorghum, commonly expressed with juice brix degree, but the relation between sugar content and brix degree has not been very clear due to different varieties during their growth. The results revealed a scientific basis for the arrangement in their sowing dates.

3.2 Characterization

Since the duration of sweet sorghum for maturation is approximately 3-5 months, 2-3 crops could be harvested annually in Thailand. Therefore, the production yields of sugar would be double or triple. We can see the possibility of reducing the world energy crisis if sweet sorghum can be converted to energy efficiently. Typically, after harvest, the leaves of the fresh crop are stripped and the stems are squeezed in a roller mill (Fig.3) to obtain sweet sorghum juice. It should be noted that the stems are stored at 4^{o} C while the juice is kept at -20^{o} C prior to use.

Fig. 3. Stems of sweet sorghum are squeezed by a roller mill for preparing sweet sorghum juice (SSJ)

Table 1 shows variations of types and sugar content in sweet sorghum collected from several areas, which depend on the strain, planting seasons, areal conditions, etc. For examples, the total sugar, analyzed by phenol-sulfuric method, was 207.43 g/l. Analysis by means of High Performance Liquid Chromatography (HPLC) has proved contents of 175.97 g/l sucrose, 12.32 g/l glucose, 5.75 g/l fructose in sweet sorghum juice. Normally, the initial sugar concentration of 30-40 g/l is suitable and sufficient for using as carbon source for microbial growth. Therefore, the juice for 1 liter can be diluted as fermentation medium for 4-5 liters (Kaewkannetra et al., 2008).

cultivation area	Harvest month	Sugar types (g/l)			Total Sugar (g/l)	Sources
		sucrose	glucose	fructose		
Thailand	July	175.97	12.32	5.75	194.04	Kaewkannetra et al (2008)
Thailand	*	124.1	20.9	16.8	161.8	Laopaiboon et al (2009)
USA	October	143.3	39.3	61.0	242.6	Liang et al (2010)
Hungary	September	75.1	25.0	18.1	118.2	Sipos et al (2009)
Greece	November	211.9	20.1	-	232.0	Mamma et al (1995)

*Not specific

Table 1. Type and sugar contents in sweet sorghum collected from several areas

4. Fermentation

4.1 Definition

Fermentation implies an intracellular biochemistry process. It is believed to have been the primary means of energy production in earlier organisms before oxygen was at high concentration in the atmosphere. Therefore, it would generate Adenosine Triphosphate (ATP) of the energy molecule of cells even in the presence of oxygen and is synthesized mainly in mitochondria and chloroplasts. In other words, it means the anaerobic enzymatic conversion of organic compounds to simple compounds producing energy in the form of ATP.

Fig. 4. Fermentation of sweet sorghum in fermentor (Kaewkannetra et al., 2008)

Fermentation occurs naturally in various microorganisms such as bacteria, yeasts, fungi and in mammalian muscle. Yeasts were discovered to have connection with fermentation as observed by Louis Pasteur and originally defined as *respiration without air*. However, it does not have to always occur in anaerobic condition. For example, starch when fermented under

anaerobic conditions gives alcohols or acids. Yeasts, in ethanol fermentation, use an anaerobic respiration primarily when oxygen is not present in sufficient quantity for normal cellular respiration. However, in large-scale fermentation, the breakdown and re-assembly of biochemicals for industry often carry on in aerobic growth conditions.

4.2 Types of fermentation

Normally, fermentation processes can be classified depending on the objective of study. For example, in terms of products fermentation is divided into 4 types, namely, microbial cell, microbial enzyme, microbial metabolite and transformation process. If considering due to its contaminating conditions, it will be classified into 3 types: septic, semi-septic and aseptic fermentation. However, in general, the fermentation processed are classified into 3 types as follows.

4.2.1 Batch fermentation

Batch fermentation means the cultivation of microorganisms, where the sterile growth medium in desired volume is inoculated with the microorganisms into the bioreactor and no additional growth medium is added during the fermentation. The product will be harvested at the end of the process. Typically, PHA's production is performed using batch fermentation because of low cost for investment and no special control. In addition, sterilization of the feed stock is easier than other fermentation processes, and operation is flexible.

Previous studies have investigated batch fermentation of both carbon sources and microorganisms. The microorganisms will accumulate PHAs after the cell reached the maximum growth coupled with the depletion of nitrogen or phosphorus (Braunegg et al 1998; Wang & Bakken, 1998; Chien et al, 2007).

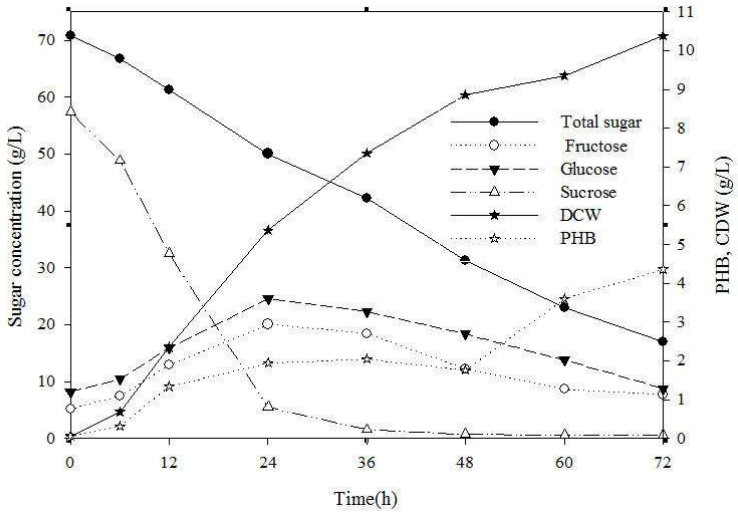


Fig. 5. Growth curve and PHAs production during batch fermentation of sweet sorghum by Bacillus arybhattai in 3 L fermentor (Tanamool et al., 2011)

Fig. 5 shows batch fermentation of sweet sorghum juice (SSJ) by *Bacillus aryabhattai* in 3 L fermentor under cultivating condition with agitation rate at 200 rpm, air rate at 1.5 l/min and temperature at 30° C. Growth monitoring and PHAs production were investigated including total sugar, dry cell weight (DCW) and variations of fructose, glucose and sucrose as functions of time. It was found that the maximum cell and PHAs product reached at about 10.38 g/l and 4.36 g/l. Both slightly increased after 72 hr. The sugar trend then changed. Sucrose was almost depleted within 36 hr while glucose and fructose were slightly increased at the beginning and reached maximum at 24 hr. Then the cells started to use both types of sugar (Tanamool et al., 2011).

Bacterium	PHAs	carbon source	Culture time (h)	Cell conc. (gl^{-1})	PHAs conc. (gl^{-1})	PHAs content (%)	Productivity ($gl^{-1}\ h^{-1}$)
Alcaligenes eutrophus	P(3HB)	Glucose	50	164	121	76	2.42
A. eutrophus	P(3HB)	CO_2	40	91.3	61.9	67.8	1.55
A. eutrophus	P(3HB)	Tapioca hydrolysate	59	106	61.9	57.5	1.03
A. eutrophus	P(3HB-co-3HV)	Glucose+ propionic acid	46	158	117	74	2.55
A. latus	P(3HB)	Sucrose	18	143	71.4	50	3.97
Azotobacter vinelandii	P(3HB)	Glucose	47	40.1	32	79.8	0.68
Chromobacterium violaceum	P(3HV)	Valeric acid	-	39.5	24.5	62	-
Methylobacterium organophilum	P(3HB)	Methanol	70	250	130	52	1.68
Protomonas extoquens	P(3HB)	Methanol	170	233	149	64	0.88
Pseudomonas olevorans	P(3HBx-co-3HO)	n-octane	$D=0.2h^{-3}$	11.6	2.9	25	0.58
P. oleovorans	P(3HBx-co-3HO)	n-octane	38	37.1	12.1	33	0.32
Recombinant *Escherichia coli*	P(3HB)	Glucose	39	101.4	81.2	80.1	2.08
Rec. *Klebsiella aerogenes*	P(3HB)	Molasses	32	37	24	65	0.75

Table 2. Production of PHAs by various bacteria and carbon sources (Yamane, 1996)

In Table 2, different carbon sources were evaluated and it was found that each strain produced different amounts of PHAs. For example, *Alcaligenes eutrophus* or *Rastonia eutropha* prefers to use fructose (Khanna & Srivastava, 2005). *A. latus* is in favour of sucrose (Grothe

et al, 1999) while *Azotobacter valandii* can use glucose (Lin & Sadoff, 1968). Currently, genetic engineering techniques can modify strains such as rec. *E. coli* and rec. *Klebsiella aerogenes* are used to produce PHAs in cheap carbon sources, such as molasses (Slater et al., 1988; Ramachander et al., 2002).

4.2.2 Fed-batch fermentation

Fed-batch fermentation process is a production technique between batch and continuous fermentation. A proper medium feed rate is required to add sequentially into the fermentor during the process and the product is harvested at the end of fermentation just like a batch type.

Fed-batch contains many advantages compared to other cultures. It can be easily concluded that under controllable conditions and with the required knowledge of the microorganism involved in the process, the feed of the required components for growth and/or other substrates are also important due to catabolic repression. The product will never be depleted, the nutritional environment can be maintained approximately constant and the extension of the operating time, high cell concentrations can be achieved and thereby, improve productivity. This is greatly favored in the production of growth-associated products.

Fig. 6 shows a fed batch fermentation of sweet sorghum juice (SSJ) by *Bacillus aryabhattai* in 3 L fermentor under cultivating condition with agitation rate at 200 rpm, air rate of 1.5 l/min, at 30° C and feeding time at 18 and 24 hr during log phase of the culture. It was found that the cell could continuously produce both biomass and PHAs. Maximum cells were obtained at about 14.20 g/l at 54 hr when PHAs content reached 4.84 g/l after 66 hr (Tanamool et al., 2011). In addition, in Table 2, fed batch fermentation by *A. latus* was used for the production of PHAs (Yamane et al, 1996; Wang & Lee, 1997). It could yield high productivity with the use of cheap carbon sources.

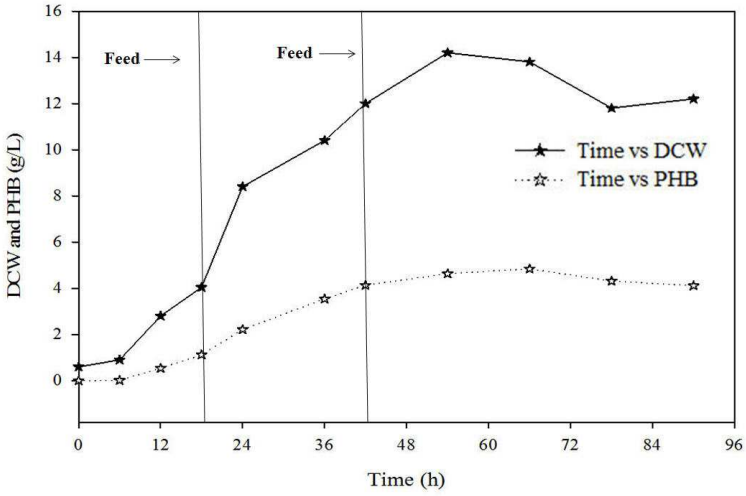


Fig. 6. Growth monitoring and PHAs production during fed-batch fermentation of sweet sorghum by Bacillus arybhattai in 3 L fermentor (Tanamool et al., 2011)

4.2.3 Continuous fermentation

Continuous fermentation is process in which feeds, containing substrate, culture medium and other requirements, are pumped continuously into the agitated bioreactor where the microorganisms are active. Final product is withdrawn from the upper part of the tank. Typically the process is performed by eliminating the inherent down time for cleaning and sterilization and the long lags before the organisms enter a brief period of high productivity.

However, it should be noted that there is a small percentage of the total time in which productivity rate is near its maximum. It is sometimes possible to maintain very high rates of products for a long time with continuous fermentation. Although it can get much more productivity from the fermentor, enhancement over batch fermentation in terms of the total volume of fermentor is not high because equipment needs to be sterilized to support the continuous tank.

Previously, Yu et al (2005) studied the increase of a co-biopolymer of PHBV by *Ralstonia eutropha* in a continuous stirred tank reactor. It was found that the productivity rate increased when sodium propionate was used as the carbon source. Later, Yezza et al (2007) investigated the use of maple sap as a carbon for PHB production by *A. latus*. The productivity of PHB reached 2.6 gl^{-1} h^{-1}.

5. Microorganisms

The majority of PHAs biosynthesis is performed by various microorganisms, especially bacteria. They can produce PHAs from a number of substrates and accumulate in their cells as carbon source and energy reserve under imbalanced growth conditions such as nutrient limitation. Fig.7 shows PHA accumulated in their cells that are characterized by transmission electron microscopic (TEM) technique.

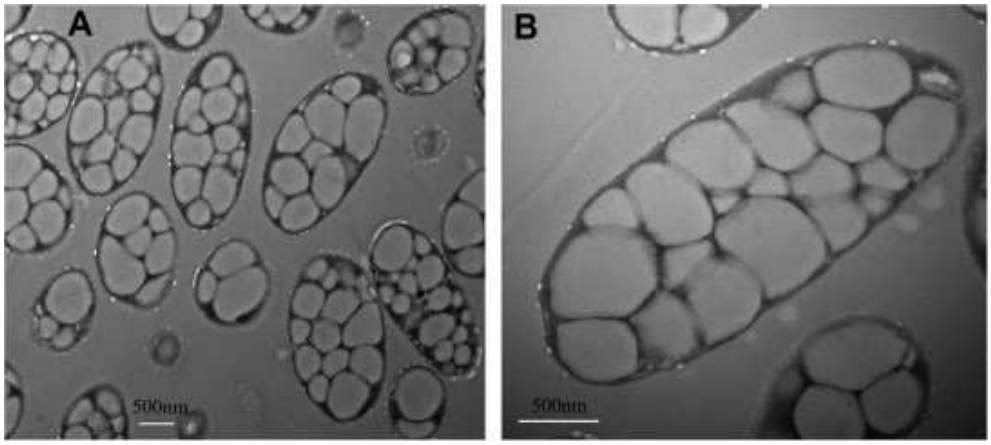


Fig. 7. (A and B) Images of PHAs accumulated in bacteria Halomonas TD01 based on transmission electron microscope (TEM) (Tan et al., 2011)

Depending on PHAs accumulation behaviour, microorganisms can be categorized into two groups. The first group requires limitation of some nutrients such as nitrogen or phosphate while there is an excess in carbon. The members of this group belong to *Alcaligenes eutrophus,* (Kaewkannetra et al, 2008), *Cupiavidus necator* (formerly known as *Ralstronia eutropha*) (Kim et al, 1994; Yu et al, 2005) and *Hydrogenophaga* sp. (Yoon & Choi, 1999; Mahmoudi et al, 2010). The second group does not depend on nutritional limitation but can accumulate PHAs during the growth. Examples are *Alcaligenes latus* (Yamane et al, 1996; Yezza, et al, 2007), *Azotobacter vinelandii*, *P. aeruginosa*, (Fernandez et al, 2005), *Bacillus mycoides* (Thakur et al, 2001) and *Escherichia coli*, etc.

5.1 Screening, isolation and identification

Although PHAs obtain interest and are widely studied by many researchers, PHAs production is limited by production cost. A major problem to the commercialization of PHAs is much higher production cost than petrochemical-based synthetic plastic (Luengo, 2003).

Many attempts have been devoted to investigate for reducing the cost of PHAs by the isolation of better bacterial strains from various sources such as sludge from wastewater (Kasemsap & Wantawin, 2007) or marine (Chien et al, 2007) and soil environment (Tanamool et al, 2011) using a simple method that can monitor the accumulation of PHAs. For example, efficient conditions for bacterial PHB production can be found in soil due to its heterogeneous nature. Nitrogen availability in soil varies with microsites. It may become a limiting factor for bacterial growth, especially in some nitrogen-poor (carbon-rich) sites (Thakur, 2001). Accordingly, some PHAs-producing bacterial strains such as *Bacillus, Arthrobacter, Corynebacterium, Curtobacterium, Pseudomonas, Micro- coccus,* and *Acinetobacter,* etc. can be isolated from soil environments.

Several simple methods used for detecting intracellular PHAs granules are applied to the screening of PHA producers such as Sudan Black staining (Anderson & Dawes, 1990) and Nile blue A staining (Wang & Bakken, 1998). The positive result shows a black color or fluorescent granules under microscope. Although these methods are feasible and easy, they are rather time- and labor-consuming due to requirement of environmental isolation. Alternative staining method has recently been developed for directly staining colonies or growing bacteria on plates containing Nile blue A or Nile red (Ostle & Holt, 1982). The dye can be directly diffused into microbial cytoplasm, resulting in fluorescent colonies that can be observed under UV illumination without microscopic observation. It is believed that the colony-staining is a suitable method for screening a large number of microbial strains.

After the samples have been collected from the environment, they are preserved at 4°C to protect against contamination prior to isolation. Recently, Tanamool et al, 2011 explained the primary isolation of soil microbes by serial dilution technique. In short, the soil sample was transferred to nutrient broth (NB) and incubated. Then the culture was diluted in normal saline and spread on nutrient agar (NA). Finally, several single colonies were picked and transferred to mineral salt agar. After incubation, PHAs producing capabilities of the microbes were confirmed by Sudan Black B and Nile blue A staining methods as described by Ostle & Holt, 1982; Wang & Bakken, 1998. Viable colonies were directly observed under UV light and fluorescence to detect the accumulation of PHAs (Spiekermann et al, 1999).

The morphology and biochemical characteristics of the isolate then are observed. The PHAs-producing strains can be identified by sequencing partial sequences of their 16S rDNA as described by Edwards et al, (1989). Fig. 8 shows phylogenetic analysis of 16S rDNA sequence of the isolate V 33. It was closely related to bacterial strain of *Hydrogenophaga* sp. (99% identity) (Tanamool et al, 2011). Previous studies have reported that *Hydrogenophaga* sp. can be isolated from soil, mud, and water. The strains were gram-negative, rod-shaped cell and yellow pigmented hydrogen-oxidizing bacteria (Willem et al, 1989). They can grow and produce PHAs from various substrates such as glucose, galactose, xylose, arabinose (Choi et al, 1995) whey lactose (Koller et al, 2008) and in fructose (Mahmudi et al, 2010).

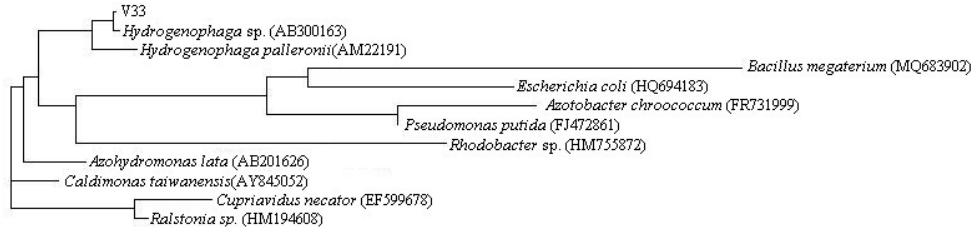


Fig. 8. Phylogenetic analysis of 16S rDNA sequence of the isolate V 33 (Tanamool et al, 2011)

6. Factors affecting fermentation process

6.1 Microbial strain

Tim & Steinbuchel (1990) studied the mechanisms of *Alcaligenes* spp. and *Rhodospirillum rubrum* for PHB synthesis by using butyric acid. Later, Steinbuchel (1991) confirmed the results for the potential of PHAs production and the compositions of PHAs. Under various microbial strains used, different contents of PHAs were obtained.

Table 3 shows PHAs contents in different weights compared to dry cell mass accumulated in various microorganisms. It was found that *Alcaligenes* spp. contains a maximum of 96% PHAs in its cell.

Microbial strain	Weight/ dry cell mass (%)
Ralstonia eutropha (reclassified Alcaligenes eutrophus)	96
Azospirillum	75
Azotobacter	73
Baggiatoa	57
Leptothrix	67
Methylocystis	70
Pseudomonas	67
Rhizobium	57
Rhodobacter	80

(Available from *www: http://members.rediff.com/jogsn/bp6.htm)*

Table 3**.** Variations of PHAs contents in various microbial strains

6.2 Nitrogen sources

Nitrogen sources are a nutrient effect on structure and the accumulation of PHAs in microbial cells. Nitrogen sources compose one limiting factor for microorganisms during cultivation. In 1982, Rhee et al studied the production of PHAs from *A. eutrophus*. Variation of nitrogen sources was also studied and the results were summarized in Table 4. Although the microorganisms can be used in the same carbon source, the cell growth and their PHAs products are different when nitrogen sources vary.

Carbon source	Nitrogen source	Cell dry Weight (g/l)	PHAs content (wt%)	PHAs	
				3HB	3HV
Glucose	Yeast extract	3.2	45.2	98.4	1.6
	Urea	1.4	14.8	85.6	14.4
	$(NH_4)_2SO_4$	1.8	29.3	93.3	6.7
Sucrose	Yeast extract	1.5	18.9	98.3	1.7
	Urea	1.3	4.0	93.5	6.5
	$(NH_4)_2SO_4$	1.2	15.1	92.0	8.0
Sorbitol	Yeast extract	3.1	44.8	93.4	6.9
	Urea	1.8	37.2	85.7	14.3
	$(NH_4)_2SO_4$	1.7	28.1	93.5	6.5
Mannitol	Yeast extract	3.4	58.7	94.1	5.9
	Urea	2.2	18.2	92.5	7.5
	$(NH_4)_2SO_4$	1.8	29.0	93.3	6.7
Na-gluconate	Yeast extract	2.3	34.5	91.9	8.1
	Urea	1.6	5.3	78.1	21.9
	$(NH_4)_2SO_4$	2.7	41.1	66.7	13.3

Table 4. Effect of nitrogen sources on PHAs production (Rhee et al, 1992)

6.3 Fermentation time

Timm & Steinbuchel (1990) revealed the production of PHAs by *Pseudomonas aeruginosa*. It was found that when the cell reached to stationary phase, the intracellular PHAs were decreased. Lageveen et al (1998) produced PHAs from n-octane by *Pseudomonas oleovorans*.

It was found that the microorganism has a specific duration for PHAs recovery. However, only some strains can accumulate PHAs along their growth or growth-associated strain.

6.4 Mineral substances

Mineral substances such as phosphorus, sulphur, magnesium, etc. could affect microbial growths in several functions. Phosphorous is assimilated only in form of orthophosphate ($H_2PO_4^-$) and it is essential for all microorganisms. Sulphur is incorporated into S-containing amino acid in protein and magnesium functions as a cofactor or effectors for many enzymes in microbial metabolism. Each microbial strain needs nutrients and minerals in different contents due to suitable limitation of nutrients for PHAs-producing microorganism.

6.5 Temperature and pH

Normally, the production of PHAs usually carries on under moderate temperature range of 30-35°C and at pH 6.5-7.0. This condition may be suitable for most PHA3s-producing microbes.

7. PHAs recovery and their properties

After fermentation, subsequent midstream to downstream processes such as cell disruption, centrifugation, extraction and drying will be carried on for product recovery. Fig. 9 shows a white sheet of PHB obtained from fermentation of sweet sorghum juice (SSJ) by *Bacillus aryabhattai*.

Fig. 9. Biopolymer of PHB obtained from *Bacillus aryabhattai*

In Fig. 10 the sheet was then characterized for the properties such as thermal properties of glass temperature (Tg) and melting temperature (Tm) analyzed by Differential Scanning Calorimetric (DSC) analysis. The Tg and Tm of the PHB sheet reached 1.11°C and 167.3°C when compared to the standard PHB (99.5%) where Tg and Tm are at 2.81°C and 176.29°C. Thermal properties of PHB sheet obtained were lower than the standard values of PHB. This implies that the PHB sheet can be easily further used to blend and form with other cheap biopolymers or raw materials by heat treatment during bioplastic production process. In Fig.11 shows the degrading temperatures analyzed by Thermo gravimetric analysis (TGA) in both standard PHB and the PHB sheet. The degradation will be completed at temperature of 450°C for the standard PHB, while the PHB sheet is completely degraded at 300°C. It means that the PHB sheet obtained containing of improved quality that was better than the standard PHB.

In addition, physico-chemical characterization, blending and forming steps need to be fulfilled to get novel biomaterials for replacing conventional plastic in a wide range of further applications. These include packing containers, bottles, wrappings, bags, thin films and disposable items (diapers or feminine hygiene products).

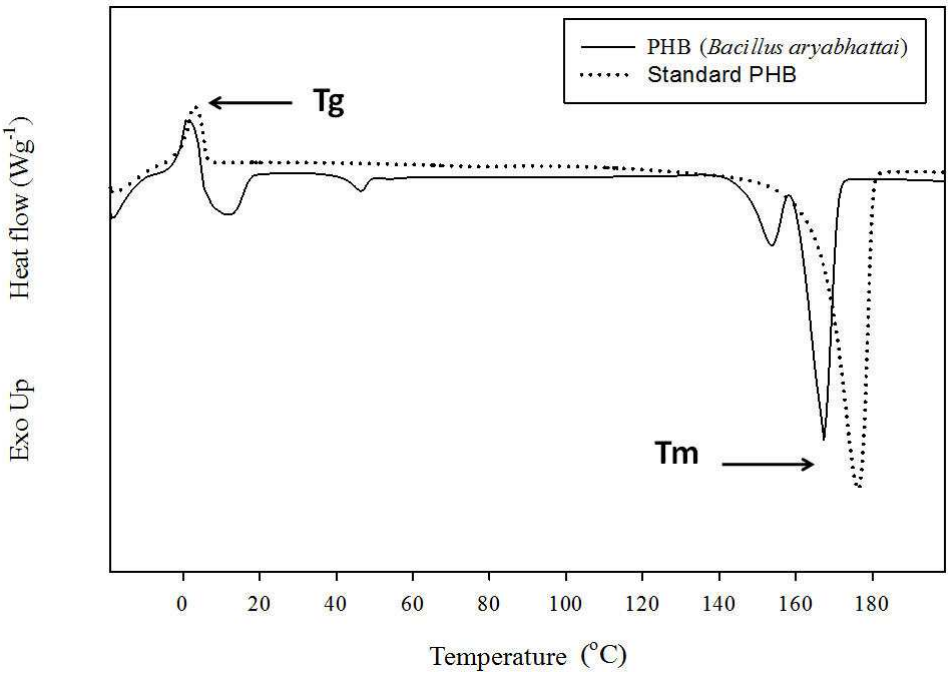


Fig. 10. Tg and Tm of standard PHB and PHB obtained from *Bacillus aryabhattai*

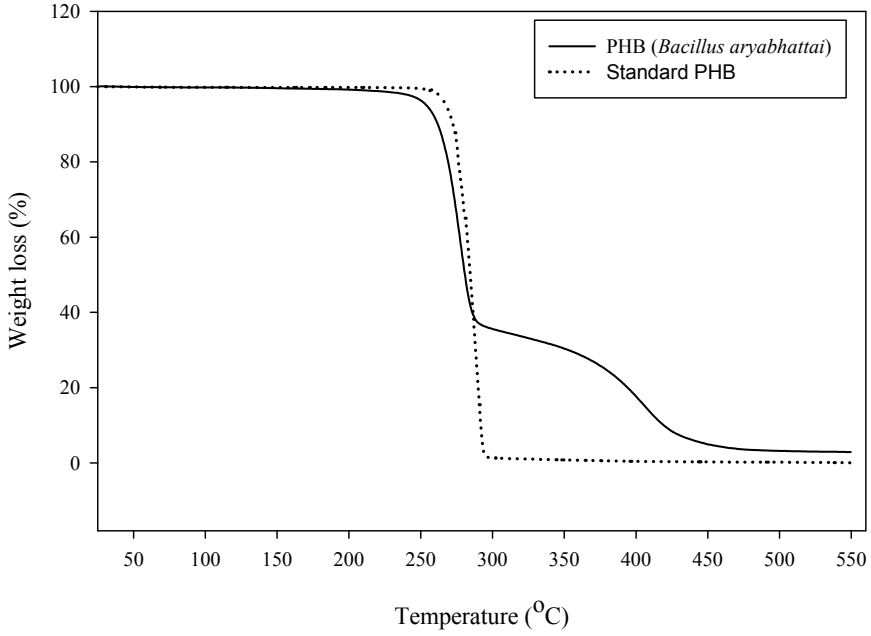


Fig. 11. Thermal degradation temperature of standard PHB and PHB obtained from *Bacillus aryabhattai* by Thermo-gravimetric analysis (TGA)

8. Summary

Although PHAs are being interested and broadly studied by many researchers for a long time, the most important obstacle to commercial application of PHAs is their price. PHAs' production cost is roughly 10 times or more compared to petrochemical-based synthetic plastic materials such as PE and PP. Much attempt has been devoted to reduce the production cost of PHAs in different ways, for example, use of isolated bacterial strains, development of improving strains by genetic techniques such as recombinant DNA of *E. coli* and *Streptomyces aureofacienc* NRRL, controlling a culture condition via various fermentation processes such as batch, fed-batch, repeated-batch/repeated fed-batch, enhancing production via optimization of fermentation process using response surface methodology (RSM), more economical recovery process, and most importantly, the use of cheaper carbon sources.

A novel non-petroleum based biodegradable plastic produced from sugar based agricultural raw materials as sweet sorghum, sugarcane and molasses, having potential properties comparable with conventional or synthetic plastics, is under development and could lower the contribution of plastic wastes to municipal landfills at about 20% of the total waste by volume and 10% by weight and can achieve a satisfactory for the environmental imperative.

The gradual transition towards the biobased economy brings opportunities for 'developing' countries to leapfrog beyond the petroleum era and into a cleaner, greener and more renewable future based on biotechnology knowledge.

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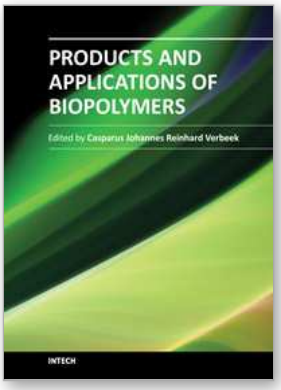

Products and Applications of Biopolymers

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It is interesting to consider that biopolymers are by no means new to this world. It is only because of our fascination with petrochemical products that these wonderful materials have been neglected for so long. Today we face a different challenge. Environmental pressure is pushing away from synthetic or petro-chemically derived products, while economic factors are pulling back from often more expensive "green" options. This book presents two aspects of biopolymers; potential products and some applications of biopolymers covering the current relevance of biopolymers.

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