Parameters Affecting Polyhydroxyalkanoate Synthesis from Wastewaters

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Abstract: Plastics used almost every manufacturing industry are resist to biodegradation. Their persistence in soil for a long time has become a major concern in terms of the environment. This promotes many investigators to search for replacement of non-biodegradable by degradable plastics. Polyhydroxyalkanoates (PHAs), known as a biodegradable plastic produced by bacteria, have received increasing attention due to the difficulties in disposal of plastics. In recent years, researchers have focused on the processes to increase PHA production which involve in biological phosphorus removal (BPR). Normally, BPR can be achieved through anaerobic- aerobic cycling by a group of bacteria known as polyphosphate-accumulating organisms (PAOs). PHA is stored within the PAO as carbon polymers under anaerobic conditions by taking up volatile fatty acids (VFAs), further it is used as energy source and phosphorus uptake under aerobic conditions. The aim of this review is to discuss recent advances in PHA production from wastewaters and parameters effecting PHA production efficiency.

1. Introduction

Plastics which are known to be widely used in almost every manufacturing industry are very much advantageous. Plastics are popular in many durable, disposal goods and as packaging materials. Beside a wide range of benefits, they are not desirable in the environment. Especially, plastics are known as hardly biodegradable even non-biodegradable due to the fact that they mainly have high molecular weights (Reedy et al., 2003). Their persistence in soil for a long time has become a major concern in terms of the environment. This promotes many investigators to search for replacement of non-biodegradable by degradable plastics. An alternative approach to conventional plastics is Polyhydroxyalkanoates (PHAs) which are known as a completely biodegradable plastic produced by bacteria, have received increasing attention due to the difficulties in disposal of plastics (Bengtsson et al., 2008). Additionally, Poly-(R)-3-hydroxybutyric acid (PHB) and hydroxyvalerate (PHV) are among the most common PHA monomers. The chemical structure of the PHAs is shown in Figure1.

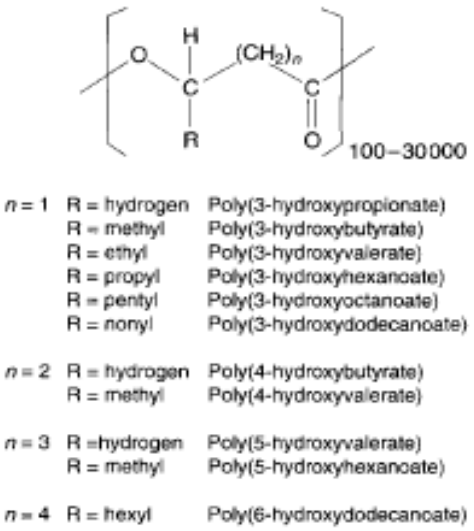

Figure 1. Chemical structure of PHAs (Lee, 1996)

2. PHA Production

As mentioned before, the PHAs are non-toxic, biocompatible, biodegradable thermoplastics that can be produced from renewable resources such as biomass. These biopolymers accumulate as storage materials in microbial cells under stress conditions (Sudesh et al., 2000; Chen et al., 2001; Kadouri et al., 2005). Many gram-positive and gram-negative bacteria are known to be able to synthesize PHAs (Reedy et al., 2003). PHAs can be produced through anaerobic- aerobic cycling by a group of bacteria known as polyphosphate-accumulating organisms (PAOs). Although PAOs are often present in a wide range of aerobic suspended growth cultures, they only have the ability to store large quantities of phosphate when they are subjected to alternating anaerobic and aerobic conditions. PHA is stored within the PAO as carbon polymers under anaerobic conditions by taking up volatile fatty acids (VFAs), further it is used as energy source and phosphorus uptake under aerobic conditions (Figure 2). Under aerobic conditions stored PHA or PHB within the cell is used as energy source for biomass growth, and glycogen synthesis.

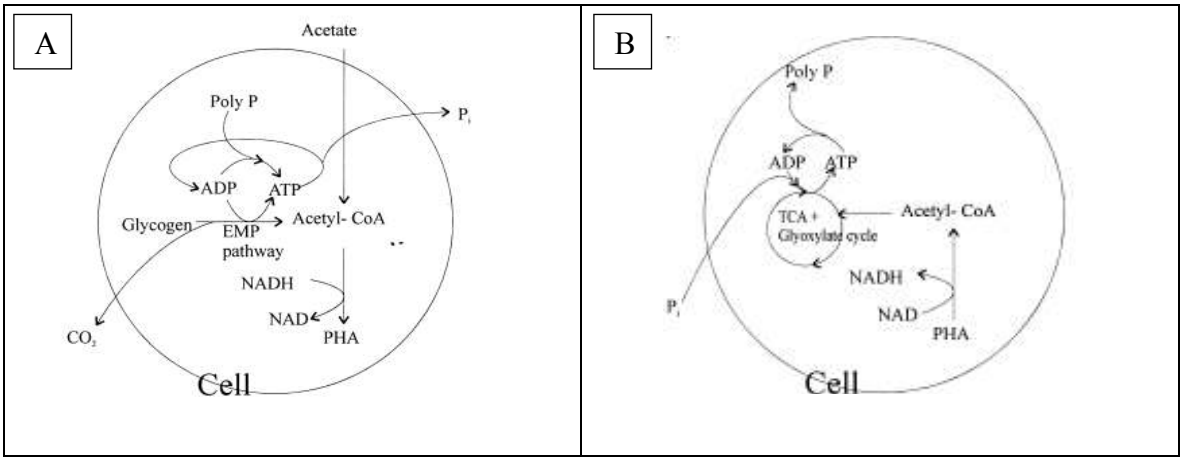

Figure 2. Anaerobic PHA production and phosphorus release (A), aerobic PHA utilization and phosphorus uptake (B) within the cell (Lee and Choi, 1999)

Recently reported research articles have been performed to understand PAO metabolism (Seviour et al., 2003). Acetate has been used almost exclusively as the carbon source in these studies, partially explained by the fact that it is typically the largest volatile fatty acid (VFA) species present in the wastewater treatment plants. Metabolic PAO models for anaerobic acetate uptake and utilization and the subsequent aerobic processes have been proposed. As depicted in Figure 2, anaerobic process is based on fermentation in which poly-P degradation and phosphate release take part. Under anaerobic conditions with acetate as the carbon source, phosphate

accumulating microorganisms can take up acetate rapidly, accumulate PHAs in the cell. The energy for this biotransformation is mainly generated by the cleavage of polyphosphate and release of phosphate from the cell. They consume previously stored intracellular carbohydrate, and release P as a result of utilization of stored poly-P. Thus, wastewater from anaerobic process is rich in inorganic phosphate. In aerobic processes, oxygen is electron acceptor and microorganism use stored PHB as their carbon and energy source.

PAOs are actually known as responsible for enhanced biological phosphorus removal (EBPR) and have a key role with respect to both PHB accumulate within the cell and phosphorous removal. The process is one of the most commonly used and environmentally sounds methods for phosphorus (P) removal from wastewater. As mentioned before, VFAs are preferable substrates for PHA production. Anaerobic fermentation converts various organic compounds to VFAs hence increasing the potential to produce PHA from the wastewater. The composition of the VFAs produced during fermentation will influence the final polymer product. However, the number of literature reports for PHA production with mixed cultures enriched with real wastewaters is limited.

- **PHAs Extraction from the Cell**

Since PHAs are accumulated within the cell, it should be extracted to be able to be used as plastic polymers. However, the extraction of bioplastics from microorganism poses yet a challenge. There are two common protocols used for PHA extraction from bacteria. The first protocol is developed by taking into consideration of solubility in chloroform and insolubility in methanol. Harvested bacterial cells are exposed to warm chloroform to make PHAs soluble. Further, residuals from harvested bacteria such as lipids and other lipophilic components are removed by reflux in hot methanol. Purified PHA production efficiency of this protocol is high. However, requirements of a large amount of hazardous solvent make it not environmentally friendly (Lee, 1996). The second protocol developed for the aim of avoiding organic solvent usage. In this protocol, a mixture of enzymes are used such as proteases, nucleases and lysozymes, additionally to remove proteins, nucleic acids, and cell walls, detergents are used.

3. Factors Affecting PHB Production

In the previous sections the importance of PAOs on PHB production and phosphorus removal from the wastewaters were emphasized. However, their production costs are much higher than the petrochemical- based plastics (Fang et al., 2009). Thus, it becomes inevitable to know parameters affecting PHA production efficiency within the cell such as; microorganisms involved, pH, substrate, solid retention time (SRT), availability of electron acceptors, and temperature. These will be briefly discussed by taking into account the published research articles that focus on increasing the cost-effectiveness of this process.

a. Microbial Population

Determination of microbial population that involve in PHB production and phosphorous removal is one of the most important factor to be able to make the process successful. It has been emphasized in so many articles that there is a competition between two microorganisms, PAOs and glycogen (non-polyphosphate) accumulating organisms (GAOs) respectively. Like PAOs, GAOs are able to proliferate under alternating anaerobic and aerobic conditions but the problem is they do not contribute to P removal hence anaerobic P release or aerobic P uptake cannot be established. This constitutes a major challenge since PHA production without any phosphorous removal cannot be convenient according to discharge regulations. Additionally, the presence of GAOs increase the anaerobic VFA requirements of these plants, thus so many investigators have focused on the ways that minimize the growth of GAOs. Factors affecting GAOs and PAOs competitions can be summarized as:

6. One factor affecting the PHA accumulation is the ratio of organic carbon to P in the influent or the so-called COD: P ratio. In so many studies it was reported that a high COD/P ratio (e.g. 450mgCOD/mgP) in the wastewater feed tends to favorable the growth of GAOs instead of PAOs while a low COD/P ratio (e.g. 10–20mgCOD/mgP) tends to favorable to the growth of PAOs (Oehmen et al., 2007).

7. Effect of pH has been reported in so many research articles. They have found that increase in pH from 6.5-7.5 is favorable for PAOs while is not favorable for GAO. Thus it is possible to eliminate GAOs by increasing pH. In a study performed by Filipe et al., (2001a) has shown that P uptake, PHA utilization and biomass growth were all inhibited by a low pH (6.5), and suggested that a higher aerobic pH (7–7.5) would be more beneficial for PAOs.

8. Effect of temperature is also investigated by so many researches and they concluded that GAOs are inhibited at 10 ºC since PAOs are the dominant microorganisms at low temperature (Carlos et al., 2009). It was also noted that high temperatures (30 ºC) can suppress the proliferation of GAO in which operating conditions for pH is high (>7) and an adequate acetate to propionate ratio (75–25%) is supplied. The experimental evidence obtained thus far suggests that GAOs tend to become stronger competitors with PAOs at higher temperatures.

b. pH

In many studies it was shown that adjusting to pH higher levels results in a higher anaerobic P release (Smolders et al., 1994; Liu et al., 1996; Bond et al., 1999; Filipe et al., 2001b). Kasemsap et al., (2007) found that increasing the pH from 6 to 8 promoted the PHA production significantly. It was also reported in other study that the ratio of anaerobic P release to acetate uptake increase from 0.25 to 0.75 P-mol/C-mol by increasing pH from 5.5 to 8.5 (Smolders,1994). Actually, by adjusting pH GAOs can be eliminated, this is the reason for why so many researchers focus on its effects.

Increase in pH makes the energy requirements for substrate uptake high. When external pH is high, more energy is needed for acetate uptake. This increased energy is generated through an increase in polyphosphate degradation. This scenario has been found ineffective for the acetate uptake, glycogen degradation and PHA accumulation rates of PAOs when the pH is over the range 6.5–8.0 (Filipe et al., 2001b). Nevertheless, this situation is rather different for the GAOs. It was reported that a higher pH results in a higher energy demand for acetate uptake, but negatively affects the ability of GAOs to take up acetate. This is obviously related to the differences between metabolic pathways of the microorganisms. The energy production pathways of GAOs and PAOs are dissimilar since PAOs use the energy required for the substrate uptake from the hydrolysis of poly-P while GAOs from the hydrolysis of glycogen (Smolders et al., 1994; Filipe et al., 2001a,b). That means PAOs have poly-P as an extra energy source as compared to GAOs and they deplete it to meet higher energy demand. In a published report performed by Chua et al.(2003) studied the effect of pH on the PHA content using acetate as the substrate. They found that, through controlling the pH at 6 or 7, the PHA content (less than 5%) was lower than at pH 8 or 9 (25–32%). Like this record, Serafim et al., (2004) was found that polymer yield per substrate and the intracellular PHB content were higher at pH 8 than at pH 7.

c. Substrate

It is a prerequisite to optimize all the fermentation conditions for the successful implementation of commercial PHA production systems. Actually there is a major challenge to reduce PHA production costs. Carbon source has a large impact on production cost of the PHA produced. Hence, recent studies have been focused on reducing costs. The price of the product ultimately depends on the substrate cost, PHA yield on the substrate, and the efficiency of product formulation in the downstream processing.

In so many studies different substrates were used to improve the predictability of the metabolism of both PAOs and GAOs. Acetate has been used almost exclusively as the carbon source. It has been well known that short chain fatty acids like acetate are favorable carbon sources for PAOs. Beside acetate, there are so many carbon sources used in order to investigate PHA production pathway, such as; lactate, propionate, sucrose, glucose, cheese whey, cane molasses, methanol, and hemicelluloses hydrolsate (Quillaguaman et al., 2007; Ahn et al., 2000; Wong and Lee, 1998; Rhu et al., 2003; Hong et al., 2000). Waste materials or industrial by-products can also be used for PHA production. However it was reported that carbohydrates are not directly stored as PHA and they are tend to be preferentially accumulated as glycogen (Dircks et al., 2001; Karahan et al., 2006). PHA production from raw materials requires a previous anaerobic fermentation step for their transformation into volatile fatty acids (VFA). This is the reason why the majority of the studies related to PHA production are based on the use of organic acids. The effect of various substrate costs, the yield on the P (3HB) and production cost are summarized in Table 1.

Wastewater contains a much more diverse mixture of substrates other than acetate and investigations were conducted with other compounds, alone or in mixtures, including: propionate, butyrate, valerate, isovalerate, formate, lactate, malate, pyruvate, glucose, citrate, succinate but glutamate but the metabolism of these organic substrates have not yet been well understood (Wang et al., 2002). Several studies concluded that glucose as sole carbon source led to deterioration of the EBPR process, as glucose promoted the growth of GAOs which do not accumulate polyphosphate and therefore capable of utilizing glucose without the release of phosphate (Cech and Hartman, 1993; Mino et al., 1994; Satoh et al., 1994; Tasli et al., 1997). However, there are also opposite research results where a stable EBPR performance could be maintained with glucose as the major organic substrate, with no appreciable proliferation of GAOs (Carucci et al., 1999; Jeon and Park, 2000; Wang et al., 2002).

Substrate	Substrate Price (US$ kg^{-1})	P(3HB) yield (g P(3HB) (g substrate)$^{-1}$)	Product cost (US$(kg P(3HB))$^{-1}$)
Glucose	0.493	0.38	1.30
Sucrose	0.290	0.40	0.72
Methanol	0.180	0.43	0.42
Acetic acid	0.595	0.38	1.56
Ethanol	0.502	0.50	1.00
Cane molasses	0.220	0.42	0.52
Cheese whey	0.071	0.33	0.22
Hemicellulose hydrolysate	0.069	0.20	0.34

Table 1. Effect of various carbon sources on PHB yield and production cost (Reddy et al., 2003)

In the past, there have been relatively few studies on EBPR systems involving propionate as a carbon source (Satoh et al., 1992,). In recent years, however, the metabolism of propionate by PAOs (Lemos et al., 2003) and its effect on EBPR performance has attracted considerable attention. Several studies have suggested that propionate could be a more favorable substrate for EBPR (Chen et al., 2004; Thomas et al., 2003), likely providing a selective advantage to PAOs over GAOs (Oehmen et al., 2004a, 2004b; Pijuan et al., 2004). In the studies used other single substrates such as lactate, ethanol, and glutamate which can be converted into PHB, very low storage yield has been obtained. When lactate used for carbon source, 0.20 g PHA g-^{1} substrate accumulation was obtained, it was 0.25 g PHA g^{-1} substrate for ethanol, and 0.058 g PHA g-^{1} substrate for glutamate (Dionisi et al., 2004; Doi et al.,1987). Lactate is taken up by PAO cells and converted to propionyl-CoA, using both poly-P and glycogen hydrolysis as energy sources. Poly-P is hydrolyzed to orthophosphate and released from the cells, while glycogen is hydrolyzed to acetyl-CoA and CO_2. Acetyl-CoA and propionyl-CoA are reduced and condensed to form PHA, with the reducing power provided by glycogen hydrolysis. This mechanism is compared to the situation in which acetate is used as sole carbon source in Figure 3.

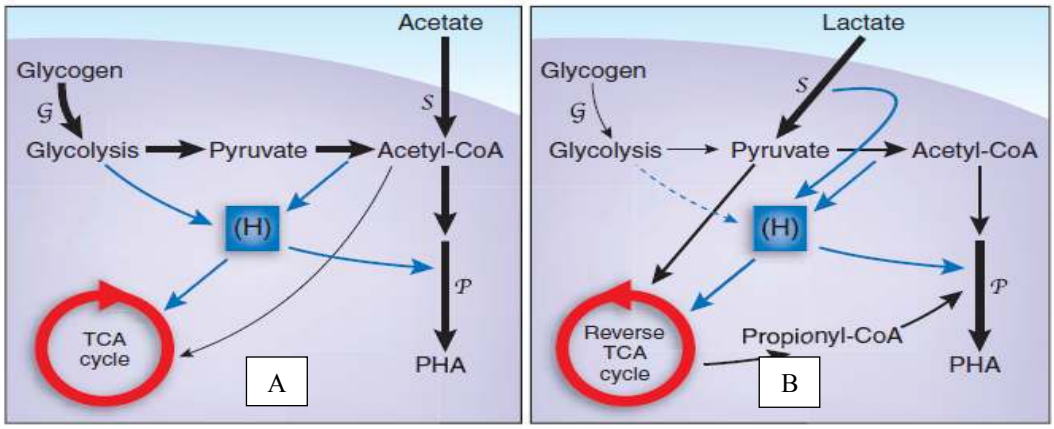

Figure 3. Control of redox balance of different carbon source in PHA production under anaerobic conditions (Mino and Satoh, 2006).

H, reducing power or hydrogen in such forms as NAD(P)H and $FADH_2$;
S, molar amount of acetate or lactate taken up;
G, molar amount of glucose unit in glycogen consumed;
P, molar amount of monomeric units of PHA produced.

In Figure 3, redox balance regulation is depicted which is the key mechanism for anaerobic carbon uptake and hence proliferation of PAOs. Glycolysis via Embden Meyerhof pathway (EMP) and acetate oxidation through the TCA cycle provides the required reducing power for the conversion of acetate into 3-hydroxybutyrate for PHA synthesis (Figure 3A). Mino reported in this study that the ratio S/G/P will be 1:(1/6):(2/3) and 1:0:(4/9) if all reducing power is supplied by glycolysis and by the TCA cycle, respectively. In Figure 3 B, lactate is taken up within the cell as carbon source. By this way, ratio of S/P is increased to 2. During conversion of lactate into acetyl-CoA the reverse operation of the TCA cycle is needed to consume excess reducing power produced.

d. Solid Retention Time (SRT) and Temperature

It is obvious from the published reports that SRT has important impact on PHA production yield for a given organic loading rate (OLR) .Short SRT sludge acquires higher PHA production capability, hence sludge acclimatization with a short SRT may also be preferable for PHA production purpose. This approach is confirmed since the sludge yield under a shorter SRT is higher than that under a longer SRT. So it can be concluded that with a short SRT can supply sufficient amount of sludge for PHA production compared to that with a long SRT. It was found that sludge with a short SRT (3 days) could achieve PHA content about 10% more than sludge with a long SRT (10 days) (Chua et al, 2003). However, it was reported that higher cell growth rates resulted in a lower PHB content higher PHB yields were produced at longer SRT when the cells were growing more slowly (Dias et al 2006). Beun et al. (2000) reported that the PHB yield per substrate and specific productivity were almost constant when vary the SRT from 3.8 to 19.8 day. Dionisi et al. (2001) obtained a relatively constant storage yield in a SRT range of 0.37–3 day.

Temperature also appears to be a factor that has an important impact on the PHA production. It was reported that temperature has actually directly affected microorganism competition which is known as GAO and PAO. In a published report, it was mentioned that a lower temperature decrease the rates of P release/uptake, acetate uptake, PHA oxidation, growth (Brdjanovic et al., 1998). Panswad et al. (2003) found that the rate of P release increased with increasing temperature from 20 to 35 ºC, while the rate of P uptake decreased. Aditionally,it was reported in a study that the increase of temperature from 15 to 35 ºC result in decrease in the yield of PHB on acetate from 0.43 to 0.072 g PHA g^{-1} substrate and a decrease in the specific productivity from 0.12 to 0.060 g PHA g^{-1} cell dry weight h^{-1} (Krishna and van Loosdrecht, 1999). The yield of biomass also decreased with temperature increase. Low temperatures (between 15 and 20 ºC) allow for a less costly process thus increasing the PHA productivity.

e. Availibity of Electron Acceptors

Since anaerobic P release based on fermentation process, availability of electron acceptors, such as; oxygen, nitrate and sulphate, is not desired since this will eliminate the fermentation process. For example availability of nitrate will result in denitrification process and nitrate reduction will take place other than fermentation process in which organic compounds are usually used as electron acceptors. Additionally, it has been observed that aerobic P uptake is inhibited by the presence of nitrite (Kuba et al., 1996). Saito (2004) also reported that the presence and accumulation of nitrite inhibits PAOs, thereby favoring the growth of GAOs. Third et al. (2003) was studied the effect of dissolved oxygen concentration (DO) on PHA production. They found that when oxygen was limited PHA yield was 0.49 g PHA g^{-1} substrate using acetate as sole carbon source. They have found that PHA yield was decreased to 0.34 g PHA g^{-1} substrate under excess oxygen.

4. Conclusion

Polyhydroxyalkanoates (PHA) have gained major importance because of their similar properties to conventional plastics and their complete biodegradability. PHA can be produced from renewable carbon sources, allowing for a sustainable process for the production and use of such polymers. PHA can be synthesized by polyphosphate-accumulating organisms (PAO) under anaerobic conditions from external carbon sources and internal glycogen. Glycogen-accumulating organisms (GAO) are also present in EBPR systems and compete for carbon substrates with PAO. They also cycle PHA and glycogen in a fashion similar to PAO, but GAO do not cycle polyphosphate. Hovewer, much more effort is required in this area to increase the production of bioplastics to successfully replace the non-degradable plastics. Thus the future of bioplastics depends on the efforts towards fulfilling requirements of price and performance. This review shows the parameters affecting PHA production efficiency. Process monitoring and control are important factors for achieving high productivity. Since carbon source has a large impact on production cost of the PHA produced recent studies have been focused on reducing its costs. Besides carbon source, some other factors such as SRT, temperature, pH, availability of electron acceptors in the anaerobic phase are proved to have important affect on PHA production yield. It can be concluded that, low SRT, temperature ranging between 15-25 ºC, pH above 7 can be preferable for higher PHA production efficiency. Indeed, the main challenge regarding the bioreactor operation and control is the development of culture selection strategies of fast growing organisms that have a high PHA storage capacity. It can be recommended to introduce the new metabolic pathways for not only to expand the utilizable substrate range but also enhance the current PHA yields.

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