

Aerobic Treatment of Oily Wastewater: Effect of Aeration and Sludge Concentration to Pollutant Reduction and PHB Accumulation

Budhi Primasari, Shaliza Ibrahim, M Suffian M Annuar and Lim Xung Ian Remmie

Abstract—This study is aimed to investigate feasibility of the aerobic biological process to treat oily wastewater from palm oil food industry. Effect of aeration and sludge concentrations are studied. Raw sludge and raw wastewater was mixed and acclimatized for five days in a stirred tank reactor. The aeration rate (no aeration, low; 1.5L/min and high rate; 2L/min) and sludge concentration (3675, 7350, and 11025mg/L of VSS) were varied. Responses of process were pH, COD, oil and grease, VSS, and PHB content. It was found that the treatment can remove 85.1 to 97.1 % of COD and remove 12.9 to 54.8% of oil & grease. The PHB yield was found to be within 0.15% to 2.4% as PHB/VSS ratio and 0.01% to 0.12% as PHB/COD removed. The higher aeration results a high COD removal and oil & grease removal, while experiment without aeration gives better PHB yield. Higher sludge concentrations (11025mg/L VSS) give higher removal of oil & grease while moderate sludge concentration (7350mg/L VSS) give better result in COD removal. Higher PHB yield is obtained in low sludge concentration (3675mg/L).

Keywords—oily wastewater, COD, PHB, oil and grease

I. INTRODUCTION

MALAYSIA is considered one of the largest palm oil exporters in the world with a total 4.3 million hectare of palm oil plantations and over 400 palm oil mills throughout the country. The palm oil sector provides an important element in the rural economy of Malaysia by providing a significant source of employment in large scale. In general, there are upstream and downstream industries which are related to palm oil sector. In the upstream industries, there are palm oil plantations and palm oil mills while the downstream industries include the food industries and non-food industries such as biodiesel industry, oleo chemicals, pharmaceutical and others. These downstream industries like mills, local services and support to the plantations and mills as well as providing the

local source of economic development, especially in those remote and rural areas.

Apart from being one of the Malaysia's main sources of income industry, this industry especially the vegetable oil (edible oil) processing plant is a main contributor of oily effluent discharge. The wastewater is high in organic content, which resulting in a chemical oxygen demand and also high in oil and grease. Approximately, 10 to 25 m³ of wastewater is generated per metric ton of product from seed dressing and edible fat and oil processing.

Among all the pollutants in the effluent, oil and grease is one of the most complicated pollutants to be removed due to the characteristics of these waste waters which contain a very high volume of oil-in-water emulsions as their basic contaminant. These emulsified oil droplets are sheltered from spontaneous coalescence into larger flocs, thus making oil separation by simple gravity a difficult and time consuming process.

Due to enormous scale of this industry, the palm oil related effluents have been increasing at an alarming rate, and the effects are detrimental to both the environment and human race. In overcoming these issues, measures to counter pollution from vegetable oil related effluents have been deployed by the Malaysia Government. In order to regulate the discharge from the palm oil related industry as well as to exercise other environmental control through the enactment of Environmental Quality Act (EQA), 1974. In addition, the Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979 which states the Maximum Effluent Parameter Limits has been incorporated in regulating the industrial effluent quality.

Usually, the problems of this oily effluent pollution have been overcome with the use of anaerobic and facultative digestion to treat the waste. This is due to the anaerobic treatment of wastewater has been considered to have a number of advantages over the conventional aerobic process. It saves the energy needed for aeration, converts organic pollutants into methane gas, a readily useable fuel, needs a low nutrient requirements and produces low biomass.

To treat oil and grease rich waste waters biologically the most important criteria to be met are stable and active biomass and proper sludge concentration. Hence, this paper is aimed at achieving the goal by using a simple anaerobic batch reactor to treat the oily wastewater through assessing the effects of

B. Primasari is with the Department of Civil and Environmental Engineering, University of Malaya, Malaysia (phone: 60-3-79677647; fax: 60-3-796753185555; e-mail: bprimasari@um.edu.my) and also affiliated with Water Laboratory, Department of Environmental Engineering, University of Andalas, Padang, Indonesia.

S. Ibrahim, is with the Department of Civil and Environmental Engineering, University of Malaya, Malaysia (phone: 60-3-79674458; fax: 60-3-796753185555; e-mail: shaliza@um.edu.my).

M.S.M. Annuar, is with the Institute of Science Biology, University of Malaya, Malaysia (phone: 60-3-79674458; e-mail: suffian_annuar@um.edu.my).

L. X. I. Remmie, is with the Department of Civil and Environmental Engineering, University of Malaya, Malaysia (phone: 60-173473489).

various sludge concentrations, pH and acclimatization periods on the oily effluent quality. Also, due to the nature of the wastewater, the microbial activity throughout the treatment will result the accumulation of biodegradable polyester, Polyhydroxybutyrate (PHB) which can be used in the manufacturing of biodegradable plastics. In short, the proposed reactor will not only reduce the detrimental effects of the effluents, but the end products of this reaction will benefit the general public.

During the wastewater treatment, bacteria can produce storage granules of PHB (poly- β -hydroxy butyric acid). PHB is microbial poly-esters, and also called as bio-plastics, as they are biodegradable polymer. PHB is stored in the cell cytoplasm by a microorganism under stress conditions caused by limitation of nutrients. Industry use pure microbial cultures and pure substrate (mostly glucose) to produce PHB. A high production cost process is caused by the substrate cost and energy for sterilization and extraction of the polymer, therefore, so far production cost of polymer. Therefore, still ten times of the conventional plastics. Using mixed cultures can reduce cost of production of PHB, because of cheap substrate and simple (non-sterile) reactors are used. Using mixed culture, selection of microorganism occurs based on its capability, to store PHB. The metabolism of PHB storage is the availability of electron donor and acceptor or because the non-continuous availability of substrate for the microorganism. Cooking oil wastewater (from palm oil factory) can be considered as an alternative, cheap substrate for PHB production. The COD moderate content (1500-5000 mg/L) and high oil and grease content (80-100 g/L) will be an advantage as a substrate.

Bioplastics are attracting much interest as alternatives to traditional plastics. They are biodegradable and can be formed from renewable resources. Among the biodegradable plastics polyhydroxyalkanoates (PHAs), and in particular, the copolymer poly β -(hydroxybutyrate-hydroxyvalerate) [P(HBHV)], are the most promising. P(HB-HV) has similar properties to polypropylene. It can be processed in the same way, and it could have the same wide range of application [1]. However, until now production of P(HB-HV) has proven to be much more costly than traditional oil-derived plastics and this has hindered its use becoming more widespread [2].

Poly β -hydroxybutyric acid (PHB) is the most extensively studied PHA, produced in nature in the presence of excess carbon by bacteria as storage granules providing food, energy and reducing power [3]. PHB has properties similar to petroleum derived synthetic plastics like polypropylene (PP) and is completely biodegradable in the environment.

Much effort has been spent in optimizing the poly- β -hydroxybutyrate (PHB) production using pure substrates and pure cultures [4] - [5]. However, the product (PHB) cost is still around ten times higher than that of conventional plastics [6]. The use of mixed cultures and cheap substrates (waste materials) can reduce costs of PHA production by more than 50%. The major costs in the PHA production are determined

by the cost of substrate (molasses, glucose and propionate) and extraction of the polymer from the cells. Furthermore, the need for sterilization and sterile fermentation systems is prevented. It is well known that activated sludge submitted to transient conditions, mainly caused by discontinuous feeding and variations in electron acceptor presence, can store large amounts of PHA. This accumulation is the key factor in the competition for substrate between microorganisms in the activated sludge processes [7].

PHA is synthesized and intracellularly accumulated in most bacteria under unfavorable growth condition such as limitation of nitrogen, phosphorus, oxygen or magnesium in the presence of excess supply of carbon source [4] and [8]. Strategies are still being developed to simulate conditions for efficient production of PHAs. [8] and [9]. Some bacteria such as *A. eutrophus*, *A. Latus* and mutant strain of *Azotobacter vinelandii* are known to accumulate PHA during growth in the absence of nutrient limitation.

Several factors need to be considered in the selection of a microorganism for the industrial production of PHA such as the ability of the cell to utilize an inexpensive carbon source, growth rate, polymer synthesis rate and the maximum extent of polymer accumulation of a particular cell based on the substrate. Some workers have derived equation that predicts the PHA yield on carbon sources [10] which could be used for the preliminary calculation of PHA yields. In order to reduce the overall cost, it is important to produce PHA with high productivity and yield.

Several methods such as fed-batch and continuous cultivations have been carried out to improve productivity [4] and [8]. Only three prominent PHAs [PHB, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and poly (3-hydroxyhexanoate-co-3-hydroxyoctanoate)] had been produced to a relatively high concentration with high productivity. Recently, workers have been exploring cultivation strategies involving inexpensive, renewable carbon substrates in order to reduce production cost and obtain high productivity [4], [11] - [14]. Recovery of PHA should also be considered because it significantly affects the overall process economics. The last stage of PHB production involves separating the polymer from the cells. To do this a solvent of aqueous extraction can be used. In the aqueous process, the cell walls are broken, and the polymer is then extracted and purified. The aqueous process is less expensive, but the process reduces the polymer molecular weight.

The research project is primarily intended to treat oily wastewater, and simultaneously to produce bio-plastic in terms of PHB (poly hydroxy butyric acid). The stirred tank reactor was used, and to enhance pollutant reduction and PHB accumulation, aeration was applied. Effect of sludge concentration, i.e. number of microbes worked for treatment, and effect of aeration, i.e. amount of aeration supplied were investigated.

II. MATERIALS AND METHODS

A. Oily wastewater and Sludge

Sample of sludge and wastewater was collected from Golden Jomalina Food Industry Sdn. Bhd. Golden Jomalina Sdn. Bhd. produces refined palm oil and palm kernel oil products which include shortening, margarine, frying palm oil, milk fat replacement, vegetable ghee and cooking oil. The industry also produces oil products for application in dairy products, coloring and as food ingredients. These products are exported to Japan, Hong Kong, Europe and United State of America. The sludge is collected from an aerobic tank, and oily wastewater is obtained directly from an outlet of the factory. Characteristics of wastewater and sludge are shown in Table 1 below.

TABLE I
CHARACTERISTIC OF WASTEWATER AND SLUDGE

Parameter	Sludge	Wastewater
TSS (mg/L)	17762.5	322.5
VSS (mg/L)	14700	215
COD (mg/L)	46246.5	3115
pH	6.48	6.30
Temperature (°C)	23.8	22.5
Oil & Grease (mg/L)	-	92.3
PHB (mg/L)	40.12	-

B. Sludge

The raw oily wastewater and SBR sludge from a palm oil refinery factory in Kuala Langat, Selangor was collected as the subjects for this study. These samples were collected using 25 liters containers and kept in the cold room (in the Environmental and Public Health Laboratory, Department of Civil and Environmental Engineering) at temperature range from three °C to four °C for preservation.

C. Experimental

Acclimatization is a process for microorganisms in the sludge to adapt to the new environment in wastewater and maintaining performance in different environmental condition. The organisms adjust their morphological, behavior, physical and chemical form so able to respond to the changes occurred. Acclimatization is carried out using a mixture of activated sludge, and wastewater collected in a 3:1 ratio in volume for five days. Every acclimatization process was carried out in a three liter stirred tank with a 6-blades up-pumping 45°-pitched blade impeller at 200 rpm.

The treatment process of oily wastewater was carried out in variation of aeration rate (zero aeration, low aeration 1.5L/min, high aeration 2.0L/min), and sludge volume (25%, 50%, and 75% equivalent to 4360mg/l, 8720mg/l, and 13080mg/l VSS respectively). All the treatment run are carried out in a three liters cylinder tank and stirred with a 6-blades up-pumping 45°-pitched blade impeller. To provide aeration, an air pump was utilized to provide different rate of air supply.

D. Analytical methods

Accumulation of PHB is measured using methods explained by Law Slepecky in Slepecky [15]. Chemical parameters, i.e., COD, VSS, oil and grease, were analyzed in accordance with methods in the standard methods (APHA, 1999).

III. RESULTS AND DISCUSSION

A. COD removal

The COD removal percentage is shown in Fig 1. COD removal was found in range of 85.1 to 97.1%. The graph shows that high aeration rate give highest removal percentage for chemical oxygen demand (COD). It can be seen that removal of COD increased with increasing aeration. The air supplied helps in degradation of organic matters in wastewater by the oxidation process.

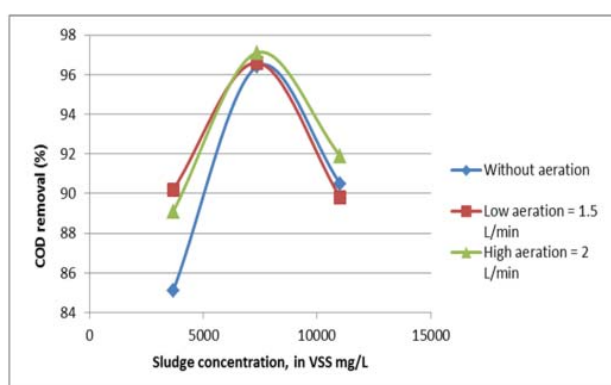


Fig. 1 Effect of aeration and sludge concentration on COD removal

Higher aeration provides more oxygen to oxidize and degrades the COD in the wastewater. The organic substances are readily degraded to simple organics by microorganisms in the treatment. Sludge concentration of 7350mg/L VSS is suitable for oily wastewater treatment as the sludge contains adequate microorganisms to remove organic substances in wastewater. It is comparable with previous study which stated 3000-6000mg/L MLSS can achieve higher treatment efficiency in activated sludge. The other operation parameters can be varied to improve the removal efficiency, and the best operational condition may apply in this study.

B. Oil and Grease removal

Fig. 2 shows oil and grease removal at different aeration rate. Oil and grease removal was found in range of 12.9 to 54.8%. It is obvious that from no aeration to higher aeration rate, the removal percentage increased. For run at sludge concentration of 7350mg/L VSS, the final effluent still contain high oil and grease concentration, as the removal percentage is low. Aerobic treatment of oil and grease is shown to be in low efficiency when oily wastewater is not diluted because oil is long chain organics and hard to be degraded. In conclusion, oxygen provided can help degrade oil and grease better.

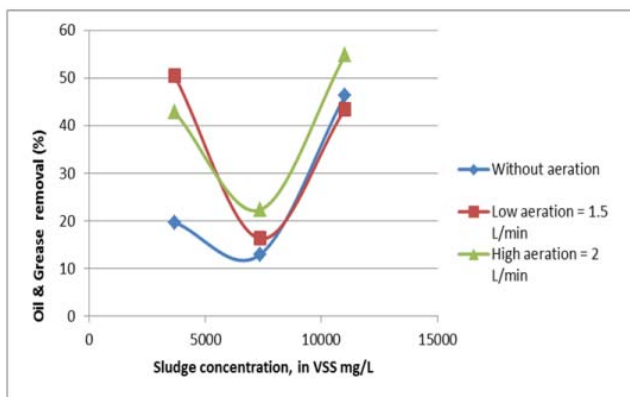


Fig.2 Effect of aeration and sludge concentration on oil and grease removal

Oil and grease degrade better when oxygen is supplied because it will react with organics and smaller of organic substances will be formed. Microorganisms can consume these simple organics for their growth and form as biomass. Higher sludge concentration indicates a high concentration of microorganisms, which reduce long chain organics better. However, the removal percentage is still low for oil and grease. Certain microorganisms can remove oil content well, but it may not exist in the sludge. Lower initial COD concentration in wastewater denotes oil and grease in wastewater has been reduced. The amount of oil is less, and a better removal can be achieved as organics to the microorganism ratio is decreased. A high sludge concentration produces a higher removal of oil and grease.

C. PHB yield

Fig 3 shown below is the graph of PHB yield at variation of aeration rate. It is indicated in the results that experiment runs without aeration supplied to get better PHB yield compared to low aeration and high aeration rate. PHB yield as PHB accumulation per mass of sludge removal was found in range of 0.15 to 2.4%. In most studies stated that with oxygen supplied, PHB production in activated sludge is higher than those without oxygen supplied. Small amount of organic substrate is oxidized and provide energy to microorganisms. However, the energy tends to utilize for cell growth rather than PHB accumulation. In addition, air supplied should be controlled as the percentage of air in activated sludge will affect the formation of PHB within the bacteria cell. Under the aerobic conditions, excess of organic carbon is required to improve the accumulation of PHB. Excess of organic carbon is available in the wastewater, but it probably is utilized for other purposes result in low yield of PHB.

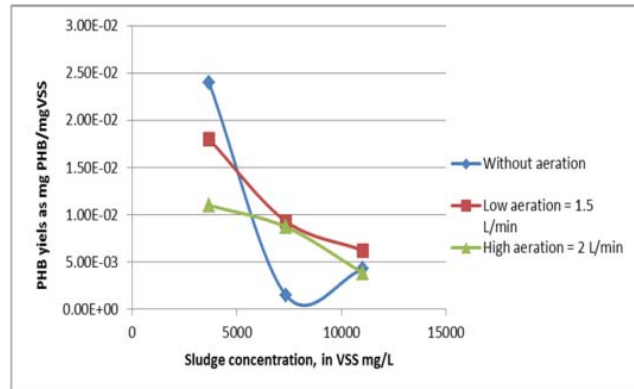


Fig.3 Effect of aeration and sludge concentration PHB yield as mgPHB/mg VSS

Fig 4 shows the PHB yield in terms of COD removal varied by aeration rate. It is shown in the graph that treatment without aeration produces a higher PHB yield in terms of COD removal. PHB yield per COD removed was found in range of 0.01 to 0.12%. When there is no aeration in treatment, microorganisms may gain energy from intracellular polyphosphate and enhance the PHB production rate. Polyphosphate degradation lead to high PHB production rate as polyphosphate can provide sustainable energy. In addition, energy also gained from oxidation by a small amount of air entered when mixing in treatment without aeration.

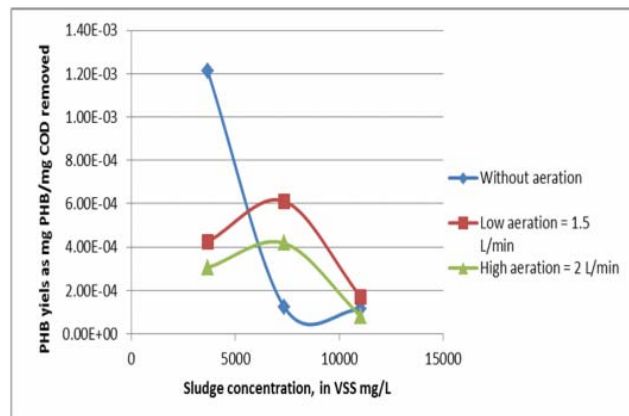


Fig.4 Effect of aeration and sludge concentration PHB yield as mgPHB/mgCOD removed

Lower sludge concentration (3675mg/L VSS) can yield more PHB per substrate provided is due to availability of excess substrate. With a low or a high aeration, sludge concentration of 7350mg/L VSS produce a higher PHB yield. Organic substrates are oxidized and provide energy for microorganisms to produce PHB. Energy supply is adequate for PHB accumulation compared to excess energy in lower sludge concentration (3675mg/L VSS), which tended to accumulate as other forms of energy.

For PHB yield in terms of PHB/VSS and PHB/COD removal, treatment without aeration can give a better result in

both terms. Microorganisms gain energy from degradation of polyphosphate to produce PHB. The rate of PHB production is higher in anaerobic condition, although higher PHB content can be accumulated in aerobic condition. High aeration can result in PHB degradation and low yield of PHB. Excess energy provided from oxidization of organics is used for other purposes such as microorganism's growth. Low sludge concentration accompanied by high concentration of wastewater as the ratio is 1:3 in volume. Thus, carbon substrate concentration is higher in smaller population of microorganisms. The substrate is enough for bacterial growth and lead to better yield of PHB. There is higher COD concentration in wastewater without dilution, so the PHB yield is higher as the organics are adequate for PHB production. The organics are utilized to produce PHB with energy provided.

IV. CONCLUSION

The experiment shows that a high removal percentage of COD and O&G can be obtained when a high aeration rate is applied in the treatment process. The COD and O&G removal increased when higher aeration rate is used. Accumulation of PHB is decreasing when higher aeration is applied. The PHB yield in process without aeration is higher than those with aeration. Low aeration can gain more PHB accumulation although the yield is low. The aerobic treatment is efficient and feasible in oily wastewater treatment but not suitable for accumulation of PHB.

Sludge concentration indicates the MLVSS of the activated sludge. It is found that sludge concentration of 7350mg/L VSS produces the highest COD removal while best result for removal of O&G obtained when the sludge concentration was 11025mg/L VSS. The yield of PHB can be seen to be highest when sludge concentration is 3675mg/L VSS. Low sludge concentration (3675mg/L VSS) can achieve a high removal of COD and O&G as well as PHB yield. However, accumulation of PHB is higher when sludge concentration is high. Further study should be carried out to investigate the treatment efficiency and PHB accumulation when a lower sludge concentration is applied,

REFERENCES

- [1] S. Lee and J. Yu. Production of biodegradable thermoplastics from municipal sludge by a two-stage bioprocess. *Resour Conserv Recy.*, vol. 19, pp. 151-164, 1997
- [2] H. Salehizadeh and M. C. M. Van Loosdrecht, "Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance," *Biotechnology Advances*, vol. 22, pp. 261-279, 2004.
- [3] M. C. M. Van Loosdrecht, J. J. Beun, and J. J. Heijnen, "Poly-[beta]-hydroxyalkanoate metabolism in activated sludge," in *Advances in Water and Wastewater Treatment Technology*, M. Tomonori, H. Keisuke, T. Satoshi, and S. Hiroyasu, Eds., ed Amsterdam: Elsevier Science B.V., 2001, pp. 239-248.
- [4] S. Y. Lee, K. S. Yim, H. N. Chang, and Y. K. Chang, "Construction of plasmids, estimation of plasmid stability, and use of stable plasmids for the production of poly(3-hydroxybutyric acid) by recombinant *Escherichia coli*," *Journal of Biotechnology*, vol. 32, pp. 203-211, 1994.
- [5] H. Shimizu, S. Tamura, S. Shioya, and K.-i. Suga, "Kinetic study of poly-d(-)-3-hydroxybutyric acid (PHB) production and its molecular weight distribution control in a fed-batch culture of *Alcaligenes eutrophus*," *Journal of Fermentation and Bioengineering*, vol. 76, pp. 465-469, 1993.
- [6] A.S.M., Chua, H. Takabatake, H. Satoh, and T. Mino. "Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent," *Water Res.* 37(15). Pp. 3602-3611.2003.
- [7] N. Azbar, (2004). A Review of Waste Management Options in Olive Oil Production. *Crit Rev Env Sci Tech.* vol. 34, no. 3, pp 209-247
- [8] G. Du and J. Yu, "Metabolic analysis on fatty acid utilization by *Pseudomonas oleovorans*: mcl-poly(3-hydroxyalkanoates) synthesis versus \square -oxidation," *Process Biochemistry* vol. 38, pp. 325-332, 2002.
- [9] G. Du, J. Chen, J. Yu, and S. Lun, "Continuous production of poly-3-hydroxybutyrate by *Ralstonia eutropha* in a two-stage culture system," *Journal of Biotechnology*, vol. 88, pp. 59-65, 2001.
- [10] J. Wang and J. Yu, "Kinetic analysis on inhibited growth and poly(3-hydroxybutyrate) formation of *Alcaligenes eutrophus* on acetate under nutrient-rich conditions," *Process Biochemistry*, vol. 36, pp. 201-207, 2000.
- [11] D. Byrom, "Production of poly-[beta]-hydroxybutyrate: poly-[beta]-hydroxyvalerate copolymers," *FEMS Microbiology Letters*, vol. 103, pp. 247-250, 1992.
- [12] T. Tsuge, K. Tanaka, and A. Ishizaki, "Development of a novel method for feeding a mixture of L-Lactic Acid and Acetic Acid in Fed Batch Culture of *Ralstonia eutropha*," *Journal of Bioscience and Bioengineering*, vol. 91, pp. 545-550, 2001.
- [13] Y. Poirier, "Production of new polymeric compounds in plants " *Current Opinion in Biotechnology*, vol. 10, pp. 181-185, 1999.
- [14] P. Kahar, T. Tsugea, K. Taguchi, and Y. Doi, "High yield production of polyhydroxyalkanoates from soybean oil by *Ralstonia eutropha* and its recombinant strain," *Polymer Degradation and Stability*, vol. 83, pp. 79-86, 2004.
- [15] J. H. Law and R. A. Slepecky, "Assay of Poly-b-hydroxybutyric acid," *Journal of Bacteriology*, vol. 82, pp. 33-36, 1961.
- [16] G. Braunegg, G. Lefebvre, and K. F. Genser, "Polyhydroxyalkanoates, biopolyesters from renewable resources-Physiological and engineering aspects," *Journal of Biotechnology*, vol. 65, pp. 127-161, 1998.
- [17] E. Y. Lee and C. Y. Choi, "Biosynthesis and Biotechnological Production of Degradable Polyhydroxyalkanoic Acid," *Biotechnol. Bioprocess Eng*, vol. 2, pp. 1-10, 1997.
- [18] B. Qu and J. Liu, "Determination of optimum operating conditions for production of polyhydroxybutyrate by activated sludge submitted to dyna[1]mic feeding regime," *Chinese Science Bulletin*, vol. 54, pp. 142-149, 2009.
- [19] G.-C. Du, J. Chen, H.-J. Gao, Y.-G. Chen, and S.-Y. Lun, "Effects of environmental conditions on cell growth and poly-b-hydroxybutyrate accumulation in *Alcaligenes eutrophus*," *World Journal of Microbiology & Biotechnology*, vol. 16, pp. 9-13, 2000.
- [20] L. S. Serafim, P. C. Lemos, M. G. Albuquerque, and M. A. Reis, "Strategies for PHA production by mixed cultures and renewable waste materials," *Applied microbiology and biotechnology*, vol. 81, pp. 615-28, Dec 2008.
- [21] K. Johnson, R. Kleerebezem, and M. C. van Loosdrecht, "Influence of the C/N ratio on the performance of polyhydroxybutyrate (PHB) producing sequencing batch reactors at short SRTs," *Water research*, vol. 44, pp. 2141-52, Apr 2010.
- [22] S. Shahhosseini, "Simulation and optimisation of PHB production in fed-batch culture of *Ralstonia eutropha*," *Process Biochemistry*, vol. 39, pp. 963-969, 2004.