EFFECT OF FAMINE-PHASE AERATION ON POLYHYDROXYALKANOATE ACCUMULATION IN AEROBIC GRANULES

VJAYAN THRUMAKUNASAGRAN

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by

VJAYAN THRUMAKUNASAGRAN

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LIST OF ABBREVIATIONS

ADF	Aerobic dynamic feeding
$AgNO_3$	Silver nitrate
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid assay
BOD	Biochemical oxygen demand
BPA	Bisphenol A
CDW	Cell dry weight
$CHCl_3$	Chloroform
COD	Chemical oxygen demand
DO	Dissolved oxygen
DOE	Department of Environment Malaysia
EPS	Extracellular polymeric substances
FFB	Fresh fruit bunch
GC	Gas chromatography
H/D	Height to diameter ratio
H_2SO_4	Sulphuric acid
HB	Hydroxybutyrate
$HgSO_4$	Mercury sulphate
HHx	Hydroxyhexanoate
HRT	Hydraulic retention time
HV	Hydroxyvalerate
$K_2Cr_2O_7$	Potassium dichromate (VI)
MCL-PHA	Medium chain length PHA

MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NADH	Nicotinamide adenine dinucleotide hydrogenase
NaOCl	Sodium hypochlorite
Na_2SO_4	Sodium sulphate
OLR	Organic loading rate
P3(HB-co-HHx)	Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate)
P3(HB-co-HV)	Poly (3-hydroxybutyrate-co-3-hydroxyvalerate)
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHHx	Polyhydroxyhexanoate
PHV	Polyhydroxyvalerate
PN	Protein
POME	Palm oil mill effluent
PS	Polysaccharide
SBB	Sudan Black B
SBR	Sequencing batch reactor
SCL-PHA	Short chain length PHA
SVI	Sludge volume index
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
VER	Volume exchange ratio
VFA	Volatile fatty acids

LIST OF SYMBOLS

$K_L a$	Oxygen diffusion rate
$Power_{air\ compressor}$	Maximum power of the air compressor
$-q_{Acetic}$	Acetic acid utilization rate
$-q_{Propionic}$	Propionic acid utilization rate
$-q_{Butyric}$	Butyric acid utilization rate
$-q_{Valeric}$	Valeric acid utilization rate
V_f	Final VFA concentrations
V_i	Initial VFA concentrations
$Y_{PHA/CDW}$	PHA content

KESAN PENGUDARAAN DALAM FASA SUBSTRAT TERHAD BAGI PENUMPUKAN POLIHIDROKSIALKANOAT DALAM BUTIRAN AEROBIK

ABSTRAK

Polihidroksialkanoat (PHA) adalah polimer biodegradasi yang telah menarik minat dalam kalangan para penyelidik akhir-akhir ini. PHA boleh dihasilkan dengan menggunakan butiran aerobik yang merawat air sisa. Walau bagaimanapun, kos pengeluaran yang tinggi, terutamanya disebabkan oleh penggunaan pengudaraan yang tinggi, telah mengehadkan aplikasi biopolimer ini. Dalam kajian ini, kesan pengudaraan yang berubah-ubah dalam fasa substrat terhad ke atas keupayaan penumpukan PHA dan morfologi butiran aerobik telah disiasat. Empat reaktor penjujukan berkelompok telah diinokulasi dengan butiran aerobik dan dikendalikan pada kadar pengudaraan yang berbeza menggunakan air sisa kilang minyak sawit sebagai substrat tunggal. Kadar pengudaraan berterusan sebanyak 2.0 L/min digunakan dalam fasa substrat berlebihan (FSB) dan fasa substrat terhad (FST) untuk Reaktor 1 (kawalan). Sementara itu, kadar pengudaraan semasa FSB dalam Reaktor 2, 3 dan 4 adalah sama dengan Reaktor 1 manakala kadar pengudaraan semasa FST dikurangkan kepada 1.0 L/min, 0.5 L/min dan 0 L/min masing-masing dalam Reaktor 2, 3 dan 4. Keputusan Reaktor 1 menunjukkan bahawa lebih daripada 90% suapan COD berjaya disingkirkan secara purata dan kandungan PHA maksimum sebanyak 56% berat kering sel telah ternumpuk di dalam butiran aerobik. PHA yang terkumpul adalah kopolimer poli (3-hidroksibutirat-co-3-hidroksivalerat) (P3 (HB-co-HV)) dengan kandungan hidroksivalerat (HV) 21%. Butiran aerobik

mempunyai saiz butiran purata, indeks isipadu enapcemar dan kandungan substrat polimerik sel (EPS) masing-masing sebanyak 1030 μm, 41 mL / g dan 829.50 μg / g biojisim. Sementara itu, kajian pengudaraan yang berubah-ubah mendedahkan bahawa semua butiran aerobik mencapai penyingkiran COD dan kandungan PHA yang sama, tanpa mengira kadar pengudaraan yang digunakan dalam FST. Terutamanya, apabila kadar pengudaraan yang rendah digunakan semasa FST, masa yang diambil untuk mencapai kandungan PHA maksimum telah menjadi singkat dengan ketara. Tambahan pula, perubahan komposisi PHA berlaku, di mana kandungan HV telah meningkat. Tambahan lagi, pengurangan kadar pengudaraan telah meningkatkan jumlah kandungan EPS dalam butiran aerobik, secara tidak langsung meningkatkan keupayaan pengenapan butiran. Pengurangan kadar pengudaraan dari 2.0 L/min ke 0.5 L/min telah mengakibatkan peningkatan saiz butiran dengan peratusan jumlah tertinggi. Sebaliknya, ketebalan lapisan mikroorganisma penumpukan PHA pada permukaan butiran didapati telah menurun apabila kadar pengudaraan dalam FST dikurangkan. Secara keseluruhan, hasil kajian menunjukkan bahawa keperluan pengudaraan dalam FST untuk penumpukan PHA dalam butiran aerobik adalah kurang penting. Penghasilan PHA dalam butiran aerobik di bawah pengudaraan sifar dalam FST boleh mengakibatkan pengurangan tenaga sehingga 74%, yang pada dasarnya dapat mengurangkan kos pengeluaran keseluruhan biopolimer ini.

EFFECT OF FAMINE-PHASE AERATION ON POLYHYDROXYALKANOATE ACCUMULATION IN AEROBIC GRANULES

ABSTRACT

Polyhydroxyalkanoate (PHA) is a biodegradable polymer which has attracted a lot of interests among researchers lately. PHA can be feasibly produced using aerobic granules treating wastewater. However, high production costs, particularly due to the high aeration used, have limited the applications of this biopolymer. In this study, the effect of variable aeration rates in the famine period on the PHA accumulating capability and morphology of aerobic granules was investigated. Four sequencing batch reactors were inoculated with aerobic granules and operated at different aeration rates using palm oil mill effluent as the sole substrate. A feast-famine constant aeration rate of 2.0 L/min was used for Reactor 1 (control). Meantime, the feast period aeration rate of Reactor 2, 3 and 4 was similar to that of Reactor 1 while the famine period aeration rate was decreased to 1.0 L/min, 0.5 L/min and 0 L/min in Reactor 2, 3 and 4, respectively. The findings of Reactor 1 show that more than 90% of the influent chemical oxygen demand (COD) was removed on average and a maximum PHA content of 56% cell dry weight was accumulated in the aerobic granules. The PHA accumulated was a copolymer of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (P3(HB-co-HV)) with 21 % hydroxyvalerate (HV) content. The aerobic granules had an average granular size, sludge volume index and extracellular polymeric substance (EPS) content of 1030 μm, 41 mL/g and 829.50 μg/g biomass, respectively. Meanwhile, the variable aeration study reveals that regardless of the

aeration rates used in the famine period, all aerobic granules achieved a similar COD removal and PHA content. Notably, under a famine period with low aeration rate, the time taken to reach the maximum PHA content had decreased significantly. Moreover, PHA compositional changes occurred, where the HV content had increased. Further, the decrease in the famine period aeration rate enhanced the total EPS content of the aerobic granules, indirectly improving the settling ability of the granules. The reduction in aeration rate from 2.0 L/min to 0.5 L/min has resulted in an increased size of granules with the highest volume percentage. On the other hand, the thickness of the layers of PHA accumulating microorganisms at the surface of the granules was found to have decreased when the famine period aeration rate was reduced. Overall, the results indicate that the requirement of aeration for PHA accumulation in aerobic granules is highly insignificant in the famine period. PHA production in aerobic granules under zero aeration in the famine period may result in an energy input reduction of up to 74%, which could essentially reduce the overall production cost of this biopolymer.

CHAPTER ONE

INTRODUCTION

1.1 Plastics in Malaysia

The plastic industry is one of the booming industries in Malaysia's manufacturing sector. It can be divided into mainly seven sub-sectors with the packaging sub-sector being the largest market, consuming approximately 45% of Malaysia's plastic production (Rahim and Raman, 2017). The extensive development of petrochemical based plastic packaging industry has made plastics an everyday use material and is indispensable in modern life. Most manufactured plastics are derived from petroleum resources. This results in conventional plastics having non-biodegradability nature. However, the increased use of packaging plastics due to easy availability and low cost have caused more of it to be disposed by landfilling. Malaysians generate almost 1.2 million tonnes of solid wastes annually in which 11.2% of it consists of plastic wastes (Rahim and Raman, 2017). Over the years, the non-biodegradable nature of plastics will cause a net accumulation of it, eventually taking up more space on the landfill area.

One possible long term solution would be to ensure complete disposal of plastics without being piled up on the landfill area. In that case, biodegradable plastics are the safest option to ensure pollution free elimination of plastic waste from landfill areas. These plastics can be broken down by microorganisms through anaerobic degradation, producing methane gas as the end product which are typically captured as "landfill gas" for power generation (Fazeli et al., 2016). As a result, the accumulation of biodegradable plastic would not cause a significant environmental concern as it can degrade similar to other organic solid wastes in landfills. One particular biodegradable plastic that has been investigated extensively is polyhydroxyalkanoate (PHA) (Jiang et al., 2011, Marang et al., 2013, Chen et al., 2015).

PHAs are polyesters of various hydroxyalkanoates that are naturally synthesised by selected microorganisms as carbon and energy source in the presence of excess carbon source under conditions of limiting nutrients (Salehizadeh and Van Loosdrecht, 2004). The enrichment of PHA-accumulating microorganisms is generally achieved through an aerobic dynamic feeding (ADF) strategy, which incorporates feast–famine regimes in a sequencing batch reactor (SBR). This strategy involves the intermittent feeding of substrates, where microorganisms are alternately exposed to an external substrate for consumption and intracellular PHA storage during the feast period and to starvation during the famine period where the accumulated PHA will be oxidised for growth and maintenance purpose. Commercial production of PHA involves the supply of sugar-based compounds for consumption by pure cultures of single strain microorganisms and subsequent intracellular PHA accumulation. The microorganisms are then subjected to PHA recovery process by solvent extraction. However, the use of pure cultures and sugar based substrates increases the production cost of PHA. Thus, attentions have shifted towards the use of mixed culture and waste based substrates.

One such potential substitute for sugar would be fatty acids. Fatty acids could be produced via acidogenesis digestion of agro-based industrial wastewater. In the context of Malaysia, one of the largely produced agro-based industrial wastewater is palm oil mill effluent (POME). POME usually contains large amount of organic components that requires biological treatment prior to discharge into receiving body. Part of the biological treatment involves anaerobic treatment where POME undergoes hydrolysis, acidogenesis and methanogenesis. During the acidogenesis process of the POME, volatile fatty acids (VFA) are produced. Thus, acidogenically digested POME could be used as substrate for PHA production.

The use of mixed culture for PHA production has been a subject of interest in recent times due to its lower production cost. It eliminates the need for sterile conditions. Conventionally, activated sludge is used as the mixed culture inoculum for PHA production. However, the volumetric productivity of the PHA using activated sludge is uncompetitive compared to pure culture in terms of total PHA yield. This could be potentially solved by transforming the sludge into aerobic granules. Aerobic granules are agglomeration of aerobically grown microorganisms in a spherical shape (Arrojo et al., 2004). These granules are compact, dense and have clear shape boundary with excellent settling ability (Liu et al., 2004). Primarily, aerobic granules are developed and used in biological treatment process of wastewater treatment plant. Thus, aerobic granules have been successfully developed in acidogenically digested POME using SBR (Gobi et al., 2011). Interestingly, cultivation of aerobic granules in wastewater treatment systems also uses ADF strategy. The feast-famine period is useful in initiating the cell-to-cell interaction process (McSwain et al., 2004). The use of the common ADF strategy to accumulate PHA in microorganisms and to develop aerobic granules has led to successful demonstrations of PHA accumulation in such granules in recent times (Gobi and Vadivelu, 2014, Wang et al., 2014). The implementation of aerobic granular PHA synthesis ensures high biomass density, which may result in the high volumetric productivity of the biopolymer.

1.2 Problem Statement

Despite the intrinsic potential of using aerobic granules to accumulate PHA, the major setback in its commercial application is the high production cost, particularly due to the high aeration used in the process, which limits the growth of this novel PHA production method. Aeration contributes to the highest energy input in ADF-based PHA production using aerobic granules (Akiyama et al., 2003) since it is important for providing dissolved oxygen and hydrodynamic shear force. Whereas oxygen is needed for substrate degradation and PHA accumulation, the hydrodynamic shear force is needed for aerobic granulation. A high hydrodynamic shear force has been proven to produce compact, dense, and strong granules (Liu and Tay, 2002). In current production processes, aerobic granules are subjected to a high hydrodynamic shear force through provision of a high aeration rate. The failure to do so results in the formation of fluffy flocs, which disturbs the system and results in low treatment efficiency (Tay et al., 2004). On another note, information about the aeration requirements for PHA production in mixed cultures is limited.

However, current aerobic granulation and PHA accumulation processes imposed constant aeration during both the feast and famine periods. During the feast period, the microorganisms oxidize the available external substrate using oxygen and convert it into storage products such as PHA and extracellular polymeric substance (EPS) (Serafim et al., 2004). EPS will be mainly used to initiate cell-cell interactions and facilitate aerobic granulation (Tay et al., 2001b) while PHA acts as a storage product to provide carbon and energy source. On the other hand, during the famine period, the storage products will be oxidized to provide energy for growth and maintenance purposes of the cells (Salehizadeh and Van Loosdrecht, 2004). Previous studies clearly showed that the feast period takes up to only 20% of the overall cycle length during PHA accumulation, while the famine period can take up to 80% (Carta et al., 2001, Coats et al., 2016, Wang et al., 2017). Furthermore, in a typical SBR operation involving ADF PHA production using aerobic granules, a high oxygen demand was encountered during the feast period due to oxygen consumption for external substrate oxidation, whereas the oxidation of internally stored PHA required minimal oxygen during the famine period (Gobi and Vadivelu, 2014). Taking this into consideration, the reduction in aeration during the famine period can contribute to significant energy and cost reductions for commercial PHA production. Ironically, the impact of reduced aeration during the famine period of aerobic granular PHA production is a specific area of study that has not been explored until now. Thus, the significance of famine-period aeration on morphology of aerobic granules and PHA accumulation remains relatively unknown.

Consequently, a comprehensive investigation is needed to claim that PHA could be produced in aerobic granules developed from POME under lower aeration energy inputs in terms of reduced aeration in the famine period. In that regard, the understanding of the PHA accumulation process in aerobic granules is required. The behavior of the PHA accumulation in response to varying famine period aeration rate is unknown. Simultaneously, the morphological changes of the aerobic granules in response to reduced aeration rate are worth studying. Apart from that, the distribution of PHA accumulating microorganisms in the aerobic granule is yet to be reported. The location of PHA accumulating cells within the granules is worth studying for inclusive understanding of the process. By addressing these knowledge gaps, the PHA accumulation in aerobic granules under reduced famine-period aeration rate could be evaluated for its feasibility in upscaling it from lab to industrial scale.

1.3 Objectives

The primary aim of this study is to accumulate PHA in aerobic granules developed using POME in SBR at a lower aeration energy input in the famine period. The specific objectives are:

- To assess the PHA accumulation process in aerobic granules under constant aeration.
- To study the effect of reduced aeration during the famine period on the PHA accumulation process in aerobic granules.
- To investigate the morphological changes of aerobic granules operated under reduced famine-period aeration rate.
- To determine the location of PHA accumulating microorganisms in the aerobic granules under reduced aeration in the famine period.

1.4 Scope of study

This entire research centers on the accumulation of PHA within the aerobic granules at lab scale. A SBR containing aerobic granules were fed with POME as the sole substrate at a constant organic loading rate and aeration rate. The PHA accumulation process and the morphology of aerobic granules were evaluated. The duration of feast and famine period, chemical oxygen demand removal capability, PHA content, composition of the PHA, granule size distribution, settling ability and EPS content were identified.

Thereafter, the effect of reduced famine-period aeration rate on the PHA accumulation process in the aerobic granules was investigated. The aerobic granules were aerated at a lower rate in the famine period as compared to the feast period.

Upon achieving stable SBR operations, the quantity and quality of PHA accumulated in the aerobic granules was evaluated.

Besides that, the morphological changes of the aerobic granules at lower famine period aeration rates were assessed. The scope was limited to evaluating the granule size distribution, EPS content, sludge volume index and structure (surface morphology) of the aerobic granules.

Subsequently, the aerobic granules operated under various famine-period aeration rates were subjected to histological analysis to obtain cross sections of aerobic granules at respective famine-period aeration rates. The cross sections were stained to identify the location of PHA accumulating cells. The identification of the distribution of PHA accumulating cells in the granules develops better understanding of the correlation between substrate diffusion and PHA accumulation in the granules.

The entire findings of this research are limited to lab scale only. Further research is required to implement this technology at pilot plant or real plant scale. However, the parameters studied in this work could be used to develop a more cost and energy effective PHA production plant.

1.5 Organization of thesis

In this thesis, there are five major chapters. Chapter 1 (Introduction) introduces PHA, POME and aerobic granules briefly. Besides that, the necessity of this project (Problem statement) to be done is explained in this chapter. Following that, objectives of this research are given in detail. Together with this information, scope of this research and organization of this thesis are given in Chapter 1.

Meanwhile in Chapter 2 (Literature review), literature review on the topic of research is given. In this chapter, extensive literature reviews are given based on the previous research works. This chapter is vital in introducing the technical terms and ideas regarding the topic of this research.

In Chapter 3 (Methodology), materials and methods used for this entire research is given. Materials used such as chemicals, reactors and analytical tools are explained thoroughly in this chapter. Additionally, the methods used for operating the SBR to accumulate PHA in aerobic granules at various famine period aeration rates, PHA extraction, morphological and histological analysis are explained elaborately in this chapter.

Next, in Chapter 4 (Results and Discussion), the findings of this entire research are given. First, the PHA accumulation process in the aerobic granules is evaluated. The composition of PHA produced and the morphology of the granules are determined. In the next subchapter, the effect of reduced aeration in the famine period on the PHA accumulation process is investigated. Subsequently, the morphological changes of the aerobic granules at reduced aeration rate are analysed. In the final sub chapter, the distribution of PHA accumulating cells in the aerobic granules is identified and discussed for its effect on the PHA accumulation process.

In Chapter 5 (Conclusions and Recommendations), which is the final chapter of this thesis. In this chapter, the conclusions of this work are given in detail. Subsequently, the outcome of this research is used as a basis to recommend future research opportunities in this area of study.