UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

Development and Application of Granular Biomass in Palm Oil Mill Effluent (POME) Treatment

By
Dr. Vel Murugan Vadivelu

2014

UNIVERSITI SAINS MALAYSIA

FINAL REPORT OF EXTERNAL GRANT
Laporan Akhir Geran Agensi Luar

Confidentiality of the report : ☐ **Confidential** ☐ **Not Confidential**
Kerahsian laporan : *Sulit* *Tidak Sulit*

A.	**PARTICULARS OF RESEARCH** / *MAKLUMAT PENYELIDIKAN:*
(i)	**Title of Research: DEVELOPMENT AND APPLICATION OF GRANULAR BIOMASS IN PALM OIL MILL EFFLUENT (POME) TREATMENT**
(ii)	**Account Number: 304/ PJKIMIA/6050233/I100**
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader: Dr. Vel Murugan Vadivelu**
	Name of Co-Researcher : -
(ii)	**School/Institute/Centre/Unit : Pusat Pengajian Kejuruteraan Kimia** *Pusat Pengajian /Institut/Pusat/Unit :*
C.	**Research Platform (Please tick (/) the appropriate box):** *Pelantar Penyelidikan (Sila tanda (/) kotak berkenaan):*

- ☐ **A. Life Sciences** *Sains Hayat*
- ☐ **B. Fundamental** *Fundamental*
- ☑ **C. Engineering & Technology** *Kejuruteraan & Teknologi*
- ☐ **D. Social Transformation** *Transformasi Sosial*
- ☐ **E. Information & Communications Technology (ICT)** *Teknologi Maklumat & Komunikasi*
- ☐ **F. Clinical Sciences** *Sains Klinikal*
- ☐ **G. Biomedical & Health Sciences** *Bioperubatan Sains Kesihatan*

Tutup Geran.

21/2/15

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D.	**Duration of this research :** *Tempoh masa penyelidikan ini :* ***Duration : 2 years** *Tempoh :* **From** : **01 April 2012** **To** : **31 Mac 2014** *Dari:* *Ke :*
E.	**ABSTRACT OF RESEARCH** (An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English**. This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large) Palm oil mill effluent (POME) discharged without proper treatment could cause severe environmental problem. The conventional biological treatment method using activated sludge is considered obsolete. The high COD and turbidity level has been the driving force to find an efficient treatment. Hence, in this work, aerobic granular biomass was developed and utilized for the treatment of POME in the sequencing batch reactor (SBR). The mean diameter of the developed aerobic granules was 0.9 mm (with a maximum value of 3.1 mm). The aerobic granules are able to remove 88% of the influent COD at organic loading rate (OLR) of 3.0 kg COD/m^3day. The increase of OLR to 6.0 kg COD/m^3day did not affect the COD removal efficiency and the aerobic granules immediately responded to the OLR increase. The sludge volume index (SVI) of the biomass reduced from 80 to 30 ml/g. Aerobic dynamic feeding (ADF) strategy was applied to accumulate polyhydroxyalkanoate (PHA) in aerobic granules. The volatile fatty acids (VFAs) in the POME are the sole source of the PHA accumulation. In this work, 100% removal of propionic and butyric acids in the POME were observed. The highest amount of PHA produced was 0.6833 mg PHA/mg biomass. Efluen kilang kelapa sawit (POME) yang dilepaskan tanpa rawatan yang sewajarnya boleh menyebabkan masalah alam sekitar yang teruk. Kaedah rawatan biologi konvensional yang menggunakan enapcemar teraktif dianggap usang. Nilai COD dan tahap kekeruhan yang tinggi menjadi penggerak untuk mencari rawatan yang lebih cekap. Dalam kajian ini, butiran aerobic telah dihasilkan dan digunakan bagi rawatan POME dalam reaktor penjujukan (SBR). Garis pusat min butiran aerobik yang dihasilkan adalah 0.9 mm (dengan nilai maksimum 3.1 mm). Butiran aerobik tersebut dapat menyingkir 88% COD influen bagi kadar bebanan organik (OLR) sebanyak 3.0 kg COD hari/m^3. Peningkatan OLR kepada 6.0 kg COD hari/m^3 tidak menjejaskan kecekapan penyingkiran dan butiran aerobik segera bertindak balas kepada peningkatan OLR itu. Indeks isipadu enapcemar (SVI) biojisim menurun dari 80 ke 30 ml/g. Strategi aerobic dynamic feeding (ADF) telah digunakan untuk mengumpul polyhydroxyalkanoate (PHA) dalam butiran aerobik. Volatile fatty acids (VFA) dalam POME adalah satu-satunya sumber pengumpulan PHA. Dalam kajian ini, 100% penyingkiran asid propionik dan butyric dalam POME berjaya dicapai. Jumlah tertinggi PHA yang dihasilkan adalah 0.6833 mg PHA/biomass mg.
F.	**SUMMARY OF RESEARCH FINDINGS** *Ringkasan dapatan Projek Penyelidikan* • **Please refer attachment**

RECEIVED 20 JAN 2015 Pej. Pengarah RCMO

G. **COMPREHENSIVE TECHNICAL REPORT**

Laporan Teknikal Lengkap

Applicants are required to prepare a comprehensive technical report explaining the project.
(This report must be attached separately)
Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Laporan ini mesti dikepilkan]

- **Please refer attachment**

List the key words that reflect your research:
Senaraikan kata kunci yang mencerminkan penyelidikan anda:

English	Bahasa Malaysia
Granular Biomass	Biomas Butiran
Palm oil mill effluent	Efluen Kilang Kelapa Sawit
Polyhydroxyalkanoate (PHA)	Polyhydroxyalkanoate (PHA)

H. **a) Results/Benefits of this research**
Hasil Penyelidikan

No. *Bil:*	Category/Number: *Kategori/ Bilangan:*	Promised	Achieved
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)*	-	3
2.	**Human Capital Development**		
	a. Ph. D Students	-	-
	b. Masters Students	-	-
	c. Undergraduates (Final Year Project)	-	-
	d. Research Officers	-	-
	e. Research Assistants	-	2
	f. Other: Please specify		
3.	**Patents** *Paten*	-	-
4.	**Specific / Potential Applications** *Spesifik/Potensi aplikasi*	-	-
5.	**Networking & Linkages** *Jaringan & Jalinan*	-	-
6.	**Possible External Research Grants to be Acquired** *Jangkaan Geran Penyelidikan Luar Diperoleh*	-	-
7.	**Others** *Lain-lain*	-	-

- Kindly provide copies/evidence for Category 1 to 7.

b) Equipment used for this research.
Peralatan yang telah digunakan dalam penyelidikan ini.

Items *Perkara*	Approved Equipment	Approved Requested Equipment	Location
Specialized Equipment Peralatan khusus		Sharp 510L 2 Door Plasmacluster Refrigerator	School of Chemical Engineering, USM
Facility Kemudahan			
Infrastructure Infrastruktur			

- Please attach appendix if necessary.

I.	**BUDGET** / *BAJET* **Total Approved Budget** : RM **Total Additional Budget** : RM **Grand Total of Approved Budget** : RM **36, 070.00** **Yearly Budget Distributed** Year 1 : RM **36, 070.00** Year 2 : RM Year 3 : RM **Additional Budget Approved** Year 1 : RM Year 2 : RM Year 3 : RM **Total Expenditure** : **RM 35150.72** **Balance** : **RM 919.28** • **Please attach final account statement from Bursary**
J.	**RECOMMENDATIONS** / *CADANGAN* (if appropriate, provide recommendations on the activities or other steps that may be taken to further develop, disseminate or to exploit commercially the results of the project Jika berkenaan, sila beri cadangan aktiviti atau langkah-langkah lain yang boleh diambil untuk membangun dan mengembangkan hasil projek ke peringkat seterusnya.) I've applied for second (and final) continual IFS grant.

Vemo Vadivelu

Signature of Researcher
Tandatangan Penyelidik

13 January 2015
Date
Tarikh

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K.	**COMMENTS OF PTJ'S RESEARCH COMMITTEE** *KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ*

General Comments:
Ulasan Umum:

Good publication record

PROFESSOR DR AHMAD ZUHAIRI ABDULLAH
Deputy Dean (Research)
School of Chemical Engineering
Universiti Sains Malaysia, Engineering Campus
14300 Nibong Tebal, Penang, Malaysia.

Signature and Stamp of Chairperson of PTJ's Evaluation Committee
Tandatangan dan Cop Pengerusi Jawatankuasa Penilaian PTJ

Date : .....
Tarikh :.....

Signature and Stamp of Dean/ Director of PTJ
Tandatangan dan Cop Dekan/ Pengarah PTJ

Date : .....
Tarikh :.....

17/1/15

PROFESOR AZLINA HARUN @ KAMARUDDIN
Dekan
Pusat Pengajian Kejuruteraan Kimia
Kampus Kejuruteraan
Universiti Sains Malaysia, Seri Ampangan
14300 Nibong Tebal, Seberang Perai Selatan
Pulau Pinang.

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UNIVERSITI SAINS MALAYSIA

JABATAN BENDAHARI
PENYATA PERBELANJAAN SEHINGGA 11 NOVEMBER 2014

Projek :

No. Akaun : 304.PJKIMIA.6050233.I100

Vot	Nama Vot	Peruntukan Projek	Perbelanjaan Terkumpul Sehingga Thn Lalu	Baki Peruntukan Tahun Lalu	Peruntukan Thn Semasa	Pendahuluan	Pendapatan Semasa	Jumlah Peruntukan Thn Semasa	Tanggungan Semasa	Bayaran Thn Semasa	Jum Belanja Thn Semasa	Baki Projek
111	GAJI	-5,737.20	0.00	-5,737.20	0.00	0.00	0.00	-5,737.20	0.00	3,763.03	3,763.03	-9,500.23
221	PERJALANAN DAN SARA HIDUP	-1,268.60	0.00	-1,268.60	0.00	0.00	0.00	-1,268.60	0.00	0.00	0.00	-1,268.60
224	SEWAAN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.03	78.03	-78.03
227	BEKALAN DAN BAHAN	-20,585.49	0.00	-20,585.49	0.00	0.00	0.00	-20,585.49	0.00	0.00	0.00	-20,585.49
229	LAIN PERKHIDMATAN IKTISAS & HOSPITALITI	-72.00	0.00	-72.00	0.00	0.00	0.00	-72.00	0.00	894.62	894.62	-966.62
335	HARTA MODAL	-1,850.00	0.00	-1,850.00	0.00	0.00	0.00	-1,850.00	0.00	0.00	0.00	-1,850.00
442	PEMBERIAN DALAM NEGERI	-901.75	0.00	-901.75	0.00	0.00	0.00	-901.75	0.00	0.00	0.00	-901.75
777	SUMBANGAN	36,070.00	0.00	36,070.00	0.00	0.00	0.00	36,070.00	0.00	0.00	0.00	36,070.00
	Jumlah	5,654.96	0.00	5,654.96	0.00	0.00	0.00	5,654.96	0.00	4,735.68	4,735.68	919.28

Penyata ini adalah cetakan komputer tiada tandatangan diperlukan
Penyata ini adalah dianggap tepat jika tiada maklumbalas dalam tempoh masa 14 hari dari tarikh penyata
Kawalan perbelanjaan bagi akaun ini adalah berasaskan pendapatan

INTERNATIONAL FOUNDATION FOR SCIENCE (IFS),

RESEARCH GRANT

FINAL REPORT

VEL MURUGAN VADIVELU

UNIVERSITI SAINS MALAYSIA, MALAYSIA

DEVELOPMENT AND APPLICATION OF GRANULAR BIOMASS IN PALM OIL MILL EFFLUENT (POME) TREATMENT

Grant Agreement No. W/5071-1

01 April 2012 – 31 Mac 2014

SUM0MARY

Palm oil mill effluent (POME) discharged without proper treatment could cause severe environmental problem. The conventional biological treatment method using activated sludge in series of ponds is considered obsolete. The high COD and turbidity level of POME has been the driving force to find an efficient treatment system. Hence, in this work, aerobic granular biomass was developed and utilized for the treatment of POME in the sequencing batch reactor (SBR). The mean diameter of the developed aerobic granules was 0.9 mm (with a maximum value of 3.1 mm). The aerobic granules are able to remove 88% of the influent COD at organic loading rate (OLR) of 3.0 kg COD/m^3 day. The increase of OLR to 6.0 kg COD/m^3 day did not affect the COD removal efficiency and the aerobic granules immediately responded to the OLR increase. The sludge volume index (SVI) of the biomass reduced from 80 to 30 ml/g.

Aerobic dynamic feeding (ADF) strategy was applied to the aerobic granules in sequencing batch reactor (SBR) to accumulate polyhydroxyalkanoate (PHA) in aerobic granules. The volatile fatty acids (VFAs) in the POME are the sole source of the PHA accumulation. In this work, 100% removal of propionic and butyric acids in the POME were observed. The highest amount of PHA produced in aerobic granules was 0.6833 mg PHA/mg biomass.

INTRODUCTION

Palm oil mill effluent (POME) is one of the enormous waste discharges from the oil palm producing mill. At present, almost 85% of the palm oil companies are using biological treatment to treat the POME prior to its release to the receiving bodies (Vijayaraghavan et al., 2007). However, the microorganism involved in this treatment is fragile in nature. Hence, the performance of the biological treatment system could be varying throughout the treatment process, subsequently leads to variable effluent quality. Generally, the POME will undergo neutralization and cooling process before the biological treatment. It was done to safeguard the microorganisms from the fresh POME, which is acidic and discharged at high temperature.

In order to totally offset this problem, researchers have been enthusiastically looking for a solution to treat the POME. Several researchers managed to come up with novel technologies in POME treatment system such as membrane technology (Ahmad et al., 2009), adsorption (Ahmad et al., 2005b) and coagulation-flocculation treatment (Bhatia et al., 2007). Nevertheless, these technologies are either costly or ineffective in large scale.

Therefore, the search for the feasible technology in terms of cost and efficiency continue to attract the attention of the researchers. One of the ways to approach this problem is by upgrading the current biological treatment system of POME. It could be done by the means of digesting the POME in a sequencing batch reactor (SBR). The land area needed to build the SBR treatment system could be reduced by 80% and the pipe works required for it is minimal compared to conventional biological treatment plant (de Bruin et al., 2004). These reasons have made the researchers to shift their attention towards the SBR technology for wastewater treatment. Some

of the earlier works proven to be successful in improving the wastewater quality via SBR technology (Arrojo et al., 2004; Wang et al., 2007). In fact, several researchers have proven that the POME could be treated in the SBR and it has some commendable performance (Fun et al., 2007).

Though SBR is one of the alternatives for the treatment of POME, but the microorganism in the form of activated sludge will not provide fast and excellent separation between sludge and treated POME. To overcome that problem, the microorganisms have to be transformed into more stable and robust structure while treating the POME to enable them to have better settleability, withstand any shock organic loadings of the POME and prevent biomass washout (Tay et al., 2002; Adav et al., 2008). The transformation of the microorganism from loose into stable and robust structure under the influence of oxygen is called aerobic granulation process. Aerobic granulation happens due to the aggregation of self-immobilized microorganism present in the reactor (Shi et al., 2010). Aerobic granulation could be influenced by several factors such as hydrodynamic shear force, settling time, substrate composition, feeding strategy and dissolved oxygen (Kong et al., 2009).

Apparently, aerobic dynamic feeding strategy (ADF) is a common strategy for both the formation of aerobic granules and the accumulation of polyhydroxyalkanoate (PHA) (Serafim et al., 2004). Thus, it can be deduced that aerobic granules can accumulate PHA. However, the interaction between PHA storage and aerobic granulation technology has been overlooked and not fully explored to date. Perhaps, the complex nature of the aerobic granules has made such exploration difficult. Thus, it is interesting to scrutinize the dynamics of the aerobic granules during the accumulation of PHA.

Thus, in this study, we intend to study the development of aerobic granular biomass in POME and simultaneously treat the POME with high efficiency in the SBR. This study is expected to give some hindsight of the aerobic granulation in POME treatment process and accumulation of PHA inside aerobic granular biomass.

MATERIALS AND METHODS

Wastewater

The POME used in this study is taken from the facultative pond of an oil palm mill in Bagan Serai, Perak, Malaysia. The POME was then filtered to remove debris inside the POME and stored in the cold storage room at 4°C. When preparing the feed for the SBR, the POME is taken out from the cold storage room and left in the open air for it to reach room temperature. Then it was fed into the SBR by using peristaltic pump. Meanwhile, the seed sludge of the SBR was taken from the aerobic pond of the POME treatment plant and inserted into the SBR. POME characteristics are shown in Table 1.

Table 1 : Characteristics of POME

	This work	(Ahmad et al., 2005a)
pH	4.5	3.8-4.5
Chemical Oxygen Demand	29000-40000 mg/l	15500-106360
Total solid	11800 mg/l	11,450-164,950

Sample analysis

Dissolved Oxygen (DO) was monitored and controlled at 4.5 to 6.0 mg/l throughout the experiment. Besides that, the samples are analyzed for COD content, mixed liquor suspended solids (MLSS), sludge volume index (SVI) and surface morphology under microscope (Olympus, SZX9). All these analysis were done according to the Standard Methods (APHA, 1998).

SBR operation

SBR used in the experiment is shown in Figure 1. The working volume of the SBR is 8 L. All the processes in the SBR are controlled ON/OFF controller. The DO probe and pH probe are connected to the computer and the reading is taken online. SBR operates in a 6 hours cycle. The filling, aeration, settling and decanting period are set at 10 minutes, 340 minutes, 0.5 minutes and 9.5 minutes, respectively. The exchange ratio of the SBR is 25% of the working volume for each cycle. The pH and temperature of the SBR is not controlled throughout the experiment. The temperature recorded in the SBR was 30°C. Whereas, the pH increased gradually from acidic to neutral range in each cycle, given the fact that the POME is acidic in nature. Meanwhile, the air is introduced flow rate of 5 L/min. The effluent is withdrawn from the SBR through the sampling port on the SBR. POME is the sole feed for the SBR in operation.

PHA extraction process

PHA was extracted at the end of the feast period of each cycle. Sodium hypochlorite-chloroform method adapted from Hahn et al. (1994) was used for the PHA extraction process. The amount of PHA produced in the resultant precipitate after drying was recorded. The amount of PHA produced is calculated using Eq. (1).

$$Y_{PHA/CDW} = \frac{Total\ amount\ of\ PHA,mg}{CDW\ of\ aerobic\ granules,mg} \qquad \text{Eq. (1)}$$

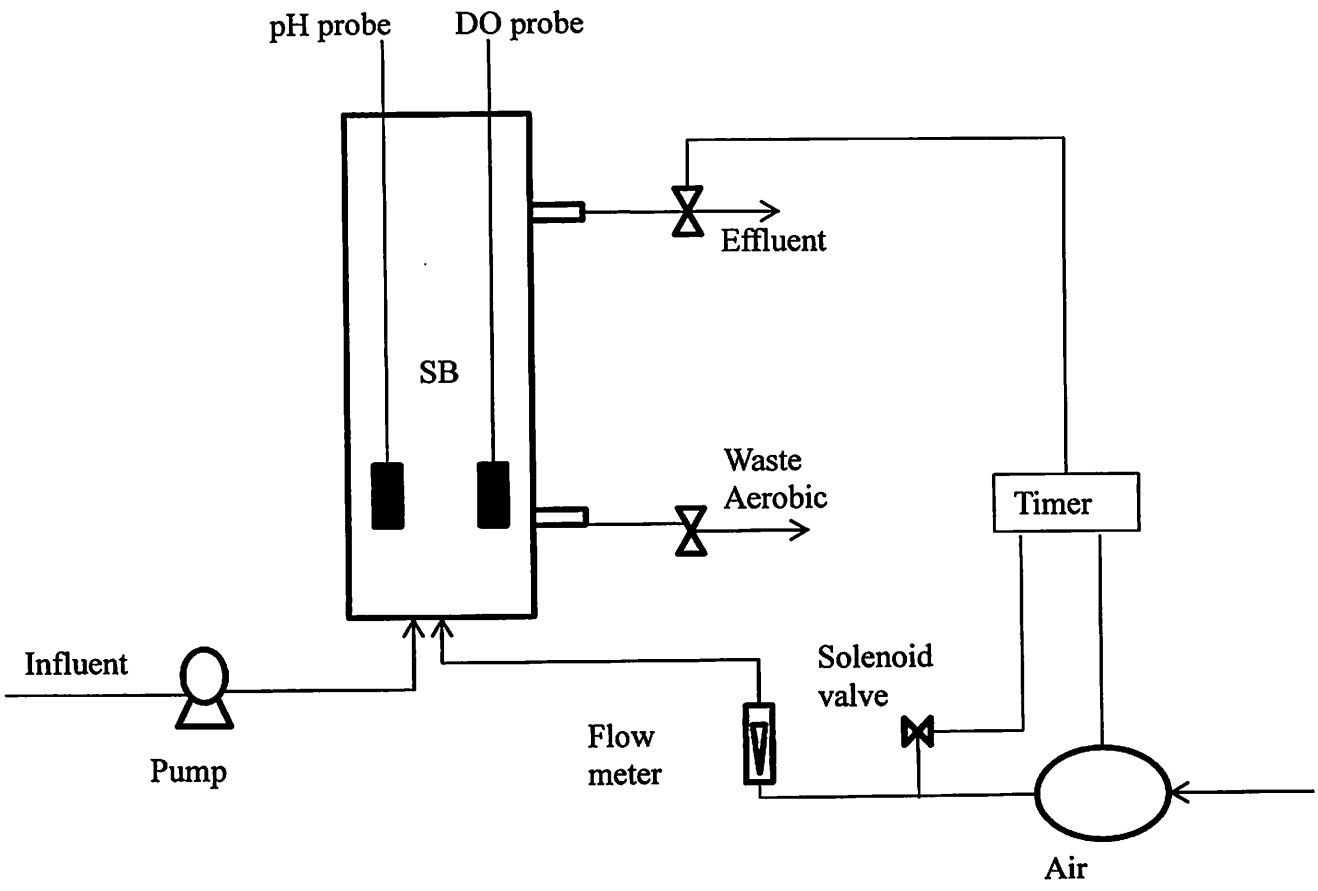

Figure 1: Experimental setup of SBR

RESULTS AND DISCUSSION

Development of aerobic granules

The seed sludge used in the SBR is activated sludge taken from the facultative pond in the POME treatment plant. On the initial day of the SBR operation, the activated sludge was seen to be in loose, individual entity and shapeless (Figure 2 (a)). In the SBR, POME provides the carbon source for the microorganisms and simultaneously reducing the COD content of the POME. The organic loading of the POME applied in the SBR is 1.47 kg COD/day.m^3.

Nevertheless, the fast settling activated sludge was not in the form of granules for the next 112 days. After 70 days, the activated sludge began to form flocculate but the shape of granules was nowhere to be seen (Figure 2 (b)). Only after 112 days, the transformation of fast settling sludge into aerobic granules was completed and visible. However, the initial size of the aerobic granules formed was in between 0.3 to 0.5 mm in average. Figure 2 (c) shows the images of the aerobic granules after 120 days. After the SBR operation reached 120 days, the size of the aerobic granules was observed to be increasing and the average value was 0.7 mm. Even though the aerobic granules are grown, the aerobic granules were dominated by the filamentous growth

around the granules. Hence, round but fluffy aerobic granules were seen during that period. On 130th day of the SBR operation, the regular, non-fluffy and round shape of the aerobic granules was dominant in the SBR (Figure 2 (d)). The filamentous growth on the granules (120th day) quickly disappeared as the fast feeding in the SBR does not favor the filamentous growth of microorganism. Besides these matured granules (seen on 130th day), new granules were kept on appearing even after 130 days. The diameter of these new granules increased in a similar trend to the pioneer aerobic granules.

Aeration rate is one of the contributing factors towards the aerobic granulation. High aeration rate will provide high shear force to the microbial community in the SBR. Under high shear force influence, the secretion of extracellular polymeric substrate (EPS) is enhanced (Tay et al., 2001). As a result, the high EPS value promotes the cell to cell adhesion and subsequently forms the compact and robust aerobic granules (Liu and Tay, 2004). According to (Wana et al., 2009), high aeration rate not only imposes hydrodynamic condition but also controls the oxygen transfer. These factors are considered to enhance the aerobic granulation process (Wana et al., 2009).

Apart from the aeration rate, the settling period of the aerobic granules is a crucial factor in developing the aerobic granules. Commonly, short settling time is chosen to enable the small loose sludge flocs to be removed from the SBR (Lemaire et al., 2008). This process enhances the growth of the aerobic granules in the SBR. At the same time, there are some developed granules that could not settle within the settling period of the SBR, subsequently they were washed out of the SBR. However, the matured granules washout will be compensated by the growing granules in the SBR.

The period for the aerobic granules to appear is quite long compared to the earlier researches (Sheng et al., 2010). This could be due to the acidic real wastewater (POME) being used to cultivate the aerobic granules. The activated sludge underwent the adaptation period before it start to aggregate to form granules. Besides that, the absence of additional chemicals in the SBR could be another reason for the late appearance of the aerobic granules. However, the addition of chemicals was deliberately prevented to ensure this treatment to be sustainable and cost saving.

Performance of aerobic granules in treating POME

After aerobic granules were formed, the COD, MLSS and SVI measurements were carried out regularly to analyze the feasibility of using aerobic granules in treating POM wastewater. OLR of about 3.0 kg COD/day.m^3 (same as in the before granulation period) was maintained throughout this period.

The percentage of COD removal after granulation in the SBR and COD content of the effluent are shown in Figure 3. It can be deduced that the highest percentage of COD removal recorded after aerobic granules formed was 93%. It should be noted that the MLSS concentration decreased after the formation of aerobic granules. However, the ability of aerobic granules to

oxidize COD was not affected. The average COD removal remained stable at about 88% even after the decrease in MLSS concentration.

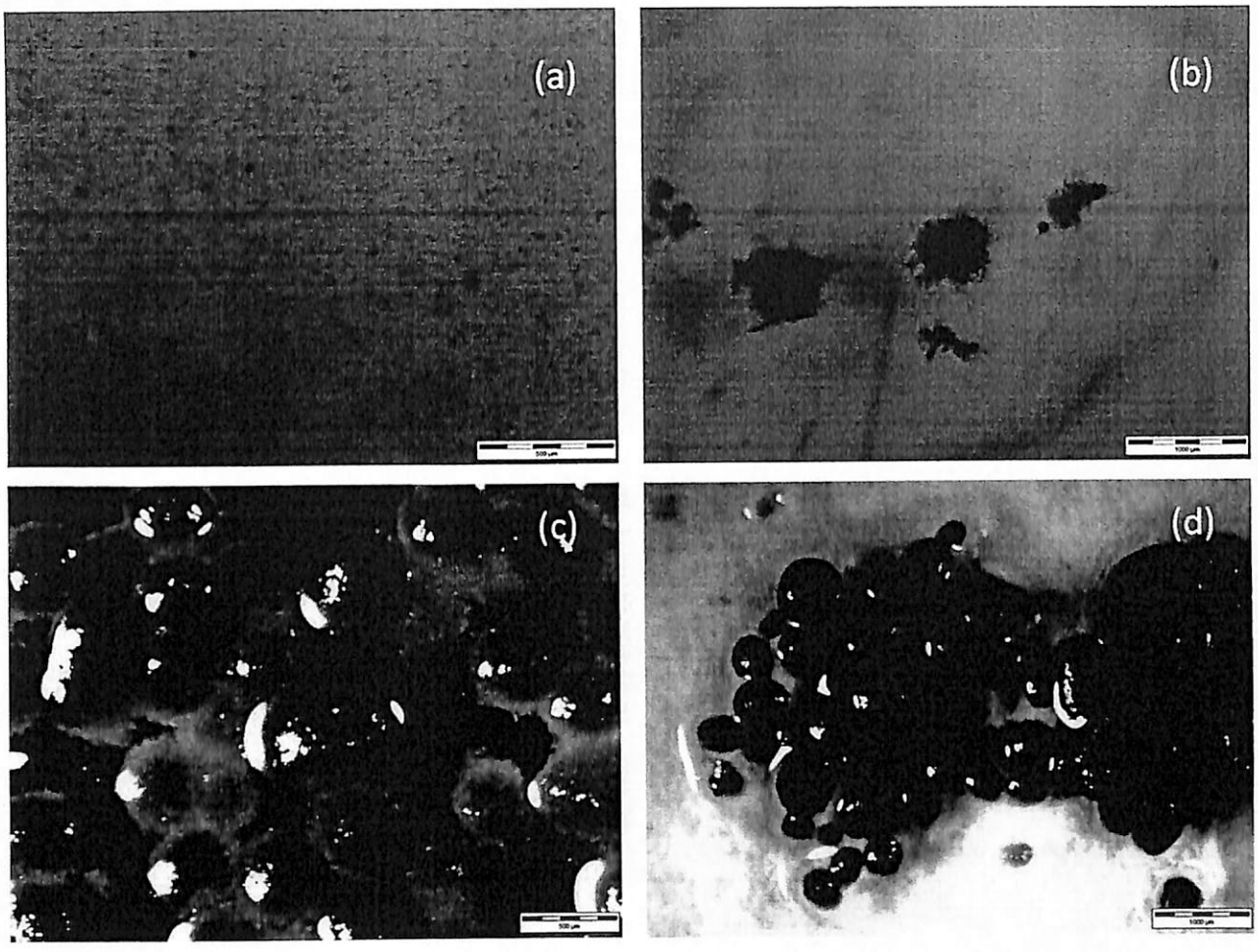

Figure 2 Aerobic granules on (a)1st day, (b) 70th day, (c) 120th day and (d) 130th day.

The MLSS concentration in the reactor after granulation is shown in Figure 4. It can be observed that the average MLSS value decreased slightly, after the formation of aerobic granules. This phenomenon could be due to the filamentous growth around the aerobic granules during the initial period of aerobic granules development. These filamentous aerobic granules affected the settling ability of the granules and subsequently some of them were removed from the SBR. It is obvious from Figure 2b, where the filamentous growth is seen around the aerobic granules. After 120th day, the MLSS started to increase gradually. This is due to the newly developing aerobic granules in the SBR while the earlier formed aerobic granules were getting bigger. At the same time, the filamentous growth around the aerobic granules disappeared gradually after 120th day (Figure 2c). It can be inferred that the aerobic granules started to became compact and denser without being fluffy after 120th day. The grown aerobic granules are shown in Figure 1d, where

the developed granules was seen to be around 3.1 mm and without the filamentous growth around it. It is further supported by the decrease in the SVI value as observed in Figure 4.

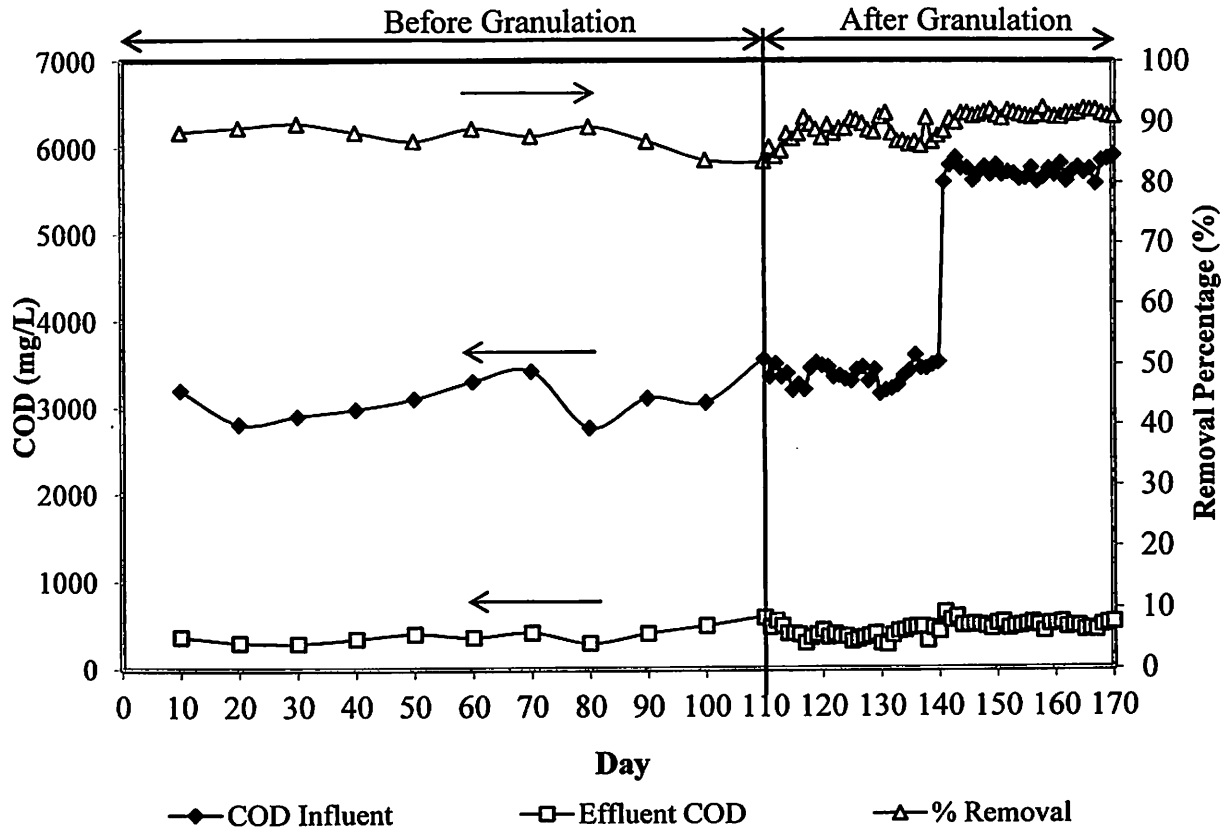

Figure 3: COD in influent and effluent, and percentage of COD removal

Figure 4 shows the plot of SVI against time. In the initial days after the formation of the aerobic granules, the SVI slightly increased due to the poor settling characteristics of the granules. However, after 120th day till 140th day, the SVI value decreased notably from 50 to 30 ml/g. This SVI value of aerobic granules is much smaller compared to the conventional activated sludge used in the work done by Chan et al., (2010), where they treated the POM wastewater using the SBR. As the aerobic granules have good settling characteristic compared to conventional activated sludge, it results in shorter settling time. Thus, good separation of biomass from the treated effluent was observed.

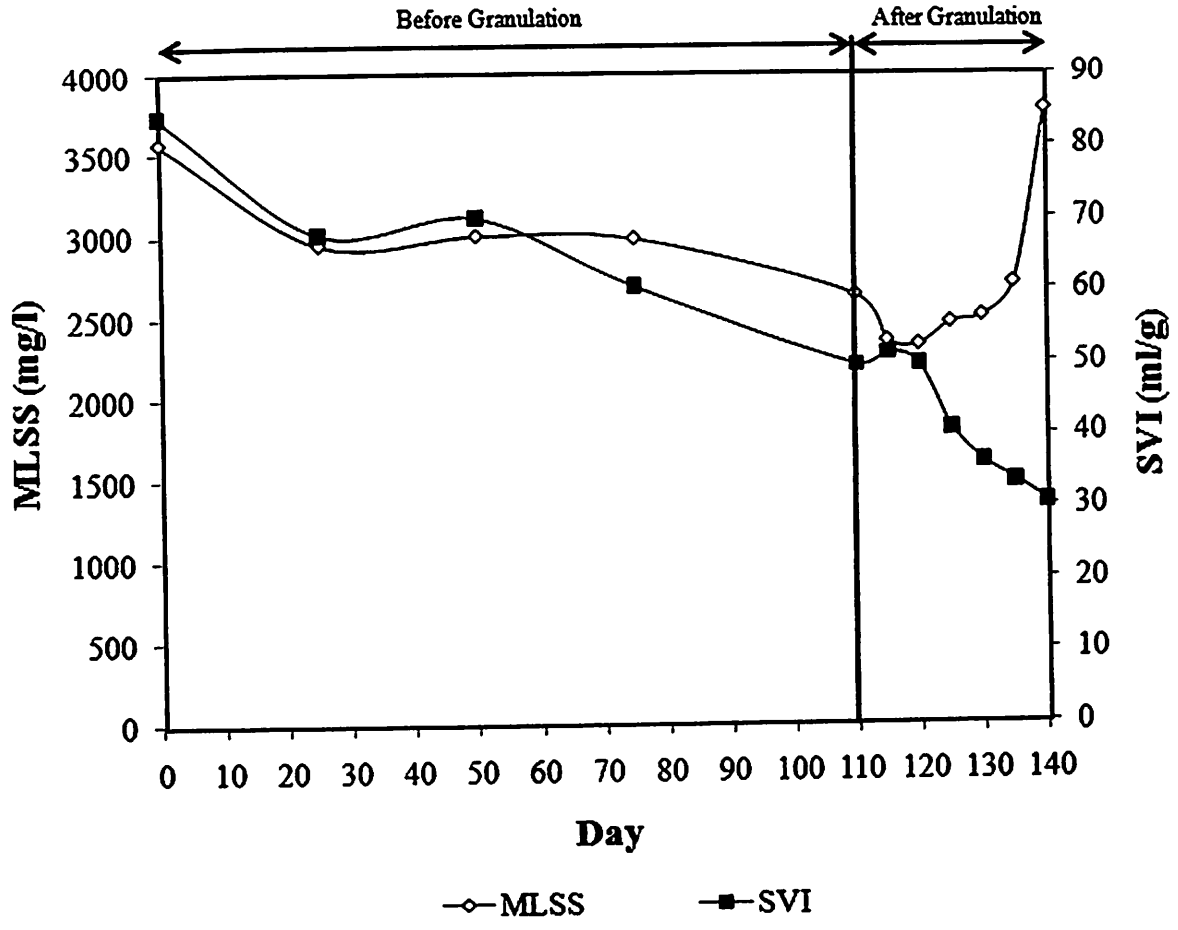

Figure 4: MLSS and SVI of the reactor

Accumulation of PHA inside aerobic granules

Figure 5 shows the PHA accumulation profile in the aerobic granules in a cycle of 6 hours. In the first two hours (feast period), the amount of PHA accumulated increases. Thereafter, the amount of PHA in the aerobic granules started to decrease sharply during the famine period. Meanwhile, for the first two hours of the reaction, a rapid decrease in COD can be observed as well, and the COD concentration reaches a minimum value of 95 mg/L. This result indicates that approximately 90% of the influent was removed in the first 2 hours of the reaction. The remaining 10% of the COD in the SBR is either slowly biodegradable or non-biodegradable COD. By correlating the end of the COD uptake and the decrease in PHA during the famine phase, it could be concluded that PHA is used as the carbon source for maintenance energy needs when the external carbon source is no longer available for the aerobic granules.

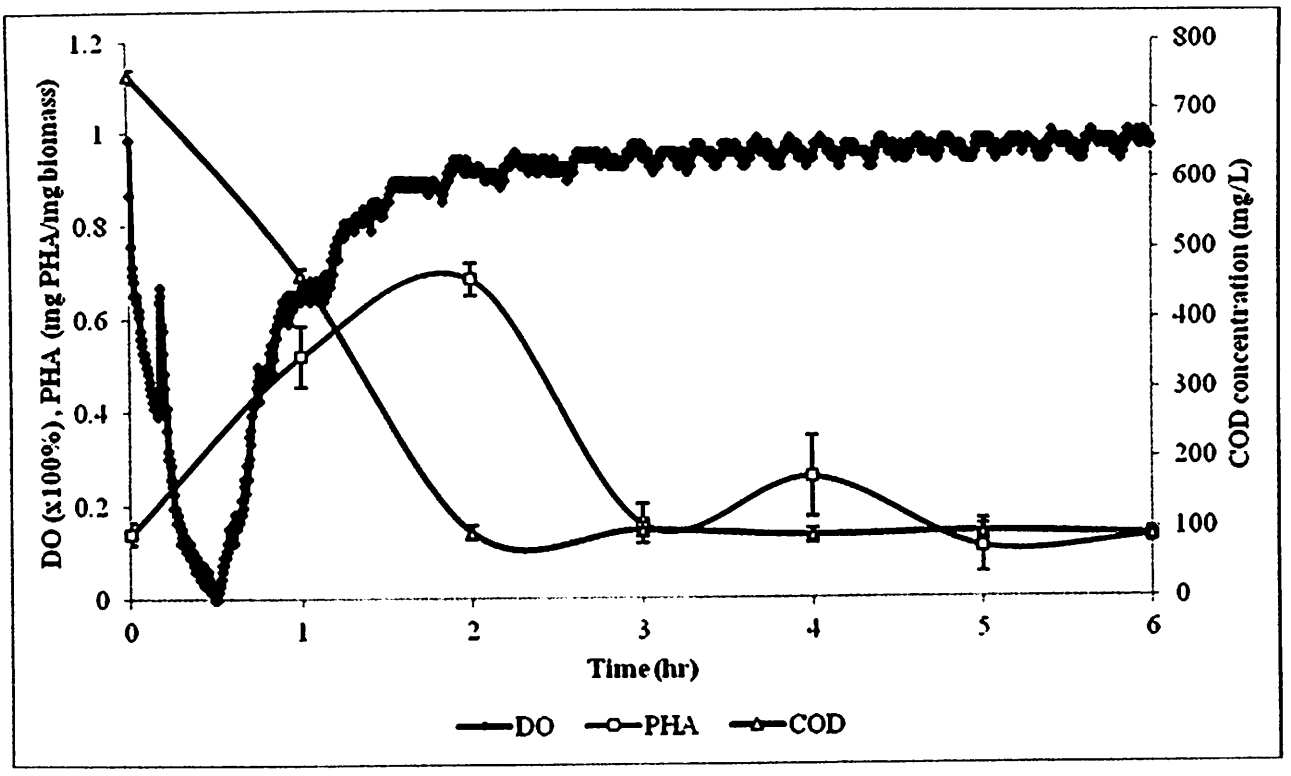

Figure 5: Dissolved Oxygen, total PHA accumulation and COD concentration profile of aerobic granules treating POME for one complete cycle.

CONCLUSION

In this study, for the first time, aerobic granules were successfully developed using POM wastewater (which is acidic in nature), without any pH adjustment. The aerobic granules formation in the POM wastewater took 140 days to achieve non-fluffy, regular and round granules with largest diameter of 3.1 mm. The developed aerobic granules were able to remove around 88% (at OLR of 3.0 kg COD/m3.day) of the influent COD and about 90% at OLR of 6.0 kg COD/m^3.day. Further, this study demonstrated that aerobic granules can accumulate PHA in their cells.

REFERENCES

Adav, S.S., Lee, D.J. & Lai, J.Y. (2008). Intergeneric coaggregation of strains isolated from phenol-degrading aerobic granules. *Applied Microbiology and Biotechnology,* **79** (4), 657-661.

Ahmad, A.L., Ismail, S. & Bhatia, S. (2005a). Membrane treatment for palm oil mill effluent: Effect of transmembrane pressure and crossflow velocity. *Desalination,* **179** (1-3), 245-255.

Ahmad, A.L., Bhatia, S., Ibrahim, N. & Sumathi, S. (2005b). Adsorption of residual oil from palm oil mill effluent using rubber powder. *Brazilian Journal of Chemical Engineering,* **22** (3), 371-379.

Ahmad, A.L., Chong, M.F. & Bhatia, S. (2009). A comparative study on the membrane based palm oil mill effluent (POME) treatment plant. *Journal of Hazardous Materials,* **171** (1-3), 166-174.
APHA. 2012. Standard Methods for the Examination of Water and Wastewater. in: *22th ed. American Public Health Association*. Washington, DC, USA.
Arrojo, B., Mosquera-Corral, A., Garrido, J.M. & Mendez, R. (2004). Aerobic granulation with industrial wastewater in sequencing batch reactors. *Water Research,* **38** (14-15), 3389-3399.
Bhatia, S., Othman, Z. & Ahmad, A.L. (2007). Coagulation-flocculation process for POME treatment using Moringa oleifera seeds extract: Optimization studies. *Chemical Engineering Journal,* **133** (1-3), 205-212.
Chan,Y.J. M.F. Chong, C.L. Law, Biological treatment of anaerobically digested palm oil mill effluent (POME) using a Lab-Scale Sequencing Batch Reactor (SBR), *J. Environ. Manage.* 91 (2010) 1738-1746
de Bruin, L.M.M., de Kreuk, M.K., van der Roest, H.F.R., Uijterlinde, C. & van Loosdrecht, M.C.M. (2004). Aerobic granular sludge technology: an alternative to activated sludge? *Water Science and Technology,* **49** (11-12), 1-7.
Fun, C.W., Haq, M.R.U. & Kutty, S.R.M. (2007). Treatment of palm oil mill effluent using biological sequencing batch reactor system. *River Basin Management Iv,* **104**, 511-518.
Hahn, S.K., Chang, Y.K., Kim, B.S., Chang, H.N. 1994. Optimization of microbial poly (3-hydroxybutyrate) recovery using dispersions of sodium-hypochlorite solution and chloroform. *Biotechnol. Bioeng.*, 44(2), 256-261.
Kong, Y.H., Liu, Y.Q., Tay, J.H., Wong, F.S. & Zhu, J.R. (2009). Aerobic granulation in sequencing batch reactors with different reactor height/diameter ratios. *Enzyme and Microbial Technology,* **45** (5), 379-383.
Lemaire, R., Yuan, Z., Blackall, L.L. & Crocetti, G.R. (2008). Microbial distribution of Accumulibacter spp. and Competibacter spp. in aerobic granules from a lab-scale biological nutrient removal system. *Environmental Microbiology,* **10** (2), 354-363.
Liu, Y. & Tay, J.H. (2004). State of the art of biogranulation technology for wastewater treatment. *Biotechnology Advances,* **22** (7), 533-563.
Serafim, L.S., Lemos, P.C., Oliveira, R., Reis, M.A.M. 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. *Biotechnol. Bioeng.*, **87**(2), 145-160.
Sheng, G.P., Li, A.J., Li, X.Y. & Yu, H.Q. (2010). Effects of seed sludge properties and selective biomass discharge on aerobic sludge granulation. *Chemical Engineering Journal,* **160** (1), 108-114.
Shi, X.Y., Sheng, G.P., Li, X.Y. & Yu, H.Q. (2010). Operation of a sequencing batch reactor for cultivating autotrophic nitrifying granules. *Bioresource Technology,* **101** (9), 2960-2964.
Tay, J.H., Liu, Q.S. & Liu, Y. (2001). Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor. *Journal of Applied Microbiology,* **91** (1), 168-175.
Vijayaraghavan, K., Ahmad, D. & Aziz, M. (2007). Aerobic treatment of palm oil mill effluent. *Journal of Environmental Management,* **82** (1), 24-31.
Wana, J.F., Bessiere, Y. & Sperandio, M. (2009). Alternating anoxic feast/aerobic famine condition for improving granular sludge formation in sequencing batch airlift reactor at reduced aeration rate. *Water Research,* **43** (20), 5097-5108.
Wang, S.G., Liu, X.W., Zhang, H.Y. & Gong, W.X. (2007). Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor. *Chemosphere,* **69**, 769-775.

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Treatment of agro based industrial wastewater in sequencing batch reactor: Performance evaluation and growth kinetics of aerobic biomass

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ABSTRACT

A sequencing batch reactor (SBR) with a working volume of 8 L and an exchange ratio of 25% was used to enrich biomass for the treatment of the anaerobically treated low pH palm oil mill effluent (POME). The influent concentration was stepwise increased from 5000 ± 500 mg COD/L to 11,500 ± 500 mg COD/L. The performance of the reactor was monitored at different organic loading rates (OLRs). It was found that approximately 90% of the COD content of the POME wastewater was successfully removed regardless of the OLR applied to the SBR. Cycle studies of the SBR show that the oxygen uptake by the biomass while there is no COD reduction may be due to the oxidation of the storage product by the biomass. Further, the growth kinetic parameters of the biomass were determined in batch experiments using respirometer. The maximum specific growth rate (μ_{max}) was estimated to be 1.143 day^{-1} while the half saturation constant (K_s) with respect to COD was determined to be 0.429 g COD/L. The decay coefficient (b_D) and biomass yield (Y) were found to be 0.131 day^{-1} and 0.272 mg biomass/mg COD consumed, respectively.

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1. Introduction

Various effluent treatment schemes have been proposed and used to treat palm oil mill effluent (POME). Although many studies on physical–chemical methods (such as membrane separation, adsorption and coagulation–flocculation treatments) for the treatment of POME have shown great performance, previous works have been limited to the laboratory scale (Ahmad et al., 2009; Chaiprapat and Laklam, 2011). The application of these treatments in industry is limited due to the high equipment cost and mechanical energy consumption required for treatment. Thus, more than 85% of POME is treated by biological systems (Jalani et al., 2000). Generally, biological treatment carried out in open pond system which consists of an anaerobic ponds followed by an open tank digester, coupled with extended aeration in the pond to further reduce the amount of COD (Poh and Chong, 2009).

The open pond system used in the treatment of POME possesses several drawbacks, including its requirement of large land area, poor sludge settling ability, the sensitivity of biomass to temperature and pH changes and the emission of unpleasant odor (Poh and Chong, 2009). Furthermore, the biological treatment pond for POME has a relatively high hydraulic retention time (HRT) of approximately 10–120 days (Igwe and Onyegbado, 2007). Considering the amount of POME generated during this period, effective treatment requires a very large land area to contain the wastewater. Additionally, the biomass in the aerobic pond needs an adequate supply of oxygen in order to effectively degrade the organic contents of POME. Therefore, the depth of the pond must be shallow so that oxygen can penetrate to the bottom of pond to ensure effective treatment. Thus, the overall area of the pond needs to be increased to compensate for the loss of depth (Henze et al., 2008). Due to the large size and configuration of the treatment ponds, it is difficult to control and maintain the efficiency of the treatment process.

In addition to the problem of the large land area required, conventional biological pond systems tend to have a poor separation between the treated effluent and biomass (Liu et al., 2003). The flush-out of biomass from the treatment system reduces the treatment efficiency and can affect the ecosystem of the effluent receiving water bodies. Therefore, bioreactors such as the upflow anaerobic sludge blanket reactor (UASB) or the UASB integrated with upflow fixed film (UFF) have been used to improve the separation of solid (biomass) and liquid (treated effluent) (Borja and Banks, 1994). Besides, due to the acidity and unique composition of POME, biological treatment systems' performance is variable, leading to inconsistent effluent quality. To improve the performance of biological treatment, POME usually undergoes a neutralization process before being transferred to the biological treatment system. The use of additional chemicals might increase the operating cost of the treatment system.

Alternatively, the sequencing batch reactor (SBR) has gained the attention of researchers studying POME treatment due to its proven performance in treating both domestic and industrial wastewater. SBR technology has been reported to achieve organic carbon, phosphorus (P) and nitrogen (N) removal and, in some cases, biological removal of heavy metals (Pijuan et al., 2004; Sirianuntapiboon and Hongsrisuwan, 2007). Owing to its flexible reactor operation, highly effective performance and relatively reduced land area requirement, SBR technology is being used increasingly for the biological treatment of industrial wastewater. The viability of the utilization of SBR for POME treatment was investigated in some works and achieves high percentage of COD removal (Chaiprapat and Laklam, 2011; Chan et al., 2010; Gobi and Vadivelu, 2014). Detailed studies of the aerobic biomass treating POME in an SBR, however, have not yet been performed. Therefore, the performance of the biomass and growth kinetics involved in the aerobic treatment of POME using an SBR was investigated in the present study.

2. Materials and methods

2.1. Palm oil mill effluent and seed sludge

The POME used in this study was collected from the anaerobic pond of Elegant Palm Oil Mill, Bagan Serai, Perak and stored in a cold room at a temperature of 4 ± 1 °C. The required volume was warmed to room temperature (28 ± 2 °C), filtered to remove debris and diluted to the desired concentration using tap water before it was fed into the SBR. The seed sludge was obtained from the aerobic pond of the same palm oil mill.

2.2. Reactor setup and operation

A 10 L SBR with an effective working volume of 8 L was used to enrich the biomass. The SBR operates in a 6-h cycle with 4 phases: filling (10 min), aerobic reaction (340 min), settling (1 min) and decanting (9 min). In each cycle, 2 L of POME were fed into the reactor for an HRT of 1 day. The reactor was operated at room temperature (28 ± 1 °C), and the pH was not controlled. The DO concentration in the reactor was maintained within the range of 4.0–5.5 mg/L with an ON/OFF controller. The acidic POME was the only feed to the reactor, and the pH of the wastewater was not adjusted. The feed concentration to the SBR was increased stepwise from 5000 ± 500 mg COD/L to 11,500 ± 500 mg COD/L.

The operation of the POME treatment using the SBR was monitored by long-term reactor performance and weekly cycle studies. Liquid and solid mixture samples were taken from the SBR to evaluate the performance of the reactor. The performance of the SBR was determined on the basis of COD removal as well as biomass concentration (MLVSS) and sludge volume index (SVI).

Analytical determinations of COD and SVI were carried out in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were determined according to Standard Methods 2540E.

3. Growth kinetic parameters determination

The biomass from parent SBR operating under steady state condition (stable MLVSS concentration, constant COD removal and repeatable cycle profiles) treating POME with the influent COD concentration of 5000 mg/L was used to evaluate the growth kinetic parameters. Batch experiments were carried out using respirometer (BM-Advance, Surcis). The data collected were analyzed using mathematical equation to determine the growth kinetic parameters, namely, maximum growth rate, half saturation constant, biomass yield and decay coefficient. Each of the experiments was repeated at least three times in order to calculate the mean values and the standard deviation.

3.1. Determination of decay coefficient (b_D)

At the beginning of each batch test, the respirometer was filled with a known amount of mixed liquor (consisting of biomass) taken from parent SBR (just before the settling phase to ensure there is no remaining soluble COD from the feed) and filled with distilled water to give a total operating volume of 1 L. Samples were taken to determine the initial biomass and COD concentration in the reactor. The DO concentration of the respirometer reactor was maintained within 4.0–5.5 mg/L. The batch test was conducted for a period of 6 h and samples were taken out at an interval of 30 min for COD concentration measurement. DO concentrations throughout the test were measured and OUR was calculated. The value of decay coefficient (b_D) can be obtained by performing calculation on the change of oxygen uptake rate (OUR) over time.

In a batch bioreactor where there is only biomass with no soluble substrates input, the usage of oxygen is solely due to the decay of biomass. Hence, the correlation between OUR and decay can be stated as in Equation (1) (Grady et al., 2011):

$$OUR_D = (1 - f_D) \cdot b_D \cdot X_{H,t} \quad (1)$$

where OUR_D = oxygen uptake rate due to biomass decay; b_D = decay coefficient of biomass; $X_{H,t}$ = MLVSS concentration at t time; f_D = fraction of active biomass contributing to biomass debris.

The OUR change over time at a prolonged cultivation period (where all the biodegradable soluble COD is completely consumed) with no addition of external substrate is considered as the endogenous respiration (Grady et al., 2011). The decrease of respiratory oxygen uptake with time corresponds to the decay of the biomass since there is no substrate supplement for the growth of biomass. By performing a mass balance on the active biomass in the bioreactor at this stage, the changes of biomass due to time are stated in Equation (2):

$$\frac{dX_H}{dt} = -b_D \cdot X_H \quad (2)$$

$$\int_0^{X_{H,t}} \frac{1}{X_H} dX_H = \int_0^t -b_D dt$$

$$\ln\left|\frac{X_{H,t}}{X_{H,0}}\right| = -b_D t$$

$$X_{H,t} = X_{H,0} \cdot e^{-b_D t} \quad (3)$$

By substituting Equation (3) into Equation (1), the resultant Equation (4) is obtained:

$$OUR_D = (1 - f_D) \cdot b_D \cdot X_{H,0} \cdot e^{-b_D t} \quad (4)$$

By performing log natural on both sides of Equation (4) and rearranging the oxygen uptake rate as function of time can be defined as in Equation (5) (Grady et al., 2011):

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$$\ln \mathrm{OUR_D} = -b_D \cdot t + \ln\{(1 - f_D) \cdot b_D \cdot X_{H,0}\} \quad (5)$$

where t = time; $X_{H,0}$ = initial reactor MLVSS concentration.

Using linear regression method, the value of decay constant (b_D) and fraction of active biomass contributing to biomass debris (f_D) can be obtain from the slope of the plot of ln OUR versus time and the y-axis interception point, respectively.

3.2. Determination of biomass yield constant (Y)

In this study, POME with a predetermined initial COD concentration was fed to the reactor with initial biomass concentration of 0.2 g/L. POME was the sole substrate feed to the reactor. The batch reactor was continuously aerated and the oxygen concentration was kept at range of 4.0–5.5 mg O_2/L for 6 h. The value of real biomass yield constant (Y) was determined based on the Equation (6):

$$Y = \frac{\Delta X}{\Delta S}\left[\frac{\text{mg COD}}{\text{mg COD}}\right] \quad (6)$$

where ΔX = net change of biomass concentration, mg/L; ΔS = net change of COD concentration, mg/L.

3.3. Determination of half saturation coefficient (K_s) and maximum growth rate (μ_{max})

The respirometric method proposed by Grady et al. (2011) was used to determine the half saturation coefficient (K_s) and maximum growth rate (μ_{max}). It relies on the OUR measurement taken at a set time interval in an aerated batch test reactor. Several experiments at different range of initial COD (feed) concentrations were conducted. The respirometer was pre-aerated to saturation at the beginning of each test. At the beginning, 40 mL POME of 20,000 mg COD/L was introduced to the 960 mL biomass of 0.8 g biomass/L in the respirometer. The organic loading at the beginning of this study was approximately 0.7 g COD/g biomass COD and it was sufficient to provide the required time to obtain reliable OUR values (Grady et al., 2011).

The earlier determined decay coefficient (b_D) and biomass yield constant (Y) of the biomass were used to calculate the half saturation constant (K_s) and maximum growth rate (μ_{max}). The overall OUR of biomass includes the oxygen uptake for growth and oxygen uptake during decay as presented in Equation (7):

$$\mathrm{OUR_{total}} = \left(\frac{1 - Y}{Y}\right) \cdot \mu_x \cdot X_H + (1 - f_D) \cdot b_D \cdot X_H \quad (7)$$

where $\mathrm{OUR_{total}}$ = Overall oxygen uptake rate, mg/L; μ_x = specific biomass growth rate.

By rearranging the equation, the heterotrophic growth rate can be described as:

$$\mu_x = \mathrm{SOUR} \cdot \left(\frac{Y}{1 - Y}\right) - (1 - f_D) \cdot b_D \cdot \left(\frac{Y}{1 - Y}\right) \quad (8)$$

where SOUR is the specific oxygen uptake rate (mg O_2/mg biomass COD h).

Meanwhile, the maximum biomass growth rate, μ_{max} can be determined using rearranged Monod model equation where by:

$$\frac{1}{\mu_x} = \frac{K_s}{\mu_{max}} \cdot \frac{1}{S_l} + \frac{1}{\mu_{max}} \quad (9)$$

where μ_{max} = maximum biomass growth rate; S_l = COD concentration in respirometer; K_s = half saturation constant.

From Equation (9), μ_{max} can be obtained from the intercept point of plot ($1/\mu_x$) versus ($1/S_l$) while K_s can be calculated from the slope of the plot.

Further, in order to validate the K_s value obtained above using the experimental data and Equation (9), a direct experimental method which determines the K_s value was carried out. The K_s value of the biomass aerobically treating POME was determined by measuring the OUR at different COD concentrations. This study was carried out using respirometer where the experimental conditions were kept identical to the parent SBR except the feed COD concentration. Several tests were conducted at different COD concentrations. Samples were taken at a prefixed time interval for COD analysis. OUR was monitored and recorded continuously for each test with different COD concentration. The K_s value with respect to feed COD was determined from the plot of OUR versus COD concentration.

4. Results and discussion

4.1. Performance of the SBR treating POME

4.1.1. COD removal

The characteristics of the anaerobically treated POME used as the feed in this study are presented in Table 1. It is generally accepted that the minimum nutrient requirement for aerobic biomass growth in terms of COD:N:P ratio is 100:5:1 (Willey et al., 2009). The COD:N:P ratio for the POME used in this study was approximately 125:5:1 (Table 1), which is within the recommended range for active biomass growth. Fig. 1 shows the COD concentrations of the influent and effluent of the SBR, together with the COD removal percentage. Long-term monitoring of the reactor's performance shows that the COD removal efficiency was stable at approximately 90% throughout its operation.

During the first 120 days, the influent COD to the SBR was approximately 5000 mg/L, which provided an organic loading rate (OLR) of approximately 5000 mg/L day and a COD concentration inside the SBR of approximately 1250 mg/L. The average MLVSS concentration of the SBR was about 4900 mg/L. During this period, the effluent COD was approximately 480 mg/L, for an average COD removal of approximately 90%. From day 120 until day 180, the influent COD concentration was increased to approximately 8000 mg/L (OLR of 8000 mg/L day; COD concentration within the SBR approximately 2000 mg/L). Meanwhile, the MLVSS of the SBR was increasing and reached a steady value of about 5900 mg/L and the effluent COD was approximately 850 mg/L. However, the COD removal remained approximately 90%. At day 180, the influent COD into the SBR was increased to approximately 11,500 mg COD/L (OLR of 11,500 mg/L day; COD concentration within the SBR approximately 2900 mg/L) to evaluate the SBR's performance with a higher influent COD. The MLVSS in the reactor reach a steady value of about 12,000 mg/L. At this stage, the SBR was still able to achieve approximately 90% COD removal at the highest influent concentration.

From these observations, it can be inferred that the biomass in the SBR was able to degrade approximately 90% of the COD when the influent COD increased up to 11,500 mg/L. Furthermore, the remaining 10% of the organic content (COD), which remains in the effluent of the SBR, is believed to be the slowly degradable or non-biodegradable COD. A more detailed study on the COD removal pattern by the biomass was performed during the cycle study and is presented in the following section.

Table 1
Characteristic of POME used in this study.

Criteria	Value
COD, mg/L	12,000–25,000
BOD, mg/L	7200–15,000
TSS, mg/L	11,300–12,300
Nitrogen content, mg/L	850–950
Phosphorus content, mg/L	150–200
pH	4.8–5.5

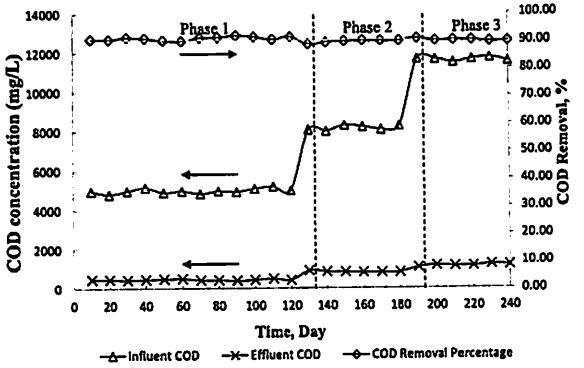

Fig. 1. COD removal performance of SBR in treating POME.

4.2. Outcomes of cycle studies

Cycle studies were carried out on a regular basis on the SBR to evaluate the biomass phenotypes and performance of the reactor. Figs. 2 and 3 show the typical DO and COD concentration profiles in the SBR during a complete treatment cycle. It was observed that the DO level in the reactor was low during the first 50 min. In this stage, the influent (POME) was fed into the reactor. As a result, the COD (organic content) in the reactor increased drastically, and the biomass was exposed to an organic carbon source. The biomass then oxidized the COD source and continuously consumed oxygen. The high consumption of oxygen during this period kept the DO level below 2 mg/L. The COD concentration profile of the SBR (Fig. 3) clearly shows that during the first 50 min (stage 1), the COD concentration decreased sharply, indicating a high COD oxidation rate and consequently, a high oxygen uptake rate (OUR).

After 50 min of operation, the DO level reached its upper set point (5.5 mg/L) and remained within the set points (4.0–5.5 mg/L). This shows that oxygen consumption by the biomass had decreased compared to the first 50 min. This is well supported by the data on the COD concentration in the SBR (Fig. 3). At this point, the concentration of COD in the SBR was below 610 mg/L. The slope of the COD profile is decreasing, which suggests that the COD consumption rate is getting lower as the available biodegradable organic carbon in the reactor is depleted (Fig. 3).

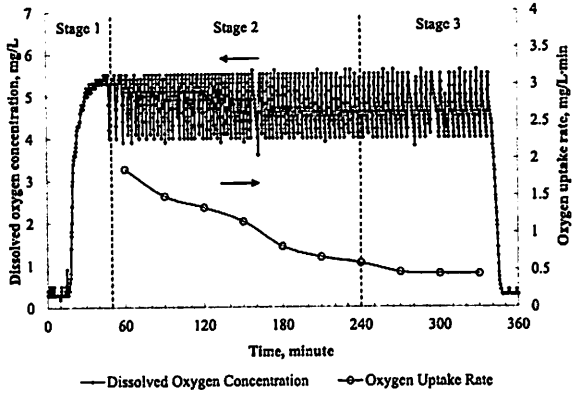

Fig. 2. A typical dissolved oxygen concentration and oxygen uptake rate profile of SBR during a treatment cycle.

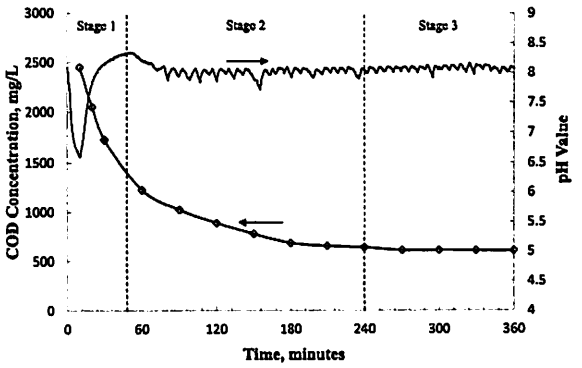

Fig. 3. A typical COD concentration profile of SBR during a treatment cycle.

Analysis of the DO profile (Fig. 3) reveals that the drop in DO during Stage 2 becomes slower with time. This indicates that the OUR of the biomass in the reactor is decreasing during this period. Fig. 3 illustrates that the decrease in the DO profile (50–360 min, Stage 2) and 240–360 min (Fig. 3, Stage 3) based on the COD concentration. At 50 min onward, the COD concentration drops gradually until it reaches a constant value at approximately 240 min. Beyond this point (240–350 min), no clear drop in COD concentration was observed, suggesting that there was no COD consumption by the biomass in the reactor during this period. However, the DO profile in Fig. 3 clearly shows that during this period (240–350 min), DO was consumed by the biomass in the reactor. Although the OUR during this period is lower compared to that of during the oxidization of COD, a considerable amount of oxygen was consumed, which cannot be attributed to the COD degradation. The biomass keeps utilizing oxygen until the end of the aerated reaction phase, when the aeration and mixing is stopped so that biomass can settle before decanting.

It should be noted that a similar trend in the DO and COD profiles was observed in all the cycle studies carried out in this study. Generally, it is believed that in the presence of biodegradable COD, heterotrophic bacteria consume oxygen to oxidize the biodegradable COD, allowing them to generate energy and build new cells (Liang et al., 2012). However, this study reveals that the biomass still consumes oxygen even when no observable COD consumption occurs in the reactor. In the absence of external COD (organic content in wastewater), biomass still needs energy to maintain cell structure integrity and other non-growth related functions. It is believed that in the absence of external COD, the biomass will consume the cells' internal carbon to generate energy to maintain cell structure integrity (Ritton, 2011). This process, which occurs during the absence of external biodegradable COD, is known as the endogenous process. Thus, the oxygen consumption beyond the 240th minute (Figs. 2 and 3), which is not attributable to external COD degradation, could be associated with the endogenous process (Van Loosdrecht and Henze, 1999). Another possibility is the consumption of biomass by microbial predators present in the reactor, which might result in the observed oxygen consumption. However, weekly microscopic observations of the reactor biomass showed no observable predators (or protozoa). To clarify this, further investigation was carried out to observe the changes in the biomass's internal energy reserves during a treatment cycle.

4.3. Storage product

Generally, microorganisms store energy within the cell in the form of polyhydroxybutyrate (PHB), which is a type of polyhydroxyalkanoate (PHA) (Wang et al., 2012). In this study, the quantity of PHB in the biomass was monitored throughout a treatment cycle. The method used for PHB extraction and quantification was adopted from the gravimetric method of Kim et al. (1994). Fig. 4 shows a typical profile of PHB in the biomass, together with the substrate COD concentration in one cycle of the SBR. Fig. 4 clearly shows that the biomass PHB content increased while the COD concentration decreased during the first 50 min of the cycle (Stage 1). This indicates that the biomass is consuming the COD and storing its energy in the cell in the form of PHB.

In Stage 2 of Fig. 4, the COD concentration continues to decrease at a much slower pace than that observed in Stage 1. Meanwhile, the PHB content has reached its peak and starts to decrease. This observation suggests that during Stage 2, the biodegradable components of the feed were nearly depleted, and the biomass started to use internal energy reserves for cell maintenance processes. As the treatment proceeded to Stage 3, in which there is no more COD reduction (but oxygen is still consumed, Fig. 4) the PHB content in the biomass continues to decrease, reaching its minimum value at the end of the cycle.

The results obtained here indicate that microorganisms oxidize PHB when deprived of an external COD supply to obtain energy. Oxygen is consumed for the conversion of PHB to the energy needed for cell maintenance processes. Thus, it can be concluded that the occurrence of oxygen consumption while there is no COD reduction in the SBR could be due to PHB oxidation. However, it should be noted that PHB oxidation alone might not be the sole contributor to the observed oxygen consumption.

4.4. Determination of growth kinetic parameters

4.4.1. Decay coefficient

The decay coefficient of the biomass treating POME obtained in this study was computed based on the experimental data presented

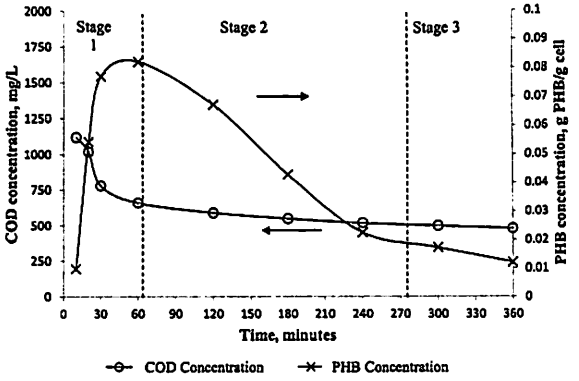

Fig. 4. Typical graph of COD concentration versus time with the tested amount of PHB at each sample point in a cycle study.

in supplementary document (Supplementary Table 4 and Supplementary Fig. 5) and Equation (5). The decay coefficient is evaluated to be 0.031 day^{-1}. This value is relatively close to the decay coefficient values previously reported for heterotrophic bacteria treating similar strength industrial wastewater (Makinia, 2010). In these previous works, the COD content of the wastewater studied is almost similar to the COD of POME used in this current study. Thus this might well contribute to the similar decay coefficient obtained.

In this study, the decay coefficient has been determined by measuring the decrease in respiration activity (consumption of oxygen) over time when the biomass was kept under the absence of organic carbon source (or commonly known as starvation). Although this method has been used in this study and also in numerous earlier studies, the decay coefficient determined is not necessarily representing the in-situ cell decay which occurring during the normal wastewater treatment processes. Generally, in the starvation method used, it is assumed that there is no growth under starvation conditions and the decrease in respiration activity (OUR) is caused by cell decay due to absence of food sources. However, the decay coefficient obtained using this method may over estimate the value due to the application of an unusual environmental condition (in this case is the absence of food). The actual biomass decay rate in the wastewater treatment plant during the normal treatment condition (with the presence of food) may be lower than in this study. Nevertheless, to our knowledge, the experimentally determined in-situ decay rate during normal growth condition with presence of food has not been reported to date.

Also, it should be noted that from the engineering point of view, the term decay is generally defined as the reduction in number and/or weight of microorganism and their specific activities in treatment system (Van Loosdrecht and Henze, 1999). Further, it is divided into internal decay and external decay. Internal decay is normally caused by the cell internal activity such as consumption of storage product to maintain the cell integrity during the starvation conditions. Besides, external decay is caused by the external factor such as predation (Ritton, 2011). In this study, the decay coefficient value obtained for the biomass treating POME could be mainly due to the internal decay. This is because the regular microscopy observation of the mixed-liquor sample taken from the parent SBR shows that there is no detectable amount of predator presence in the reactor.

4.4.2. Biomass yield

During the steady-state operation, the MLVSS in the parent SBR is approximately 4900 mg/L while the effluent MLVSS is approximately 1500 ± 150 mg/L at organic loading rate of approximately 5000 ± 500 mg/L day. Since the SBR manages to maintain a stable level of MLVSS, theoretically the biomass yield in the system must be higher or equal to 0.30 ± 0.03 mg VSS/mg COD. In order to determine the biomass yield, a series of experiments were carried out with different biomass concentration at fixed initial substrate concentration (1250 ± 150 mg COD/L, the substrate concentration in parent SBR). The experimental data is presented in the supplementary document (Supplementary Table 2).

The biomass concentrations used were 110 mg VSS/L, 210 mg VSS/L, 420 mg VSS/L, 1220 mg VSS/L, 1610 mg VSS/L and 4270 mg VSS/L. The biomass yield was calculated based on equations (1) and (2). It also shows that the variation on the initial MLVSS in reactor does not have great influence on the biomass yield. The result shows that the biomass in the reactor has an average biomass yield of 0.272 ± 0.014 mg VSS/mg COD. The average value of the biomass yield obtained is lower than the generally reported values of some typical heterotrophic microorganism (0.68–0.72 mg VSS/mg COD) (Eberl et al., 2006; Karahan et al., 2008; Wanner and Reichert, 1996). However, there are several researches which also reported yield values lower than 0.3 mg VSS/mg COD (Beltran et al., 2008; Carta-Escobar et al., 2005; Pelillo et al., 2006). It should be noted that all of these studies are conducted using similar type of wastewater (in term of COD concentration) under aerobic treatment as in this study.

Batch experiments were carried out at different COD concentration to observe the effect of COD concentration to the biomass yield. For all the tested COD concentration (1040 mg COD/L, 2310 mg COD/L, 4620 mg COD/L), the initial MLVSS of the batch tests was fixed at approximately 80 ± 10 mg VSS/L and the data is tabulated in supplementary document (Supplementary Table 3).

The experiments show that the biomass has demonstrated different yield value at different initial COD concentration region. In experiments which have COD region closer to the parent SBR (1250 ± 150 mg/L), the biomass has showed that the yield obtained is similar to the previous experiment. On the other hand, when the initial COD is at 2310 mg/L, the biomass yield has increase to about 0.369 mg VSS/mg COD. Meanwhile, at initial COD of 4620 mg/L, the biomass yield has drop to approximately 0.266 mg VSS/mg COD.

In this research, the initial COD concentration was found to affect the biomass yield greatly. Based on this observation, it can infer that the initial COD concentration of 2310 mg/L gives higher yield value compared to the 1040 mg COD/L (the concentration at which the biomass grown in the SBR). However, further increase of the substrate concentration to 4620 mg COD/L results in decrease in the biomass yield. This result is in line with the outcome of the substrate affinity study (presented in Section 4.4.3). The affinity study shows that the activity of biomass (measured in term of OUR) is at the maximum value at approximately 2000 mg COD/L and beyond this substrate concentration, the activity is decreases. This is highly believed due to the substrate inhibition towards the biomass activities.

The lower yield value obtained at higher substrate concentration suggests that the biomass might use a greater portion of its energy (obtained from the substrate oxidation) to overcome the inhibition. High fraction of the substrate consumed by biomass may be used to generate energy for cell maintenance processes in order to preserve the cell integrity rather than using the substrate for cell growth. This could have possibly results in the lower yield value at higher substrate concentration. It should be noted that in this study and other previous studies on POME, the biomass yield was calculated assuming that the substrate consumption is totally resulting in biomass growth.

However, in reality, biomass might spend a portion of the substrate consumed for non-growth related processes such as the cell maintenance processes as well (Bitton, 2011). Therefore by assuming all the substrate consumed will contribute to biomass growth, higher value of substrate may use for yield calculation. By definition yield is the biomass produced per unit of substrate consumed. Therefore, by assuming all the substrate consumed is used for growth (neglecting the substrate used for cell maintenance processes) the yield value might be underestimated. The yield value calculated using this method is more to apparent yield than the true yield in the wastewater treatment plant.

Apparently, a high yield of biomass is not necessarily preferred in a biological wastewater treatment plant. For example, if the yield of biomass is very high, it is possible that the biomass will bulk up in the biological wastewater treatment system and released to the downstream of the treatment process. Therefore, a minimal biomass concentration for optimum treatment efficiency is preferred. In this research, although the yield of biomass is not high, the biomass in SBR manages to treat the high strength wastewater (about 90% COD removal) within a short time period (less than 1 day). As long as the biomass retains in the SBR can successfully treat the POME, it is favored to have a lower biomass yield. However, more investigation about true yield is needed to describe and predict the biomass concentration changes using mathematical model.

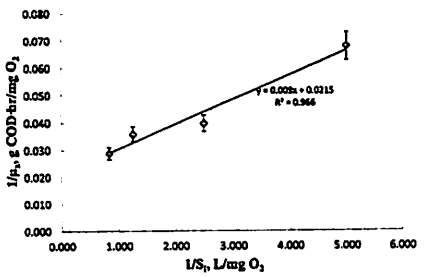

Fig. 5. Correlation between $1/S_s$ versus $1/\mu_x$.

4.4.3. Maximum specific growth rate and half saturation coefficient

Table 2 shows the experimentally obtained SOUR from 5 repetitive experiments together with the calculated μ_x using Equation (8) along with the average values and the associated standard errors. Based on Equation (9) and the values in Table 2, Fig. 5 was plotted to obtain the μ_{max} and K_s values of the biomass. The intercept point of the plot represents the μ_{max} and K_s value was calculated from the slope of the plot.

The maximum specific growth rate (μ_{max}) determined in this study is 47.625 mg O_2/g h (1.143 day^{-1}), which is higher than the previously reported values for biomass treating POME (Table 3). However, it should be noted that the values reported in the previous studies were obtained from biomass treating POME anaerobically. There are rarely any reported values from literature regarding biomass treating POME aerobically. It is commonly recognized that biomass growing in aerobic condition has higher growth rate compared to that of growing in anaerobic condition (Tchobanoglous et al., 2004).

The maximum growth rate of biomass in the aerobic biological treatment of olive mill wastewater (wastewater with similar COD strength with POME) was found to be relatively high compared to the biomass treating the same wastewater anaerobically (Hoyos et al., 2002). The biomass treating olive mill wastewater aerobically was reported to have of 0.912 day^{-1} (Hoyos et al., 2002). The maximum growth rate of biomass in anaerobic treatment of olive mill effluent is 0.315 day^{-1} (Borja et al., 1995). In this study, for the first time the μ_{max} of biomass treating POME aerobically was determined using the well-recognized Monod kinetic model and linear regression analysis of the double reciprocal plot. The obtained μ_{max} value may vary depending on the growth condition and microbial species involved in the process. Nevertheless, the growth activity of biomass is closely related to the condition of environment. Variables such as pH, temperature, F/M ratio, DO level and microbial population may affect the specific growth rate indefinitely. Therefore, more thorough works are required to obtain more precise values of growth kinetics of the biomass treating POME.

The K_s value obtained in this study is 0.429 g COD/L. The K_s value in this study is slightly higher than the values from other experimental works reported which have similar COD content (Damayanti et al., 2010). K_s value is normally depends on the nature of substrate provided to the biomass. Generally, substrate with both readily and slowly biodegradable organic matter will lead to higher K_s value compared to substrate associated with a single compound. Apart from that, the morphology of biomass could influence the substrate diffusivity into the biomass and affect the K_s value.

Generally, the value of $1/K_s$ is used to reflect the affinity of the bacteria toward substrate (Kovárová-Kovar and Egli, 1998). When the value of K_s is high, it indicates that the biomass has low affinity towards substrate. However, the ($1/K_s$) interpretation is not suitable to describe the affinity of biomass at different substrate concentration range. Therefore, the ratio of (μ_{max}/K_s) was introduced as an indicator of the biomass affinity towards substrate at different substrate concentration (Healey, 1980). Although the K_s value of this study is higher than that of average activated sludge, but the (μ_{max}/K_s) ratio in this study is 2.664 (higher than unity) which indicates that the biomass in this study has good affinity towards the substrate (COD) (Healey, 1980).

On the other hand, the substrate half saturation constant K_s of the biomass was determined independently (without using Fig. 5) by measuring OUR at different COD concentrations in a different set of experiments. These batch experiments were carried out with operational conditions identical to the parent SBR, except that COD concentration was varied in each test. OUR was continuously monitored and the maximum OUR at each COD concentration recorded. Fig. 6 shows the dependency of ($SOUR/SOUR_{max}$) on the substrate concentration.

Theoretically, the specific oxygen uptake rate is directly proportional to the activities of biomass. Fig. 6 indicates that the activities of the biomass in reactor are increasing rapidly towards the maximum value as the COD concentration increases up to 1500 mg COD/L. The increase of COD concentration beyond approximately 2000 mg COD/L to reactor will start to inhibit the biomass growth since it reduces the oxygen uptake rate. When the ($SOUR/SOUR_{max}$) ratio is at 0.5, the concentration should indicate the value of half saturation constant (K_s). From Fig. 6, the K_s value determined is approximately 400 mg COD/L, which is similar to the value obtained using Monod kinetic model. That verifies the validity of μ_{max} and K_s of the aerobic biomass determined using the Monod kinetic model. Further, it indirectly confirms the accuracy of k_d and Y determined in this study which were used in the calculation of μ_{max} and K_s using Equation (9).

Table 2
Specific OUR from 5 repetitive experiments and the calculated μ_x.

Experiment	Substrate (COD), g/L							
	0.2		0.4		0.8		1.2	
	SOUR, mg O_2/g h	μ_x, mg O_2/g h	SOUR, mg O_2/g h	μ_x, mg O_2/g h	SOUR, mg O_2/g h	μ_x, mg O_2/g h	SOUR, mg O_2/g h	μ_x, mg O_2/g h
I	40.76	15.17	64.88	24.16	72.36	26.94	94.31	35.15
II	41.55	15.46	69.22	25.78	77.31	28.79	89.88	33.50
III	37.59	13.98	70.97	26.44	69.73	25.96	95.43	35.56
IV	39.28	14.61	63.88	23.79	78.75	29.32	97.26	36.25
V	38.31	14.25	68.64	25.57	75.89	28.26	87.98	32.78
Average	39.50	14.69	67.52	25.15	74.81	27.85	92.97	34.65
Standard deviation	1.65	0.62	3.01	1.13	3.70	1.38	3.90	1.45

Table 3
Kinetic parameter in different type of reactor for POME treatment.

Type of reactor	K_s (g/L)	μ_{max} (d^{-1})	References
Upflow anaerobic sludge fixed film bioreactor (UASFF)	0.582	0.207	Zinatizadeh et al. (2006)
Continuous stirred tank reactor (CSTR)	0.100	0.780	Damayanti et al. (2010)
Aerobic sequencing batch reactor (SBR)	0.429	1.143	This study

5. Conclusion

In this study, anaerobically pretreated, POME was treated biologically without any pH adjustment in an SBR. The aerobic biomass successfully removed approximately 90% of the COD content from the POME up to an organic loading rate (OLR) of 11,550 mg COD/L day (the highest value tested in this study). Analysis of the stored energy (PHB) of the biomass shows that oxygen uptake when there is no significant reduction of COD may due to the oxidation of storage product. It is believed that the biomass uses PHB as an

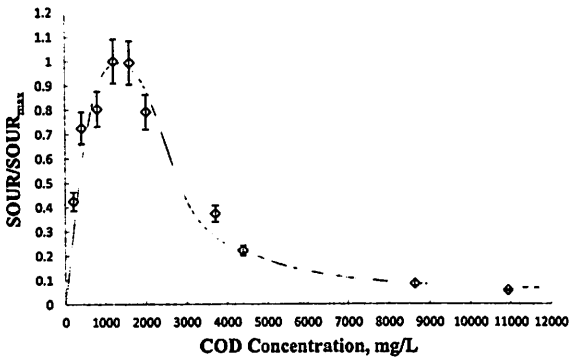

Fig. 6. Ratio of $SOUR/SOUR_{max}$ of biomass as the function of COD concentration in the feed.

energy source when deprived of external substrate. Further, the growth kinetic parameters of aerobic biomass treating POME were determined. The μ_{max} of the biomass was determined to be around 1.143 day^{-1}. The K_s was estimated to be 0.429 mg/L with respect to the COD. Meanwhile, the decay coefficient and biomass yield were found to be 0.131 day^{-1} and 0.272 mg biomass/mg COD consumed, respectively.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jenvman.2014.07.023.

References

Ahmad, A.L., Chong, M.F., Bhatia, S., 2009. A comparative study on the membrane based palm oil mill effluent (POME) treatment plant. J. Hazard. Mater. 171, 166–174.
APHA, Rice, E.W., Association, A.P.H., Association, A.W.W., Wef, Federation, W.E., 2012 Standard Methods for Examination of Water and Wastewater 2012. American Public Health Association.
Beltran, J., Gonzalez, T., Garcia, J., 2008. Kinetics of the biodegradation of green table olive wastewaters by aerobic and anaerobic treatments. J. Hazard. Mater. 154, 839–845.
Bitton, G., 2011. Wastewater Microbiology. Wiley.
Borja, R., Banks, C.J., 1994. Treatment of palm oil mill effluent by upflow anaerobic filtration. J. Chem. Technol. Biotechnol. 61, 103–109.
Borja, R., Martín, A., Alonso, V., García, I., Banks, C.J., 1995. Influence of different aerobic pretreatments on the kinetics of anaerobic digestion of olive mill wastewater. Water Res. 29, 489–495.
Carta-Escobar, F., Pereda-Marín, J., Alvarez-Mateos, P., Romero-Guzmán, F., Durán Barrantes, M.M., 2005. Aerobic purification of dairy wastewater in continuous regime: part II: kinetic study of the organic matter removal in two reactor configurations. Biochem. Eng. J. 22, 117–124.
Chaiprapat, S., Laklam, T., 2011. Enhancing digestion efficiency of POME in anaerobic sequencing batch reactor with ozonation pretreatment and cycle time reduction. Bioresour. Technol. 102, 4061–4068.
Chan, Y.J., Chong, M.F., Law, C.L., 2010. Biological treatment of anaerobically digested palm oil mill effluent (POME) using a lab-scale sequencing batch reactor (SBR). J. Environ. Manag. 91, 1738–1746.
Damayanti, A., Ujang, Z., Salim, M.R., Olsson, G., Sulaiman, A.Z., 2010. Respirometric analysis of activated sludge models from palm oil mill effluent. Bioresour. Technol. 101, 144–149.
Eberl, H., Morgenroth, E., Noguera, D., Picioreanu, C., Rittmann, B., Loosdrecht, M.V., Wanner, O., 2006. Mathematical Modeling of Biofilms. IWA Publishing.
Gobi, K., Vadivelu, V.M., June 2014. Aerobic dynamic feeding as a strategy for in situ accumulation of polyhydroxyalkanoate in aerobic granules. Bioresour. Technol. 161, 441–445.
Grady, C.L., Daigger, G.T., Love, N.G., 2011. Biological Wastewater Treatment. Taylor & Francis Group.
Healey, F.P., 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. 5, 281–286.
Henze, M., Loosdrecht, M.C.M.V., Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment: Principles, Modelling and Design. IWA Pub.
Hoyos, S.F.G., Nieto, L.M., Rubio, F.C., Cormenzana, A.R., 2002. Kinetics of aerobic treatment of olive-mill wastewater (OMW) with *Aspergillus terreus*. Process Biochem. 37, 1169–1176.
Hung, Y.T., Wang, L.K., Shammas, N.K., 2012. Handbook of Environment and Waste Management: Air and Water Pollution Control. World Scientific.
Igwe, J.C., Onyegbado, C.C., 2007. A review of palm oil mill effluent (POME) water treatment. Global J. Environ. Res. 1, 54–62.
Jalani, B.S., Basiron, Y., Chan, K.W., Malaysia, L.M.S., 2000. Advances in Oil Palm Research. Malaysian Palm Oil Board, Ministry of Primary Industries, Malaysia.
Karahan, O., Dogruel, S., Dulekgurgen, E., Orhon, D., 2008. COD fractionation of tannery wastewaters—particle size distribution, biodegradability and modeling. Water Res. 42, 1083–1092.
Kim, B.S., Lee, S.C., Lee, S.Y., Chang, H.N., Chang, Y.K., Woo, S.I., 1994. Production of poly(3-hydroxybutyric-co-3-hydroxyvaleric acid) by fed-batch culture of *Alcaligenes eutrophus* with substrate control using on-line glucose analyzer. Enzyme Microb. Technol. 16, 556–561.
Kovarova-Kovar, K., Egli, T., 1998. Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. Microbiol. Mol. Biol. Rev. 62, 646–666.
Liu, Q.S., Tay, J.H., Liu, Y., 2003. Substrate concentration-independent aerobic granulation in sequential aerobic sludge blanket reactor. Environ. Technol. 24, 1235–1242.
Makinia, J., 2010. Mathematical Modelling and Computer Simulation of Activated Sludge Systems. IWA Publishing.
Pellillo, M., Rincon, B., Raposo, F., Martin, A., Borja, R., 2006. Mathematical modelling of the aerobic degradation of two-phase olive mill effluents in a batch reactor. Biochem. Eng. J. 30, 308–315.
Puyan, M., Saunders, A.M., Guisasola, A., Baeza, J.A., Casas, C., Blackall, L.L., 2004. Enhanced biological phosphorus removal in a sequencing batch reactor using propionate as the sole carbon source. Biotechnol. Bioeng. 85, 56–67.
Poh, P.E., Chong, M.F., 2009. Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Bioresour. Technol. 100, 1–9.
Sinanuntapiboon, S., Hongsrisuwan, T., 2007. Removal of Zn^{2+} and Cu^{2+} by a sequencing batch reactor (SBR) system. Bioresour. Technol. 98, 808–818.
Tchobanoglous, G., Burton, F.L., Metcalf, Eddy, Stensel, H.D., 2004. Wastewater Engineering: Treatment and Reuse. McGraw-Hill Education.
Van Loosdrecht, M.C.M., Henze, M., 1999. Maintenance, endogeneous respiration, lysis, decay and predation. Water Sci. Technol. 39, 107–117.
Wang, B., Sharma-Shivappa, R., Olson, J., Khan, S., 2012. Upstream process optimization of polyhydroxybutyrate (PHB) by *Alcaligenes latus* using two-stage batch and fed-batch fermentation strategies. Bioprocess Biosyst. Eng. 35, 1591–1602.
Wanner, O., Reichert, P., 1996. Mathematical modeling of mixed-culture biofilms. Biotechnol. Bioeng. 49, 172–184.
Willey, J.M., Sherwood, L.M., Woolverton, C.J., Prescott, L.M., 2009. Prescott's Principles of Microbiology. McGraw-Hill Higher Education.
Zinatizadeh, A.A.L., Mohamed, A.R., Najafpour, G.D., Hasnain Isa, M., Nasrollahzadeh, H., 2006. Kinetic evaluation of palm oil mill effluent digestion in a high rate up-flow anaerobic sludge fixed film bioreactor. Process Biochem. 41, 1038–1046.

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Short Communication

Aerobic dynamic feeding as a strategy for in situ accumulation of polyhydroxyalkanoate in aerobic granules

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HIGHLIGHTS

- For the first time aerobic granules developed in POME are used for PHA accumulation.
- PHA accumulation in aerobic granules matches pure culture PHA accumulation.
- Even with lower concentration of VFA, commendable PHA accumulation were obtained.
- Butyric and propionic acids were preferred over acetic acid for PHA accumulation.

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ABSTRACT

Aerobic dynamic feeding (ADF) strategy was applied in sequencing batch reactor (SBR) to accumulate polyhydroxyalkanoate (PHA) in aerobic granules. The aerobic granules were able to remove 90% of the COD from palm oil mill effluent (POME). The volatile fatty acids (VFAs) in the POME are the sole source of the PHA accumulation. In this work, 100% removal of propionic and butyric acids in the POME were observed. The highest amount of PHA produced in aerobic granules was 0.6833 mg PHA/mg biomass. The PHA formed was identified as a P (hydroxybutyrate-co-hydroxyvalerate) P (HB-co-HV).

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1. Introduction

Aerobic dynamic feeding (ADF) is a fundamental feeding strategy for polyhydroxyalkanoate (PHA) accumulation in pure and mixed cultures (Serafim et al., 2004). ADF works on the basis of feast-famine phase establishment in a wastewater treatment system. PHA-accumulating microorganisms will produce and store PHA (as energy storage material) in their cells when an external carbon source is available in abundance (feast) (Serafim et al., 2004). PHA is a biodegradable plastic and shares similar properties to polypropylene (Marang et al., 2013). However, current hindrance in expanding the PHA production is the high substrate cost and utilization of pure culture fermentation (Moita and Lemos, 2012). Thus, waste products (as substrate) and mixed culture (as inoculums) were used as a measure of reducing the overall PHA production cost (Bengtsson et al., 2008; Davis et al., 2013; Huang et al., 2012; Mumtaz et al., 2010).

The major challenge in mixed culture is to produce PHA as much as pure culture strain for a given volume (Campos et al., 2014). It is mainly because not all the myriad types of microorganism in a mixed culture can accumulate PHA. Thus, in order to optimize the PHA accumulation in a mixed culture, the ADF strategy was adapted (Johnson et al., 2009; Serafim et al., 2004). ADF functions as a selection process to enrich the PHA-accumulating bacteria such as *Ralstonia* genus (Huang et al., 2012) or *Gammaproteobacteria* group (Johnson et al., 2009) in a mixed culture system. The successful implementation of this strategy was reported in the work of Johnson et al. (2009), in which 89% of the cell dry weight (CDW) of the mixed culture consists of PHA.

Concomitantly, ADF is also being applied as one of the basic strategies in the formation of granules under aerobic conditions (Tay et al., 2001). Essentially, during the formation stages of aerobic granules, ADF is used to induce hydrophobicity between the cells (Tay et al., 2001). Moreover, extracellular polymeric substances (EPSs) secreted by the bacteria functions to bind the cells to form the aerobic granules and act as a shield for the aerobic granules when they are exposed to shock conditions (Wang et al., 2005). Aerobic granules are superior to activated sludge in terms of settling ability, robustness, density and ability to withstand shock loadings (Tay et al., 2001).

Apparently, ADF is a common strategy for both the accumulation of PHA and the formation of aerobic granules. Thus, it can be deduced that aerobic granules can accumulate PHA. However, the interaction between PHA storage and aerobic granulation technology has been overlooked and not fully explored to date. Perhaps, the complex nature of the aerobic granules has made such exploration difficult. The cross-sectional area of the aerobic granules exhibits the existence of an oxygen concentration gradient across the granule. The core of the granule it was reported to be an anaerobic zone (Li et al., 2008). Conventionally, PHA accumulation occurs preferentially under aerobic conditions. Only a few researchers agree that PHA accumulation can occur under anaerobic conditions (Satoh et al., 1999). Thus, it is interesting to scrutinize the dynamics of the aerobic granules during the accumulation of PHA.

The aim of this study is to analyze the in situ accumulation of PHA in aerobic granules during the treatment of agro-based industrial wastewater. The total PHA accumulation, production yield and the PHA content with respect to CDW were evaluated. In addition, the PHA monomer produced using the readily available volatile fatty acid (VFA) in the feed is reported and discussed in this work. Regarding related literature studies, no comprehensive work has been reported on the accumulation of PHA inside aerobic granules to date. Thus, the PHA accumulation inside aerobic granules is expected to elucidate the wider application of aerobic granules in the future.

2. Methods

2.1. Cultivation of aerobic granules

Aerobic granules were cultivated in a sequencing batch reactor (SBR). The sole substrate for the cultivation process is palm oil mill effluent (POME), an agro based industrial wastewater. Activated sludge from an aerobic pond of a POME treatment plant was used as the seed sludge for the aerobic granules formation. The SBR was operated in a 6-h cycle with filling for 20 min, aeration for 330 min, settling for 1.5 min and decanting for 8.5 min. A 25% volume exchange ratio was applied for the SBR system. The height over diameter (H/D) ratio of the SBR used is 10. Meanwhile, fine aeration with a flow rate of 3 L/min was used for aerating the SBR. The SBR has been in operation for approximately 450 days.

2.2. Substrate and feeding strategy for aerobic granule cultivation

POME was used as the sole substrate for aerobic granule cultivation. The average chemical oxygen demand (COD) content and pH of the POME are 51,000 mg/L and 5, respectively. Prior to SBR feeding, the POME was filtered to remove debris and diluted with tap water to achieve an organic loading rate (OLR) of 2.25 kg COD/m^3 day. During the 330 min of aeration, the ADF strategy was adopted. The substrate was taken up within the first 50–120 min (feast period) and for the remaining period, the aerobic granules were under starvation mode (famine period). Therefore, the feast-famine ratio was maintained between 0.15 and 0.36 in each cycle.

2.3. PHA extraction process

PHA was extracted at the end of the feast period of each cycle. Sodium hypochlorite-chloroform method adapted from Hahn et al. (1994) was used for the PHA extraction process. The amount of PHA produced in the resultant precipitate after drying was recorded. The amount of PHA produced is calculated using Eq. (1).

$$Y_{PHA/CDW} = \frac{\text{Total amount of PHA, mg}}{\text{CDW of aerobic granules, mg}} \quad (1)$$

2.4. Analytical method

The chloroform layer enriched with PHA was injected into the gas chromatography (GC). An Agilent 7890A GC system equipped with a flame ionization detector (FID) and a GsBP-5MS column (length: 30 m, internal diameter: 250 μm, film thickness: 0.25 μm) were used with helium as the carrier gas. A modification of the method of Bengtsson et al. (2008) was used for the PHA analysis. The injector and the detector temperatures were set at 200 °C and 260 °C, respectively. Calibration of PHA was performed using poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (12 wt.% PHV) of natural origin as the standard (Sigma Aldrich). Meanwhile, the calculation of PHA yield in terms of the COD consumption was performed using Eq. (2).

$$Y_{PHA/COD} = \frac{\text{Total amount of PHA, mg}}{\text{Amount of COD converted, mg/L}} \quad (2)$$

The volatile fatty acid (VFA) was also analyzed using gas chromatography. The samples (influent and effluent of SBR) were centrifuged, and the supernatant was filtered using a syringe filter (ø 22 mm and mesh size of 0.45 μm) and then injected into the GC instrument. An Agilent 7890A GC system equipped with flame ionization detector (FID) and a Nukol Supelco column (length: 30 m, internal diameter: 250 μm, film thickness: 0.25 μm) were used with helium as the carrier gas. The initial oven temperature was set to 100 °C with a gradual increase at a rate of 8 °C/min to a final temperature of 200 °C, which was maintained for 2 min. Split injection at 200 °C with a 10:1 ratio was used. The injector and detector temperatures were set at 200 °C and 260 °C, respectively. Calibration of VFA was performed using a volatile free acid mix solution standard (Sigma Aldrich).

The feed concentration (in terms of COD), aerobic granules concentration (in terms of mixed liquor volatile suspended solids (MLVSS)) and settling ability (in terms of sludge volume index (SVI)) were measured using standard APHA method (APHA, 2012). Meanwhile, the presence of PHB-co-PHV was confirmed with Fourier Transmission Infrared Spectroscopy (FTIR). The Shimadzu IRAffinity spectrometer was used to record the FTIR spectra of the PHA in the range of 750–4000 cm^{-1} and 0.4 cm^{-1} resolution.

3. Results and discussion

3.1. Aerobic granules and feed characterization

POME was aerobically treated in an SBR using activated sludge as the seed sludge. The activated sludge turned into aerobic granules within nine days. The diameter of the aerobic granules slowly increased until it reached approximately 3 mm. Thereafter, the granules disintegrated, resulting in the formation of new aerobic granules. This cycle occurs in a period of 14 days. The aerobic granules cultivated in SBR have excellent settling ability with a sludge volume index (SVI) in the range of 25–40 mL/g. Meanwhile, in terms of the COD removal efficiency, the granules managed to remove approximately 90% of the influent COD in each cycle.

On average, 90% of the COD removal was achieved after 2 h of feeding, resulting in a famine period for the rest of the cycle (4 h). Fig. 1 shows a typical dissolved oxygen (DO) concentration profile of a 6-h cycle of the SBR. The establishment of two phases (feast

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and famine) can be seen in the graph based on the DO and COD profiles. The start of the famine period is indicated by the DO saturation level (close to 100%) and the end of the COD uptake process (at approximately 95 mg/L). Indirectly, the start of the famine period indicates that oxygen is no longer being used by the aerobic granules to digest the remaining organic components in the POME. Identification of the feast-famine period in a cycle helps with the collection of samples for polyhydroxyalkanoate (PHA) accumulation. It has been widely reported that the accumulation of PHA is the highest at the end of the feast period (Moita and Lemos, 2012).

Meanwhile, the feed used in this study was POME, which is a high-strength industrial wastewater. In POME, volatile fatty acid (VFA) is naturally available but in a limited amount. Fig. 1b shows the breakdown of the available VFA in the feed and effluent of the SBR. Generally, acidogenesis treatment is applied to enhance the amount of VFA in the feed for the PHA accumulation (Mumtaz et al., 2010). However, in this work, this treatment was not performed. The readily available VFAs in the POME were the sole feed for the aerobic granules to convert them into PHA.

3.2. Accumulation of PHA inside aerobic granules

Fig. 1 also shows the PHA accumulation profile in the aerobic granules in a cycle of 6 h. In the first two hours (feast period), the amount of PHA accumulated increases. Thereafter, the amount of PHA in the aerobic granules started to decrease sharply during the famine period. Meanwhile, for the first two hours of the reaction, a rapid decrease in COD can be observed as well, and the COD concentration reaches a minimum value of 95 mg/L. This result indicates that approximately 90% of the influent was removed in the first two hours of the reaction. The remaining 10% of the COD in the SBR is either slowly biodegradable or non-biodegradable COD. By correlating the end of the COD uptake and the decrease in PHA during the famine phase, it could be concluded that PHA is used as the carbon source for maintenance energy needs when the external carbon source is no longer available for the aerobic granules.

Meanwhile, the EPS content of the aerobic granules remained stable at approximately 40–45 μg/mL throughout each cycle. No drastic changes were recorded over the 4 h of the famine period. Previously, it was reported that EPS is consumed during the starvation period (Wang et al., 2005). It was reported that the EPSs present at the core of the granules were consumed for the maintenance of cells during the starvation period. Thus, this consumption of EPS makes the structure of the granules weaker, and the granules are more easily broken. However, in this study, the morphology and structure of the aerobic granules remain intact after the starvation period. It should be noted that the starvation period in this study

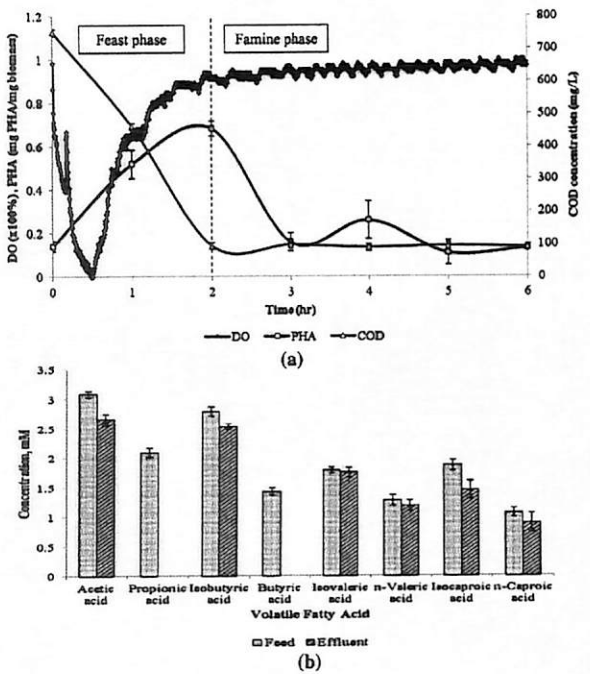

Fig. 1. Dissolved oxygen, total PHA accumulation and COD concentration profile of aerobic granules treating POME for one complete cycle. (b). Volatile fatty acid (VFA) composition of SBR feed and effluent.

Table 1
Comparison of the yield from various types of accumulator.

Type of accumulator	Substrate	Yield COD(mg PHA/mg COD)	PHA content (% CDW)	References
Activated sludge	Olive oil pomace	0.36	39.00	Waller et al. (2012)
Activated sludge	Paper mill wastewater	0.11	48.00	Bengtsson et al. (2008)
Activated sludge	Bio-oil	0.62	9.20	Moita and Lemos (2012)
Aerobic granules	Raw POME	0.66 (0.065)	68.33 (0.03)	This work

Standard deviation in brackets.

was only approximately 4 h, whereas in the work of Wang et al. (2005), the aerobic granules were exposed to a starvation period of 20 days. This difference between this study and previous studies may be one of the reasons for the non-consumption of EPS in this study and the subsequent maintenance of the structure of the granules. Moreover, the consumption of PHA during the famine period suggests that PHA is preferred over EPS as the carbon source for the maintenance needs of the aerobic granules.

The aerobic granules used in this study managed to accumulate 0.6833 mg PHA/mg biomass. Because the POME used was not optimized for VFA production, the concentration of readily available VFA was only 15 mM. For a low amount of available VFA, a yield of 0.6833 mg PHA/mg biomass is commendable. Thus, it is envisaged that optimization of the VFA production via the acidogenesis process of POME will provide a larger concentration of VFA for the aerobic granules to take up. A higher amount of VFA may provide a sufficient driving force to overcome the mass transfer resistance and thereafter diffuse across aerobic granules to form a larger amount of PHA. It appears that among all of the available VFAs, propionic and butyric acids were the most consumed VFAs. The amounts of propionic and butyric acids were 2.1 and 1.43 mM, respectively (Fig. 1b). Overall, 100% removal of both of these acids was recorded.

Nevertheless, the ability of aerobic granules to accumulate PHA, even with a small amount of VFA, is advantageous. For a similar volume of aerobic granules and activated sludge, the amount of cells present in aerobic granules is higher compared to that of activated sludge (Tay et al., 2001). Thus, in terms of volumetric productivity, the amount of PHA produced using aerobic granules is higher compared to that of activated sludge. In addition, the aerobic granules are able to accumulate PHA up to 68.33% of the CDW. Though PHA primarily functions as energy storage material in aerobic granules, the capability of aerobic granules to accumulate PHA up to 68.33% of its CDW could be used to commercialize PHA as a valuable commodity.

Table 1 shows the comparison of the PHA yield in activated sludge and aerobic granules. It is clearly seen from Table 1 that the yield of accumulated PHA in aerobic granules is higher compared to the yield in conventional activated sludge. Even when aerobic granules are fed with a limited amount of VFA, the PHA yield obtained (0.6600 mg PHA/mg COD) is commendable. However, it is more relevant to observe the amount of PHA accumulated per cell dry weight (CDW). Compared to conventional activated sludge, the amount of PHA accumulated per aerobic granule is approximately 68.33% on average. Note that the VFA in the feed was not optimized; otherwise, it is predicted that more PHA could be accumulated. The optimization of acidogenesis would enhance the VFA accumulation and subsequently used for PHA accumulation. As a result of VFAs accumulation, the pH of the wastewater would be below 5.5. This pH inhibits the growth of methanogens. Inhibited growth of methanogens would leave the VFAs intact, without turning them into methane gas. Higher amount of VFA would enhance its diffusion process across the granules for PHA accumulation process.

3.3. Composition of PHA accumulated

FTIR analysis was done to confirm the type of PHA produced. In the FTIR analysis of PHA accumulated in aerobic granules, peaks present at 2924.09 cm^{-1} and 1130.29 cm^{-1} represents the C–CH_3 and C–O–C chemical groups of the PHA, respectively. Meanwhile the peak at 1720.50 cm^{-1} indicates the presence of C=O from PHA. Meanwhile, the adsorptions band at 3429.43 cm^{-1} shows the stretching of CH_3 asymmetric (Venkateswar Reddy et al., 2012). Similarities of peaks between PHA accumulated in aerobic granules and PHB-co-PHV found in the work of Venkateswar Reddy et al. (2012) confirms that the PHA produced is a PHB-co-PHV copolymer. For the yield of 0.6600 mg PHA/mg COD, the composition of the copolymer PHB-co-PHV is 55% HB and 45% HV. As seen in Fig. 1b, almost all of the VFA exhibits a reduction in concentration after aerobic treatment. Most notably, two VFA components, namely, propionic and butyric acids, show 100% removal. The complete removal of these VFAs and the formation of the PHB-co-PHV copolymer validate that propionic and butyric acids are responsible for the PHA formed (Kulkarni et al., 2010). In fact, the PHB-co-PHV copolymer is preferred for plastic production because it is more stable and less brittle compared to the PHB monomer alone (Kulkarni et al., 2010). Meanwhile, other PHA monomers were not detected in this study. This lack of other PHA monomers may be due to the utilization of the other VFAs (apart from propionic and butyric acids) for growth and respiration (Bengtsson et al., 2010). Moreover, aerobic granules prefer to use propionic and butyric acids because they are converted to PHA via a simple pathway of β-ketothiolase (PhbA) and acetoacetyl-CoA reductase (PhbB). Meanwhile, the uptake of caproic and iso-caproic acids for the conversion into PHA requires a complex β-oxidation cycle (Ren et al., 2000).

In the meantime, the amount of acetic acid consumed is lower compared to that of butyric acid, even though acetic acid represents the highest proportion of VFA in the POME (Fig. 1b). The generation of nicotinamide adenine dinucleotide hydrogenase (NADH) in the process of butyric acid consumption may be one of the reasons for the butyric acid uptake preference. Conversion of 1 mol of butyric acid produces 0.5 mol of NADH, which, in turn, could produce excess amounts of adenosine triphosphate (ATP) via oxidative phosphorylation (Marang et al., 2013). Meanwhile, the conversion of acetic acid to PHB requires NADH (a reverse of butyric acid conversion mechanism). As a result of this NADH requirement, a sufficient amount of ATP must be generated via an external source, which reduces the utilization of acetic acid (Marang et al., 2013). Thus, it could be concluded that aerobic granules share a similar mechanism with activated sludge in taking up the available VFA.

4. Conclusion

This study demonstrated that aerobic granules can accumulate PHA in their cells. The accumulated PHA is identified as the PHB-co-PHV copolymer, which arises from the consumption of propionic and butyric acids. With the limited amount of VFA present in the feed, the highest total PHA accumulation, the PHA yield, and the proportion of PHA in terms of CDW are 0.6833 mg PHA/mg biomass, 0.6600 mg PHA/mg COD, and 68.33%, respectively. In the future, the acidogenesis treatment of raw POME is expected to enhance the amount of VFAs and subsequently increase the amount of PHA produced in aerobic granules.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.03.104.

References

APHA, 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, Washington, DC, USA.

Bengtsson, S., Pisco, A.R., Johansson, P., Lemos, P.C., Reis, M.A.M., 2010. Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures. J. Biotechnol. 147 (3–4), 172–179.

Bengtsson, S., Werker, A., Christensson, M., Welander, T., 2008. Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. Bioresour. Technol. 99 (3), 509–516.

Campos, M.I., Figueiredo, T.V.B., Sousa, L.S., Druzian, J.I., 2014. The influence of crude glycerin and nitrogen concentrations on the production of PHA by cupriavidus necator using a response surface methodology and its characterizations. Ind. Crop. Prod. 52, 338–346.

Davis, R., Kataria, R., Cerrone, F., Woods, T., Kenny, S., O'Donovan, A., Guzik, M., Shaikh, H., Duane, G., Gupta, V.K., Tuohy, M.G., Padamatti, R.B., Casey, E., O'Connor, K.E., 2013. Conversion of grass biomass into fermentable sugars and its utilization for medium chain length polyhydroxyalkanoate (mcl-PHA) production by pseudomonas strains. Bioresour. Technol. 150, 202–209.

Hahn, S.K., Chang, Y.K., Kim, B.S., Chang, H.N., 1994. Optimization of microbial poly (3-hydroxybutyrate) recovery using dispersions of sodium-hypochlorite solution and chloroform. Biotechnol. Bioeng. 44 (2), 256–261.

Huang, Y.-T., Chen, P.-L., Semblante, G.U., You, S.-J., 2012. Detection of polyhydroxyalkanoate-accumulating bacteria from domestic wastewater treatment plant using highly sensitive PCR primers. J. Microbiol. Biotechnol. 22 (8), 1141–1147.

Johnson, K., Jiang, Y., Kleerebezem, R., Muyzer, G., van Loosdrecht, M.C.M., 2009. Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. Biomacromolecules 10 (4), 670–676.

Kulkarni, S.O., Kanekar, P.P., Nilegaonkar, S.S., Sarnaik, S.S., Jog, J.P., 2010. Production and characterization of a biodegradable poly (hydroxybutyrate-co-hydroxyvalerate) (PHB-co-PHV) copolymer by moderately haloalkalitolerant halomonas campisalis MCM B-1027 isolated from lonar lake,India. Bioresour. Technol. 101 (24), 9765–9771.

Li, Y., Liu, Y., Shen, L., Chen, F., 2008. DO diffusion profile in aerobic granule and its microbiological implications. Enzyme Microb. Technol. 43 (4–5), 349–354.

Marang, L., Jiang, Y., van Loosdrecht, M.C.M., Kleerebezem, R., 2013. Butyrate as preferred substrate for polyhydroxybutyrate production. Bioresour. Technol. 142, 232–239.

Mosta, R., Lemos, P.C., 2012. Biopolymers production from mixed cultures and pyrolysis by-products. J. Biotechnol. 157 (4), 578–583.

Mumtaz, T., Yahaya, N.A., Abd-Aziz, S., Rahman, N.A.A., Yee, P.L., Shirai, Y., Hassan, M.A., 2010. Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent – a Malaysian perspective. J. Clean Prod. 18 (14), 1393–1402.

Ren, Q., Sierro, N., Kellerhals, M., Kessler, B., Witholt, B., 2000. Properties of engineered poly-3-hydroxyalkanoates produced in recombinant *Escherichia coli* strains. Appl. Environ. Microbiol. 66 (4), 1311–1320.

Satoh, H., Mino, T., Matsuo, T., 1999. PHA production by activated sludge. Int. J. Biol. Macromol. 25 (1–3), 105–109.

Serafim, L.S., Lemos, P.C., Oliveira, R., Reis, M.A.M., 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. Biotechnol. Bioeng. 87 (2), 145–160.

Tay, J.H., Liu, Q.S., Liu, Y., 2001. Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor. J. Appl. Microbiol. 91 (1), 168–175.

Venkateswar Reddy, M., Nikhil, G.N., Venkata Mohan, S., Swamy, Y.V., Sarma, P.N., 2012. Pseudomonas otitidis as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. Bioresour. Technol. 123, 471–479.

Waller, J.L., Green, P.G., Loge, F.J., 2012. Mixed-culture polyhydroxyalkanoate production from olive oil mill pomace. Bioresour. Technol. 120, 285–289.

Wang, Z.W., Liu, Y., Tay, J.H., 2005. Distribution of EPS and cell surface hydrophobicity in aerobic granules. Appl. Microbiol. Biotechnol. 69 (4), 469–473.

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By-products of palm oil mill effluent treatment plant – A step towards sustainability

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ABSTRACT

The current wastewater treatment system for palm oil mill effluent (POME) regularly fails to treat the effluent efficiently. The growing demand for palm oil has caused a substantial increase in the generation of POME. To meet the discharge limit proposed by the Department of the Environment, the POME must be treated effectively before being released into the receiving water bodies. The open pond system is presently being used to treat the POME because the open pond system is cheap and less maintenance is required. However, the failure of this technique in the current scenario has spurred the research into new technologies to explore their applicability in treating POME. Although the discovery of new technologies is commendable, the financial infeasibility of these new treatment techniques has stagnated their progress. In this work, a role for the by-products of the treatment systems in implementing the new technologies with return of investment has been revealed. A thorough review of the characteristics and recent trends for producing polyhydroxyalkanoate (PHA), a by-product, is also discussed in this work. Moreover, the opportunities available to further enhance the production of PHA in POME wastewater have been addressed and are presented in this work. Production of biohydrogen, another by-product, is also discussed in this review. In a nutshell, the enhancement of PHA production coupled with biohydrogen production as a by-product may provide a new dimension to the POME treatment plant by generating revenue. Production of PHA and biohydrogen from POME contributes significantly towards the cause of sustainability.

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Contents

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1. Introduction

An industrial wastewater treatment plant is generally considered a money depletion zone in a business operation. Little investment would therefore be made in the wastewater treatment plant. As a result, the wastewater is either insufficiently treated or not treated at all. If this trend continues, the environment will be severely polluted, and access to clean water will be limited in the future. An increase in the volume of industrial wastewater has been reported in developing countries [1]. The increase in the volume of industrial wastewater in developing countries can be related to the growth of the population. Population growth causes the demand for end products to increase. Similar developments can be expected for palm oil derivative products. An increase in the demand for end products causes the production rate to increase and concomitantly increases the amount of wastewater released into water bodies. Fig. 1 shows the projected world population growth for developing countries and industrialised countries. An exponential increase in world population is expected in developing countries in another 30 years.

Developing countries such as Malaysia, Indonesia, Nigeria and Thailand are the major force in supplying palm oil to the world [3]. Fig. 2 shows the palm oil producers in the world. In particular, the oil palm industry is the fourth largest contributor to the Malaysian economy, contributing approximately RM 53 billion of the Gross National Income of Malaysia [4]. This industry has grown in tandem with the nation's growth. The production of palm oil contributes approximately 39% of the total palm oil production in the world, and 44% of the palm oil is exported around the world [5]. Essentially, the palm oil demand grows because palm oil is cheap and has high oxidative stability. Biodiesel production from palm oil has recently escalated the demand for palm oil further. With the growing demand for palm oil, the plantation area of oil palm trees has reached approximately 5 million hectares in Malaysia [6]. The crude palm oil (CPO) production was 18.8 million tonnes in 2012. Under the Tenth Malaysian Program, the palm oil is expected to contribute approximately RM 69.3 billion through exportation [7].

In tandem with the increase in demand for palm oil, CPO production rate has escalated approximately 171% in the span of 20 years in Malaysia (as shown in Fig. 3). Higher CPO production has increased the amount of waste released from the oil extraction process, and this waste has a critical need to be addressed. Empty fruit bunches, press fibre, palm kernel endocarp, palm kernel press cake and liquid effluent (palm oil mill effluent (POME)) are the wastes generated through processing to produce CPO [9]. These wastes cause detrimental effects to environmental quality if they are left untreated. Among those wastes, POME makes up the largest portion. For every 1 t of CPO, approximately 5–7.5 t of water are necessary. More than 50% of this water that is used in the production of CPO will end up as liquid waste [10]. POME characteristics as determined by different researchers are presented in Table 1. Table 1 shows that POME is evidently an agent causing severe pollution (high chemical oxygen demand (COD) and the presence of oil and grease). The critical need to treat the POME has sparked the interest among researchers to find new

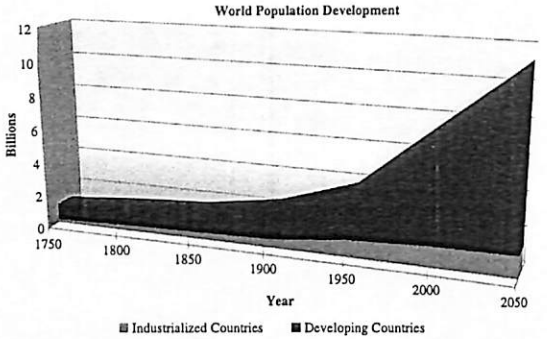

Fig. 1. Projected population of the world [2].

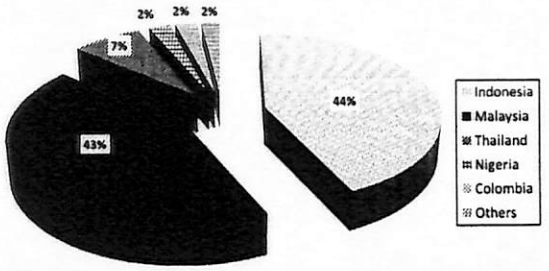

Fig. 2. World palm oil producers [8].

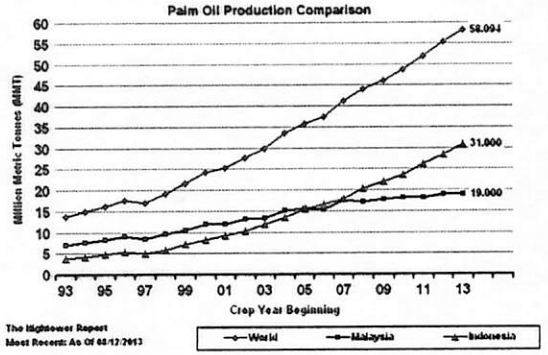

Fig. 3. Crude palm oil production amount [11].

Table 1
Characteristics of POME from various previous studies.

pH	BOD (mg/L)	COD (mg/L)	Oil and grease (mg/L)	Suspended solids (mg/L)	Nitrogen Content (mg/L)	Reference
3.5–4.5	11,000–30,000	30,000–70,000	5000–13,000	9000–25,000	500–900	[12]
5	11,000	246,000	–	–	–	[13]
4.7	25,000	50,000	4000	18,000	750	[14]
4.52	–	70,900	–	25,800	–	[15]
4.0–4.8	–	30,000–50,400	1300–4700	11,500–22,000	660–890	[16]
3.5–4.2	10,000–44,000	16,000–100,000	–	5000–54,000	–	[17]
4.15–4.45	21,500–28,500	45,500–65,000	1077–7582	15,660–23,560	300–410	[18]
5.6	–	46,000	–	42,800	–	[19]
5.5	–	35,000–50,000	–	35,000	–	[20]

efficient treatment methods. The treatment method must complement the increasing growth of the CPO production rate and comply with the demand of the Department of the Environment (DOE) to release waste to the environment within safe parameters.

POME is generally treated using series of large shallow ponds (biological treatment system). The ponds cannot be very deep, so large reaction volumes are obtained by increasing the surface area. The restriction on depth is mainly because there is difficulty in oxygen penetration to the bottom of the pond if the pond is deep. Therefore, large land areas and long HRTs (approximately 40 days) are required for the series of aeration ponds to treat the POME effectively [21]. However, this technique is considered obsolete and as a result, POME is being treated ineffectively. The failure of the treatment system is caused by the sensitivity of the microorganisms present in the treatment ponds towards climate changes (related to temperature changes) and pH fluctuation [14]. The series of shallow ponds also occupies a large land area, releases obnoxious gases such as hydrogen sulphide and methane openly to the environment and has a long hydraulic retention time (HRT) [22]. By addressing the hazards posed by POME, various efforts have contributed towards the improvement of the POME treatment system.

The obsolete open pond method has been the stimulus either to explore new technologies or to modify the existing treatment

technology. Through intensive research, a number of innovative technologies to treat the POME have been found. Physical and chemical treatment systems such as membrane technology are among the new technologies used to treat the POME [23–25]. The results of these technologies are highly encouraging (COD removal of approximately 96.5%) [24]. However, the newly invented technologies remain at laboratory scale without seeing much progress to industrial scale because a high cost is required to operate a sophisticated treatment system compared to the current ponding system. Difficulty in upscaling the laboratory-scale technologies also contributes to the lack of new technologies applied at the industrial level. A profitable and feasible technology is desired to cause new treatment technologies to be implemented on an industrial scale. Most of the innovative technologies fail in terms of financial feasibility [26].

Failure of the innovative technologies to be upscaled has led to attention reverting to biological treatment method. However, new perspectives must be found to make the biological treatment method feasible in terms of treatment efficiency as well as financial sustainability. One of the attractive new approaches is to transform the POME treatment plant into an income-generating source rather than an investment depletion zone. Income could be generated through the trading of by-products from the wastewater treatment plant. Most of the by-products are largely underutilised and their economic potential is yet to be exploited. Nevertheless, the by-products could be generated only through a systematic treatment system. A systematic and efficient treatment system would ensure that the POME is treated efficiently and simultaneously generate income through the by-products. Return of investment (ROI) from the wastewater treatment plant is a win-win situation for the mill operation as well as the environment.

With these considerations, this review will focus on the by-products from POME that have been reported in previous work. First, the available POME treatment methods and their by-products will be discussed. The current utilisation and the potential uses of the by-products will be reviewed in this work. This review will also determine the appropriate treatment method to treat POME efficiently and to simultaneously produce by-products that have return of investment value. Profitable by-products from wastewater may attract a palm oil mill to invest in a wastewater treatment plant. Production of profitable by-products would ensure that wastewater treatment could achieve its goal, suppress the operating cost to a minimal level and concurrently protect environmental quality. Current constraints in producing by-products and future work are discussed in this review.

2. POME treatment methods

2.1. Methods to treat POME

To chart a new dimension for a POME treatment plant (or for any agricultural wastewater), the available and currently used treatment methods must be understood. By understanding the current treatment methods, a relevant solution could be found and implemented in the future. POME is generally treated in various ways at industries. These treatment methods have been in use for decades, and their efficiency is widely variable.

2.1.1. Biological treatment

In the current scenario, biological treatment is the commonly used method in industries due to its low cost, high organic loading capability, and simple and low energy demand [25]. Microorganisms present in the treatment system will degrade the biodegradable substances, which in turn reduces the COD of the wastewater. The anaerobic treatment is preferred at the beginning stage of the POME treatment process due to the ability of the anaerobic treatment to reduce the COD and the BOD rapidly in the absence of oxygen [27]. As aeration is not needed, an anaerobic treatment reduces the operating costs. During the anaerobic process, a sequence of reactions occurs, namely, hydrolysis, acidogenesis and methanogenesis [28]. For anaerobic treatment, series of open ponds have commonly been used to degrade the POME. The open ponds consist of a de-oiling tank, acidification ponds, anaerobic ponds and facultative or aerobic ponds [22]. The ponding system has been in practice to treat the POME since the mid-1980s [29]. At present, 85% of the POME treatment is based on the anaerobic and facultative ponding system [10]. However, the effluent from the pond system was not able to meet the DOE discharge standards even after 80 days of retention time. The COD and biochemical oxygen demand (BOD) for the final effluent of the aerobic pond were 1725 mg/L and 610 mg/L, respectively [29].

Drawbacks of the open pond system led to other biological technologies that were tested for POME treatment. Borja and Banks [30] have treated the POME with an upflow anaerobic sludge blanket reactor (UASB). The results from the treatment showed that up to 96% of the COD was removed. The UASB reactor also has a noteworthy advantage over the ponding system because the hydraulic retention is much shorter (1.5–3 days), and the area required for the UASB reactor is smaller. One of the salient features of the UASB is the formation of granular sludge [25]. However, the UASB could not retain an adequate amount of microorganisms for the high loading treatment. Consequently, the experiment was conducted in an upflow anaerobic filtration (UAF) for POME treatment [31]. The necessity of introducing the UAF is to retain the denser microorganisms in the reactor, which eventually allows efficient treatment of the higher loading of POME. In the UAF method, almost 90% of the substrate was oxidised, and the operation of the reactor was reported to show good stability under acidic and alkaline conditions [31]. Unlike the ponding system, the UASB or UAF can capture the methane in the reactor. Methane gas can be utilised as an energy source to power the palm oil processing mill, subsequently saving the operating cost of the mill. Methane is produced in the methanogenesis step of the anaerobic reaction. The organic compounds in POME will be converted into methane and carbon dioxide by methanogens. For every 1 g of COD removed from the POME, 0.69–0.79 dm^3 of methane gas is produced in the UAF [31]. Despite the production of methane, the common problem associated with the UAF is malfunctioning at a high organic loading rate due to the presence of suspended solids in the POME [25]. To overcome this shortcoming, the integration of the UASB with the Upflow Fixed Film (UFF) reactor was proposed and used successfully to treat the POME on a laboratory scale [25]. The integrated reactor is called the upflow anaerobic sludge fixed film (UASFF) reactor. The schematic diagram of the UASFF is shown in Fig. 4. Through the UASFF reactor, the retention of solids would be higher and improve the solid/liquid/gas separation in the reactor. The 97% COD removal (reactor was operated at 3 days HRT) was achieved by using this reactor [25]. However, this technology could not be implemented at the industrial level because of the failure of the upscaling process.

Apart from the anaerobic treatment techniques for POME discussed earlier, research has been performed on aerobic treatment as well. The major drive for aerobic treatment research is to reduce the hydraulic retention time of the anaerobic POME treatment. Vijayaraghavan et al. [32] have investigated the aerobic treatment of POME using an activated sludge reactor. They reported that the COD removal achieved for aerobically treated POME was 98% for a hydraulic retention time of 60 hours. Compared to the work done by Borja and Banks [30], almost 96% COD removal could be achieved with a shorter hydraulic retention

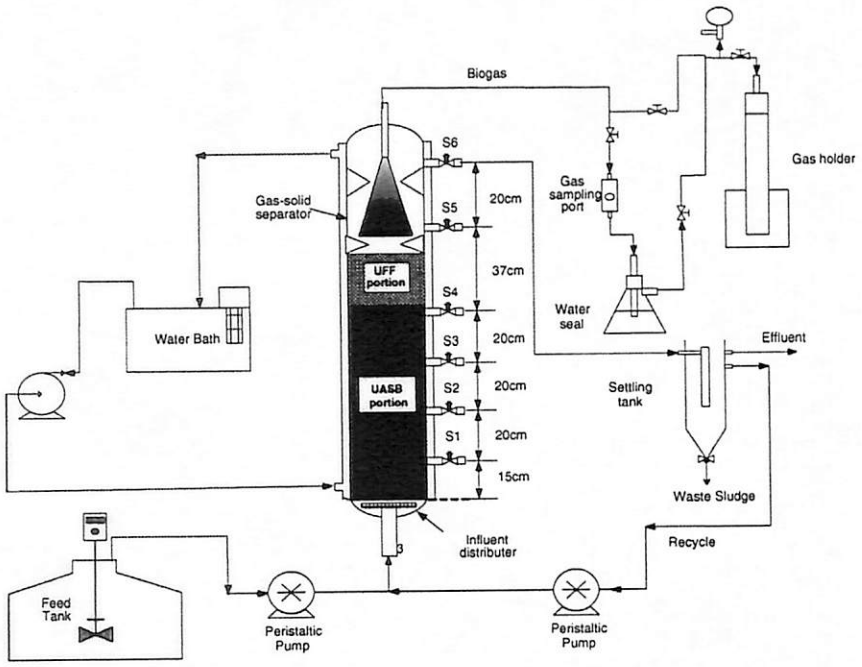

Fig. 4. Schematic diagram of UASFF [25].

time for POME treatment using an aerobic system. Meanwhile, the use of aerobic digestion of POME decreases the carbon content and inorganic nitrogen whilst changing the pH from acidic to alkaline [33]. Although aerobic treatment offers a method with a shorter retention time, the aerobic treatment also has drawbacks. In the method used by Vijayaraghavan et al. [32], the presence of biomass in the effluent poses problems for the receiving water body. These problems paved the way for the development of aerobic granules for treating POME. Gobi et al. [20] have successfully developed aerobic granules in POME and simultaneously used these aerobic granules to treat POME. Aerobic granules have good settling ability in addition to being robust in nature [34] and enable the effluent to be biomass-free. Work done by Gobi et al. [20] differs from the work of Vijayaraghavan et al. [32] in terms of the reactor setup as well as the biomass morphology. Emphasis was given to establishing a greater height over diameter (H/D) ratio in the reactor operated by Gobi et al. [20] to promote the formation of aerobic granules in the reactor. The sequencing batch reactor (SBR) system used by Gobi et al. [20] occupies a small footprint compared to the activated sludge reactor. The comparison in terms of the morphology of the biomass used is shown in Fig. 5. The conventional activated sludge (as a seed sludge for granular formation) used by Gobi et al. [20] is shown in Fig. 5(i). Meanwhile, the fully developed aerobic granules could be seen in Fig. 5(ii). Another salient feature of the SBR aerobic granule system is the hydraulic retention of POME. The SBR aerobic granule system requires only 24 h for the POME to be treated with 90% efficiency. Though the granulation could be promising, aeration could increase the overall operating cost.

2.1.2. Non-biological treatment

Apart from biological treatment methods, several alternative methods were found that could be used to treat the POME. Among these alternative methods are coagulation–flocculation, adsorption, membrane technology, and integrated technologies. In the coagulation–flocculation treatment method, several types of chemicals have been used to destabilise the colloids in the POME. Chemicals such as polyacrylamide derivatives, aluminium sulphate and polyaluminium chloride have been used as coagulants and flocculants for POME treatment [35]. In the coagulation–flocculation treatment system, only the suspended solids would be separated from the POME wastewater. The coagulation–flocculation treatment system does not reduce the COD value significantly. The adsorption technique is limited to removal of the residual oil in POME and used as a final polisher. The effectiveness of the adsorption technique in reducing the COD is evidently not reported [36,37]. Apart from low COD removal, both coagulation–flocculation and adsorption techniques could not be used comprehensively to treat the POME due to the maintenance and operating cost [17]

As far as membrane technology is concerned, the results obtained from the experimental work appear to be excellent. Ahmad et al. [14] have reported that 99% of the influent COD was removed from the raw POME. However, prior to the membrane treatment, POME must be pre-treated with several techniques. In the study done by Ahmad et al. [23], POME underwent three stages of pre-treatment. The function of these three stages was to partially reduce the turbidity, COD and BOD before the membrane treatment. In each stage of the membrane treatment, either chemical or external energy is needed to produce the final effluent, which directly raises the operating cost of a treatment plant. By acknowledging the shortcomings of a membrane treatment method for POME, a comparison of overall operating costs has been conducted with different setups of the membrane treatment system. The lowest cost of the three setups studied was RM 7.03/m^3 [38]. The membrane treatment system is still considered high end technology compared to the biological treatment method [39], but implementation of the membrane technology at the industrial level has been unsuccessful.

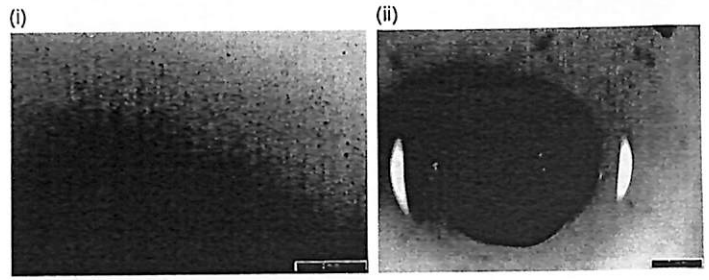

Fig. 5. Morphological comparisons between (i) activated sludge and (ii) aerobic granules for POME treatment [20].

2.1.3. Integration of biological and non-biological treatment

Zhang et al. [17] have integrated biological treatment with the membrane separation system. The first stage of the treatment consist of an expanded granular sludge bed (EGSB) and an aerobic reactor. The second stage of treatment consists of an ultrafiltration membrane and reverse osmosis membrane. This treatment system is capable of producing biogas energy as well. The effluent produced at the end of the treatment system was almost crystal clear and could be used as boiler feed water [17]. A similar concept of recycling the water has been investigated by others as well [14,23,40]. Chaiprapat and Laklam [41] have integrated an ozone pretreatment system with an anaerobic treatment system. The breakdown of recalcitrant components in POME by ozone would ease the subsequent anaerobic treatment system. The anaerobic sequencing batch reactor (ASBR) was reported to manage to treat the POME efficiently at high organic loadings (9.04 kg COD m^{-3} day^{-1}), shorter cycle times (12 h) and longer hydraulic retention times (10 days). In another work, Chan et al. [42] integrated anaerobic and aerobic technologies into a single bioreactor for the treatment of POME. This technique is indeed a fresh approach in terms of reducing the land area needed for the inefficient ponding system. Ideally, a single reactor could be used for the POME treatment because anaerobic and aerobic treatment could be carried out in the same reactor. The removal efficiency reached more than 99% in terms of BOD and TSS removal. In addition to the high removal efficiency, 0.42 L/g COD of methane gas is produced in this bioreactor. Though the long term implications and operation of this reactor are still being evaluated, the early results from this reactor look promising. However, the presence of biomass in the effluent was not addressed in their work. Choi et al. [43] used an anaerobic hybrid reactor (AHR), anaerobic baffled filter (ABF) reactor and an anaerobic downflow filter (ADF) reactor in a single system for the treatment of POME. The main purpose of this treatment method was to sustain a high ratio of effluent recycling as well as to reduce the retention time for the POME treatment. This technology has managed to remove 95.6% of influent COD at an OLR of 13 kg COD m^{-3} day^{-1}. The combination of the anaerobic reaction and the filtration process helps to maintain the effluent clear of biomass. Moreover, as a result of the anaerobic process, methane gas was produced at 0.171 and 0.269 L CH_4/g COD removed.

POME could be treated in various ways. The choice of treatment system depends on the capital cost as well as the concern of the operators about the environment. The summary of POME treatment methods and their respective COD removal is given in Table 2.

2.2. Summary of POME treatment methods

All of the methods reported exhibited various degrees of efficiency in terms of treating POME. Clear comparison in terms of advantages and disadvantages of the methods used to treat POME is given in Table 3. Nevertheless, the biological treatment is still preferred by the mill operators as it is cheap and requires less maintenance. However, considering the impact of the effluent on nature, many alternatives research efforts have been conducted, as discussed in the previous section.

Thus, to strike a fine balance between safeguarding the environment and keeping the operational cost of the POME treatment low, industrially feasible solutions must be decoded. The most obvious issues with the currently implemented biological treatment system (series of ponds) are the occupancy of large areas and the release of obnoxious gases to the environment. These issues could be resolved with either altogether new technology or improvements to the biological treatment system. However, investment is needed for the new technology or improvement to occur. Taking the necessary investment into consideration, return of investment from the new technologies or improved technologies would be an exciting prospect that can be explored. Return of investment from the treatment plant could be generated by marketing the by-products. By-products from treatment plant are an added boon for the investors in palm oil mills. Moreover, if the by-products can replace any non-sustainable raw materials for the production of end products, this type of replacement would contribute significantly towards sustainability. Production of marketable by-products could give a new dimension altogether for a treatment plant. More investment can be expected in the treatment methods as well as pledging the efficient treatment of wastewater. Ultimately, production of

Table 2
POME treatment methods and COD removal.

Treatment methods		Details	COD removal (%)	Reference
Biological treatment	• Anaerobic	• Pond	65	[20]
		• UASB	90	[30]
		• UASFF	97	[25]
	• Aerobic	• ASR	98	[32]
		• SBR	90	[20]
Physical treatment	• Membrane technology	–	99	[14]
	• Microbial fuel cell	–	96.5	[24]
	• Adsorption	• Rubber powder	NA	[36]
		• Chitosan	NA	[35]
		• Activated carbon	17.45	[37]
Chemical treatment	• Coagulation–flocculation	• Moringa oleifera seeds	52.2	[44]
Integration technology	• Biological-membrane	–	93	[17]
	• Ozone-anaerobic treatment	–	NA	[41]
	• Anaerobic–aerobic	–	99	[42]
	• Anaerobic-filtration	• AHR–ABF–ADF	96.5	[43]

NA – Not available.

Table 3
Comparison between various treatment methods of POME.

Methods	Advantages	Disadvantages	References
Membrane treatment system	• Excellent pollution removal efficiency	• Costly treatment system • Needs high maintenance • High pressure is required	[14]
Biological-membrane integrated system	• Excellent removal performance	• Costly treatment method	[17]
Microbial fuel cell	• Generation of electricity • High removal performance	• Difficult to scale up • Costly treatment method	[24]
Upflow anaerobic sludge fixed film (UASFF)	• Small footprint • Produce and capture methane gas • Able to retain biomass in the reactor for higher organic loading treatment	• Poor separation between treated effluent and biomass	[25]
Ponding system	• Low cost • Less/no maintenance needed	• Large land area required • Long HRT (> 50 days) • Direct release of methane and hydrogen sulphide to environment • Poor separation between treated effluent and biomass	[29]
Upflow anaerobic sludge blanket (UASB)	• Small footprint • Produce and capture methane gas	• Unable to retain biomass for higher organic loading treatment • Poor separation between treated effluent and biomass	[30]
Aerobic digestion	• No formation of pollution-causing gases	• High energy for aeration required to breakdown the organic content of POME • Poor separation between treated effluent and biomass	[32]
Ozone-anaerobic digestion integrated system	• Efficient in converting recalcitrant pollutants into biodegradable organics	• Costly treatment method	[42]
Aerobic-anaerobic integrated system	• Small footprint • Can treat high organic loading of POME	• Poor separation between the treated effluent and biomass	[42]
Anaerobic-filtration integrated system	• Capable of treating high organic loading of POME	• Requires frequent maintenance to sustain desirable product	[43]
Coagulation–flocculation	• Low cost	• Removes only suspended solids and residual oil • Release of residual elements from coagulant pollutes water	[45]
Sequencing batch reactor – aerobic granules	• No formation of pollution causing gases • Excellent separation between treated effluent and biomass • Small footprint • Able to be operated at higher loading rate	• High energy for aeration is required to breakdown the organic content of POME • No production of methane	[46]

marketable by-products could achieve zero discharge for the treatment plants and simultaneously contribute towards sustainability. Thus, screening of the possible by-products from the various treatment methods is a prerequisite.

3. By-products of POME treatment systems

Various types of by-products are generated while treating POME. Some of the by-products are extremely undervalued, and consequently, their economic potential remains untapped. Very little research has been devoted towards research into the further utilisation of these by-products. By-products produced to date from various forms of POME treatment methods and their potential to replace existing products are given in Table 4.

3.1. Gas by-products

From Table 4, methane gas is one of the common by-products produced from the treatment methods. Methane has been proven to be an energy source to power the mill [50]. The utilisation of methane gas to produce electricity will directly reduce the overall production cost of CPO. However, not all the palm oil mills have the facility to convert the methane energy source into electricity. As a result, the potential of the methane gas is not used at all. Apart from methane gas, hydrogen gas could also be produced from the anaerobic treatment plant. Biohydrogen is formed during the acidogenic fermentation of wastewater [46]. During the acidogenesis process, volatile fatty acids (VFAs) and biohydrogen gas are produced. However, these VFAs will not be converted into methane gas due to the inhibition of the methanogenesis process (a process to produce methane gas). Lower pH value (below 6) and higher temperature (above 60 °C) in the reactor inhibit the methanogenesis process.

3.2. Solid by-products

As depicted in Table 4, waste sludge is another by-product of the wastewater treatment plant. Research on waste sludge is quite sporadic, and the growing amount of sludge needs serious attention. Generally, waste activated sludge from various sources of wastewater has been used as adsorbent to remove textile dyes [51,52] or used as fertiliser [22]. The adsorption capacity of the waste-activated sludge is shown in Table 5. Tsai et al. [53] have reported that commercial activated carbon has an adsorption capacity of 373 mg/g for chloroform adsorption. Table 5 clearly shows that the adsorption capacity of waste-activated sludge is comparable to that of commercial activated carbon. Waste sludge is applicable as an adsorbent due to the naturally occurring functional groups on the surface [51]. Waste sludge aids the adsorption process via physical and chemical adsorption methods. Meanwhile, waste aerobic granules are produced from SBR. Excess aerobic granules in SBR are wasted to retain the balance of the food to microorganism ratio and to maintain solid retention times. The wasted aerobic granules are still active biomass that could be re-used for other purposes.

Based on the 2012 market price, PHA has the highest market value of all of the by-products (Table 4). The reason for the expense is the cost of the raw materials used to produce PHA. As a result, the applicability of PHA is limited, and the market is continually being flooded with cheaper petroleum-based plastics. The market price of PHA is approximately 20–80% higher compared to conventional petroleum-based plastics [57]. Production of PHA from waste streams is highly welcomed to reduce the market price and make the production of PHA competitive with the petroleum-based plastics. Mass production of PHA from waste streams would reduce the reliance on conventional plastics. From the perspective of an investor, PHA production from waste streams provides an additional income to the overall plant operation.

In 2012, the estimated release of POME was approximately 58 million tonnes (based on the CPO production). By envisaging the yield of PHA production to be 0.257 t PHA/t substrate (similar to Chakravarty et al. [58]), POME is capable of producing approximately 2.5 million tonnes of PHA annually (based on the average COD value of POME). Use of POME as a carbon substrate and high volume of PHA production would lower the price of PHA and eventually expand the use of PHA. The estimated PHA production and its income are summarised in Table 6. On average, the estimated annual income for each palm oil mill in Malaysia is projected to be around RM 10.75 million. This gross income looks very appealing. Apparently, the annual net revenue is expected to be around RM 4.3 million (40% of the total income) after considering the tax and operating cost. Hence, PHA production from POME can be considered as a profitable venture for the investors.

Table 5
Utilization of sludge as adsorbent.

Adsorbent	Adsorption capacity (mg/g)	Reference
Methylene Blue	66.23	[51]
Reactive Black 5	93.00	[54]
Methylene Blue	130.69	[52]
Chloroform	244.00	[53]
Toluene	350.00	[55]
Phenol	100.00	[56]
Rhodamine	33.33	[56]

Table 4
By-products of various POME treatment methods.

POME treatment method	Valuable by-product	Potential use(s) for	Market price of potential product (RM)	Reference
UASB	• Waste anaerobic granular sludge	• Activated carbon	1.68–6.01/kg[a]	[25]
	• Biohydrogen	• Power generation	0.32/kW h[b]	
	• Methane gas	• Power generation	0.32/kW h[b]	
	• Volatile fatty acid	• PHA feedstock	–	
Aerobic digestion	• Waste activated sludge	• Activated carbon	1.68–6.01/kg[a]	[32]
Sequencing Batch Reactor	• Waste aerobic granules	• Activated carbon	1.68–6.01/kg[a]	[20]
		• PHA synthesis	14.1–19.37/kg[c]	
	• Biohydrogen	• Power generation	0.32/kW h[b]	
Membrane Technology	• Water	• Boiler feed	–	[23]
	• Methane gas	• Power generation	0.32/kW h[b]	

1USD=RM 3.19 (as on 19th July 2013).
[a] Gongyi City Xianke Water Supply Material Co., Ltd. [47].
[b] Feed-in-Tariff (FIT) rates for biogas [48].
[c] Bioplastic development increases with new applications [49].

Moreover, this projected amount of PHA is estimated to reduce the dependence on petroleum-based plastics by at least 50%. However, this projected value is achievable only with proper regulation of operating parameters. As mentioned in Table 4, aerobic granules could be used to accumulate PHA inside its cells. Research attempting re-use of waste aerobic granules for PHA accumulation is discussed in the following section.

4. Waste aerobic granules

4.1. General structure of aerobic granules

Aerobic granules differ from waste activated sludge in terms of morphology, robustness and performance [34,61]. Fig. 5(i) and (ii) clearly exhibits the morphological difference between activated sludge and aerobic granules. The mechanism of formation of aerobic granules is shown in Fig. 6. Because the aerobic granules originated from activated sludge, the aerobic granules inherit the microbial behaviour of activated sludge. The aerobic granules formed are compact and denser. Hence, aerobic granules have better settling ability compared to conventional activated sludge [62]. The good settling ability of aerobic granules makes the effluent free of residual biomass. The use of clarifier could therefore be eliminated, making the treatment area smaller. This minimisation of the treatment area is one of the reasons for the application of granulation technology to increase at wastewater treatment plants in recent years.

Table 6
Estimated production of PHA and projected gross income.

Input	Parameter	Unit	Value
A	CPO production[a]	Tonne	18,785,030
B	POME production[b]	m^3	58,703,218.75
C	COD in POME[c]	kg/m^3	51
D	Estimated COD[d]	kg COD	2,544,784,533
E	PHA yield[e]	kg PHA/kg COD	0.257
F	PHA production[f]	kg PHA	654,009,624.9
G	Price of PHA[g]	RM/kg	7.00
H	Estimated gross PHA price[h]	RM	4,578,067,375.00
I	Number of palm oil mill in Malaysia		430
J	Estimated average annual income for each palm oil mill[i]	RM	10,646,668.31

[a] Based on statistics reported by Malaysian Palm Oil Board [59].
[b] Assume 3.125 m^3 of POME produced for every 1 t of CPO production.
[c] Average COD value of POME [60].
[d] Efficiency of COD conversion is assumed to be 85% (D=0.85*B*C).
[e] PHA yield is assumed to be similar to Chakravarty et al. [58].
[f] (F=D*E).
[g] Price of PHA is assumed to be half of the current price with the envisaged escalation in PHA production.
[h] (H=F*G).
[i] (I=H/I).

4.2. Mechanism of action of aerobic granules applied to the treatment of wastewater

Like conventional activated sludge, the aerobic granules will consume the biodegradable organic content of the wastewater for both growth and maintenance. In conventional activated sludge, after each cycle of substrate feeding, some portion of the consumed organic content will be stored as polyhydroxyalkanoate (PHA) inside the cells of microorganisms. PHA functions as the energy storage material which will be used during famine periods [64]. In aerobic granules, PHA accumulation has been reported previously [65,66]. However, a sole focus on extracting PHA from aerobic granules has thus far not been done.

4.3. Formation of aerobic granules in POME

Formation of aerobic granules in POME has been reported by Abdullah et al. [67] and Gobi et al. [20]. In both of the papers, the aerobic granules were reported to be formed inside the sequencing batch reactor with POME as the substrate. The maturation period of the aerobic granules as reported in the two research papers differs. A period of 120 days was required for Gobi et al. [20], while the maturation period was only approximately 60 days in the work done by Abdullah et al. [67]. The difference is presumably due to the different exchange ratios and reactor configurations. In both of the papers, the amount of COD removed was approximately 90%. These research efforts prove that aerobic granules could be developed in POME.

4.4. Generation of waste aerobic granules

The growth process of aerobic granules has led to the presence of excess aerobic granules in SBR. To maintain sludge retention time (SRT) inside the reactor, some of the excess aerobic granules must be wasted. Rather than just disposing of the waste aerobic granules, they could be re-used for some other purposes. The re-use of the excess aerobic granules is basically unexploited as only a handful of researchers have reported on the growth process of aerobic granules in the literature to date.

4.4.1. Application of waste aerobic granules

Gao et al. [68] have used inactive aerobic granules for the adsorption of Yellow 2G and Reactive Brilliant Red K-2G. The biosorption capacity of the aerobic granules has been reported as 58.50 and 66.18 mg g^{-1} for Yellow 2G and Reactive Brilliant Red K-2G, respectively. Gobi et al. [20] have used inactive aerobic granules to adsorb remaining COD and to reduce the turbidity of POME treated in SBR. The removal percentage of the remaining COD and turbidity is reported as 21% and 99%, respectively. The two papers indicate that aerobic granules can be useful as adsorbents.

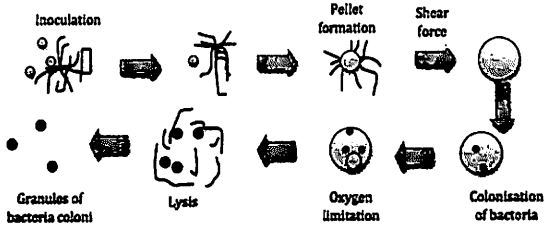

Fig. 6. Formation of aerobic granules from activated sludge [63].

Aerobic granules have also been used as seed sludge to develop aerobic granules in a new reactor. Pijuan et al. [69] have used crushed aerobic granules as seed sludge to reduce the startup time of the reactor. Only 18 days were required for the aerobic granules to develop [69]. The time required was far less compared to 120 days in the work of Gobi et al. [20] using activated sludge as the seed sludge. The time difference can probably be explained by the fact that some portion of the crushed aerobic granules remained intact. As a result, the crushed aerobic granules enhanced the attachment of the floccular sludge on the crushed aerobic granules. This phenomenon has resulted in faster development of aerobic granules, simultaneously reducing the startup time of the reactor. This novel method reduced the startup time of the reactor without altering the dimensions or changing the operating parameters of the reactor. In another research effort, Wang et al. [70] have used broken aerobic granular sludge to produce a nitrobenzene-degrading bacterium (*Klebsiella ornithinolytica NB1*). The biodegradation rate of nitrobenzene reached 9.29 mg L^{-1} h^{-1} with the use of that bacterium.

However, of all the re-utilisation schemes for aerobic granules, the most attractive use of aerobic granules is to produce PHA. The synthesis of PHA has been overlooked because PHA is an intermediate product. Thus, the challenge of the upcoming research would be finding a way to promote bulk production of PHA in aerobic granules (by using POME as the substrate) and subsequently using PHA as a substitute for the petroleum-based plastics. This substitution, if it could be accomplished, would significantly reduce the dependence on the non-sustainable petroleum-based biopolymer and simultaneously promote green technology.

5. Polyhydroxyalkanoate (PHA)

5.1. Production mechanism

Polyhydroxyalkanoate (PHA) is an energy storage molecule accumulated naturally inside microorganisms [71]. PHA is a common term that refers to the total accumulation of monomers such as hydroxybutrate (HB) and 3-hydroxyvalerate (3HB-3HV), 3-hydroxyvalerate (3HV), 3-hydroxy-2-methylvalerate (3H2MV) or 3-hydroxyhexanoate (3HHx) [72]. PHA can generally be classified according to the carbon chain length, either short chain length (SCL) or medium chain length (MCL). The general chemical structure of the PHA is shown in Fig. 7. PHA is a readily biodegradable and biocompatible polymer with properties almost similar to conventional plastics [73]. However, commercialisation of PHA is hindered by the high substrate cost as well as the non-sustainable nature of the feedstock [74]. Researchers found that waste streams rich in carbon sources could be used as a substrate to produce PHA [75]. Various types of wastes such as olive oil mill effluent, sugar molasses and food waste have been used as the substrate to produce PHA [76,77]. The PHA accumulation profile in a mixed culture is shown in Fig. 8. Fig. 8 shows that the accumulation of PHA occurs during the feast period with concurrent reduction of carbon content. A similar trend will be observed in any other mixed culture used to accumulate PHA.

In wastewater, PHA will be accumulated inside the microorganisms by taking up the available volatile fatty acid (VFA). Production of PHA has been reported to be higher in fermented substrates rather than an unfermented carbon source [77]. The fermented substrate possibly contains a higher proportion of VFA, which has a shorter chain length. The PHA accumulating microorganism can more easily take up the VFA rather than a complex substrate. Wastewater will generally undergo acidogenic fermentation to produce VFA. The VFA is then fed into a reactor that contains PHA-accumulating organisms. VFA will be transformed into the respective acyl-CoA and subsequently converted into PHA by the organisms present in the reactor [80]. The common equations for PHA production from various carbon sources are summarised in as follows [81]:

$$\Delta\text{Carbon substrate} + \Delta\text{Carbohydrate} = \Delta P(HB/HV) + \Delta\text{Active biomass} + \Delta\text{Soluble microbial products} + \Delta CO_2 \quad (1)$$

To accumulate PHA inside the microorganisms, operating conditions play a vital role. Aerobic dynamic feeding (ADF) is the conventional approach adopted by the researchers to accumulate PHA inside cells. In ADF, feast and famine phase will be established in the reactor. ADF suppresses the internal growth of the microorganism, thus forcing the microorganism to adapt itself to the limitation of nutrients arising from ADF. During the adaptation period, the substrate will be stored as PHA inside the cells of microorganism [82]. Gao et al. [83] recently illustrated the overall pathway of PHA production clearly, and this pathway is shown in Fig. 9. Although an overall idea of PHA accumulation inside the microorganisms were established, PHA accumulation inside the microorganisms is mainly applicable to pure culture methods only. With a comprehensive understanding of the accumulation in the pure culture method, approximately 86% CDW PHA was accumulated inside microorganisms [84]. However, similar amounts of PHA accumulation were not accomplished inside mixed culture microorganisms. As viewed from an economic perspective, a mixed culture method is more feasible to be applied at the industrial level rather than a pure culture method [79,85]. Current research therefore focuses on PHA production via mixed culture microorganisms [73,77].

5.2. Production of PHA via mixed culture

A mixed culture method of producing PHA offers a good solution to the high cost of a pure culture method [86]. However, the major problem of this mixed culture method is poor accumulation of PHA inside the microorganism [79]. Though myriads of microorganisms are present in the mixed culture, not all of the microorganisms can function to accumulate PHA inside their cells. The researchers therefore came up with a solution by enriching the PHA-accumulating microorganisms in the mixed culture system [76,86]. Just by altering the operating conditions of the reactor, microbial communities that are highly enriched in PHA-accumulating organisms could be cultured.

However, in a mixed culture method, the challenge would be ceasing the inhibition of the PHA accumulation process. Johnson et al. [87] recently studied the effect of ammonium on PHA accumulation in a mixed culture and found that only 69 wt% of PHA was accumulated under conditions of excess ammonium after 4.4 h.

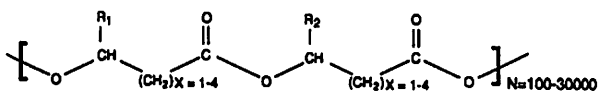

Fig. 7. General chemical structure of polyhydroxyalkanoates. R1 and R2 are alkyl groups (C1–C13) [78].

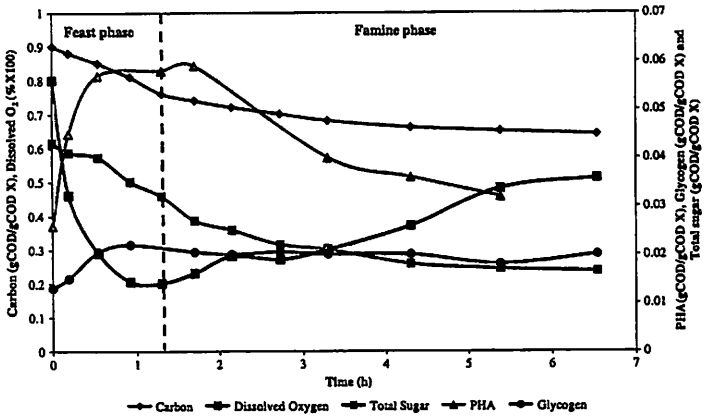

Fig. 8. PHA accumulation profile in a mixed culture [79].

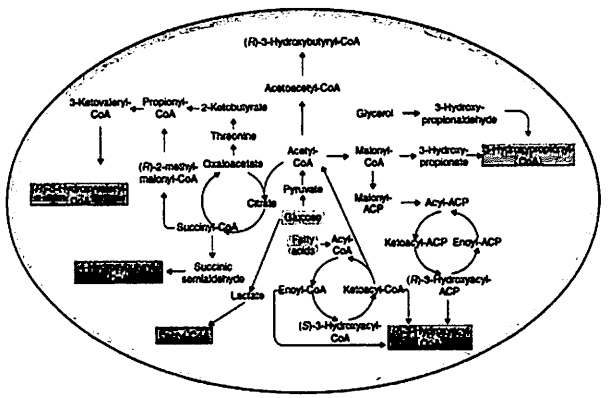

Fig. 9. Overall pathway of PHA production [83].

Serafim et al. [88] found that when 180 Cmmol/L acetate was fed in one single pulse, the acetate causes inhibition of the accumulation of PHA, and eventually only 67.5% of the cell dry weight of PHA could be accumulated. High level inhibition due to substrate is believed to result in production costs similar to the pure culture method, which nullifies the objective of using the mixed culture method. Moreover, inhibition will result in high production costs due to the recovery of small amounts of PHA accumulated inside the microorganisms. Inhibition factors ranging from operating conditions, excess nutrients, limitation of nutrients and thermodynamic effects have been studied for their effect on PHA accumulation [73,87,89,90]. These parameters were reported to inhibit the PHA accumulation to various degrees.

5.3. Recent trend of producing PHA via mixed culture

Development of a mixed culture method to produce PHA has paved the way for the utilisation of waste streams rich in organic content to be used as a carbon substrate. Wastewaters from sources such as sugar molasses [73], olive oil pomace [91], food waste [92], and POME [93] have been used successfully as carbon substrates for PHA production. One of the key issues that need to be addressed with the mixed culture method is the lower amount of PHA accumulation compared to the pure culture method [76]. To enhance the amount of PHA in the mixed culture, attention has been focused on the enrichment of the PHA-accumulating organism. As a fundamental step towards enrichment of the PHA-accumulating organism, a feast-famine period has been established. In the work done by Albuquerque et al. [94], a feast-famine ratio of 0.5 and 0.22 yields 0.18 and 0.59 Cmol PHA/Cmol VFA, respectively, clearly indicating that a lower feast-famine ratio would spur the PHA yield. This eco-biotechnology method was first reported by Johnson et al. [86] in their attempt to match the PHA yield of engineered bacteria. This breakthrough has been the stimulant to produce PHA in a continuous mode reactor. Chakravarty et al. [58] have reported the production of PHA in a continuous mode reactor, where the yield of PHA was between 0.213 and 0.257 g PHA/g acetate. Ideally, the methodology used in the continuous mode reactor is applicable to all other wastewaters as well. Enrichment of the PHA-accumulating organism and successful operation of the continuous mode reactor could be the way forward to commercialise the PHA production from wastewater in the future. The ways to apply this technology to POME must be explored. To be able to apply this technology to POME, the recent trend for PHA produced from POME has to be understood.

6. PHA accumulation using POME

6.1. Composition of POME

To date, several papers have reported the production of PHA from POME [84,93]. As Table 1 shows, POME is very rich in biodegradable organic content. Anaerobically digested POME will convert the biodegradable organic content into volatile fatty acids (VFAs). VFAs function as the carbon substrate for PHA production. Complete anaerobic digestion of POME will generally result in production of methane and CO_2 [27]. A complete anaerobic digestion consists of three theoretical stages. Hydrolysis, acidogenesis, and methanogenesis are the three stages involved in producing methane and CO_2 [27]. When the anaerobic process is stopped at the acidogenesis stage, the process will yield only VFA and biohydrogen. The common VFAs produced are acetic, propionic, butyric, and isobutyric acids [95,96]. VFA composition will eventually determine the type of PHA formation [97]. Theoretically, in POME, a wide range of PHAs such as 3-hydroxybutyrate, 3-hydroxyvalerate (3HV) and 3-hydroxy-2-methyl valerate (3H2MV) could be formed from the constituents of VFA.

6.2. PHA-accumulating microorganisms

PHA-accumulating microorganisms will take up the available VFA and store it inside the cells. These microorganisms possess the enzymes to turn the VFA into PHA by a specific pathway. Due to their high accumulating ability, the pure culture method has been the preferred method over mixed culture. PHA quantity is calculated in terms of PHA content (% of cell dry weight). In the work of Mumtaz et al. [84], *Comamonas sp. EB172* (microorganism species) has been used to produce the PHA. Cell dry weight with 90% PHA was reported in this work. *Comamonas sp. EB172* has the ability to accumulate the PHA with high purity when the operating parameters favour the accumulation of PHA. Meanwhile, in the work of Hassan et al. [98], the PHA has been produced using *Rhodobacter sphaeroides*, a photosynthetic bacterium which functions to produce the PHA from the VFAs. PHA is accumulated up to 30% of the dry cell weight of the bacteria. Meanwhile, Alias and Tan [99] were able to isolate two types of bacterium that could utilise palm olein for PHA synthesis. The FLP1 and FLP2 used have managed to accumulate PHA up to 18.6% of the cell dry weight [99]. The massive difference in PHA accumulation in the work done by Mumtaz et al. [84] and Alias and Tan [99] is the substrate used for PHA accumulation. Mumtaz et al. [84] used a higher percentage of VFA resulting from the anaerobic process. The higher percentage of organic acid enabled the *Comamonas sp. EB172* to wholly use the VFA available to convert the VFA into PHA. Apart from the pure culture method, recently Md. Din et al. [93] studied PHA production using the mixed culture method. A partially high yield (0.8 Cmol/Cmol acetate) has been reported for a mixed culture method. This development could be the 'game changer' for producing the PHA in POME commercially. However, this development is still in the beginning stages and requires more studies before commercialisation.

6.3. Quantity of PHA produced

Though numbers of research studies have been dedicated to PHA production using POME, the yield reported from these studies has yet to match other wastewaters [76]. Table 7 exhibits the microorganism used for PHA accumulation and its respective yield and PHA content. The low yield observed for pure culture method in Table 7 indicates that the available organic content was not channelled towards PHA production, possibly due to the complexity of the VFAs produced in POME or the limitation within the microorganisms used to accumulate PHA using POME. The limitation of the microorganisms could be overcome by selecting a superior microorganism with high selectively towards PHA accumulation. A higher percentage of cell dry weight would be economically feasible for an extraction process. Nevertheless, a significant surge in terms of PHA content has been found between the earlier work done by Hassan et al. [100] and the work recently performed by Mumtaz et al. [84].

7. Biohydrogen generation using POME

The literature reveals that biohydrogen has been generated successfully from the POME treatment process [101]. Biohydrogen has been generated from the POME using either pure culture strains or mixed culture bacteria. The maximum yield of biohydrogen in pure culture [101] and mixed culture [102] was 31.95 mL H_2/g COD and 4708 mL H_2/L-POME, respectively. Mixed culture appears to offer a better option for the production of biohydrogen than pure culture in POME. Previous research using POME for biohydrogen production and the results are shown in Table 8.

Biohydrogen is highly regarded as a potential fuel in the future, especially in a carbon-free energy system [103]. Attention has been given to the biohydrogen because biohydrogen can generate a higher energy yield compared to fossil fuels. Hence, the production of biohydrogen has been extensively researched from various perspectives in recent years [17,101]. These studies attempt to combat the high storage and production costs, which are the hindrance to the commercialisation of biohydrogen [104]. Biohydrogen could be generated via several pathways such as dark fermentation, photofermentation, a two-stage process (integration of dark- and photofermentation) and biocatalysed electrolysis [105]. However, dark fermentation is reported to be the most feasible pathway to produce biohydrogen because dark fermentation has a high rate of cell growth, requires no light energy, has no oxygen limitation problems and requires a lower capital cost [104]. During the dark fermentation process, the fermentation process has to be stopped at the acidogenesis process to promote the collection of biohydrogen. Failure to stop the methanogenesis step will result in further degradation of the dark fermentation product which also includes the biohydrogen gas generated in the acidogenesis stage. The production mechanism for biohydrogen is shown in the following equation:

$$C_6H_{12}O_6 + 2H_2O \rightleftharpoons 2CH_3COOH + 2CO_2 + 4H_2 \quad (2)$$

Temperature plays an important role in biohydrogen generation. Table 8 shows the microbial species specifically identified

Table 7
PHA production in POME.

Microorganism	Type of microorganism	PHA yield	PHA content (% cell dry weight)	Reference
Comamonas sp. EB172	Pure culture	0.31	85.8	[84]
Rhodobacter sphaeroides	Pure culture	0.5	67.0	[98]
Burkholderia cepacia	Pure culture	NA	57.4	[99]
Alcaligenes eutrophus	Pure culture	0.32	45.0	[100]
Heterotrophic aerobic bacterium and activated sludge	Mixed culture	0.80[a]	74.0	[93]

NA – not available.
[a] C-mol/C-mol acetic acid (HAc).

Table 8
Production of biohydrogen from POME.

Medium	H_2 production rate (mL H_2/L-POME)	Reference
Clostridium butyricum EB6	31.95	[101]
Anaerobic sludge	4708	[102]
Microflora	102.6	[106]
Thermophilic microflora	4.4[a]	[107]
Thermoanaerobacterium thermosaccharolyticum	4800	[108]
Rhodopseudomonas palustris PBUM001	1050	[109]
Thermoanaerobacterium	4200	[110]
Anaerobic mixed microflora	6700	[111]
Thermotolerant consortia	702.52	[112]
Mixed culture	144.00	[96]
Mixed culture	589.00	[113]
Mixed culture	2640[a]	[114]

[a] (mL H_2/L-POME. day).

with their temperature tag (thermophilic and mesophilic). Biohydrogen generation is highly favourable in the thermophilic region (approximately 60 °C). At this temperature, the activity of the methanogens will be inhibited, and this inhibition will promote the production of biohydrogen. In a nutshell, biohydrogen is a by-product that can be generated in large amounts by using POME. However, to date, the applications of biohydrogen are very limited. Biohydrogen generation can therefore be coupled with PHA production at the POME treatment plant for the benefit of the palm oil mill. Reliance on biohydrogen and PHA could contribute significantly towards green technology and sustainability. However, some constraints need to be addressed before implementation of these by-products could be commercialised.

8. Constraints in PHA and biohydrogen production

8.1. PHA from POME

The papers on PHA production from POME indicate some persistent problems that must be addressed before upscaling PHA production from POME to the industrial level. First and foremost, the pure culture method has been used to produce PHA inside the microorganisms. This methodology is very fragile and incurs a high cost for implementation at the industrial level [86]. Moreover, this method is very sensitive towards environmental changes as well as the operating parameters of a reactor. Though the pure culture method could give high yield in terms of PHA accumulation, the pure culture method is always susceptible to failure on a large scale. The mixed culture method is very much at the beginning stages for large scale implementation. Further studies are required for comprehensive understanding of PHA accumulation using a mixed culture. Most significantly, inhibition factors in the mixed culture must be studied extensively before overall conclusions can be drawn regarding commercialisation.

Proper modelling of the PHA production has yet to be done. Modelling of a process is essential in upscaling the process to the industrial level. Modelling of PHA production should include inhibitory effects of operating parameters on PHA production and the importance of the composition of VFAs. The interaction between these parameters would be useful in determining the yield of the PHA. Once all these factors are scrutinised, a comprehensive model might be developed for PHA production from POME.

Another obvious challenge is producing the PHA in a continuous mode rather than in batches. Currently, no attempts have been made to study the feasibility of the PHA production in a continuous mode using POME. Only a handful of papers have been produced using this technique for another source of wastewater [58]. The main problem is determining the retention time of the POME at each stage of the operation. Adequate time is needed for the organic acids to be synthesised at the acidogenesis process for PHA production in microorganisms. Perhaps, if adequate retention time could be found in the POME treatment plant for PHA production, the chances of this method to be implemented at industrial level would be enhanced.

PHA production also faces problem in terms of a non-sustainable extraction process. Thus far, a halogenated method has been in use for the extraction of PHA [115]. Hazardous chemicals such as chloroform and sodium hypochlorite, which are necessary for the halogenated method, are not feasible for commercial use. Moreover, a complex treatment system would be required to remove the hazardous solvents once PHA has been extracted. Recent work on an eco-friendly PHA recovery system was reported by Mohammadi et al. [115]. However, this method requires further study to ensure that a higher purity of PHA is recovered. Moreover, PHA accumulated inside aerobic granules might require a complex mechanism for the recovery process.

8.2. Biohydrogen from POME

The production of biohydrogen from POME is yet to be commercialised due to several factors. Most commonly, the biohydrogen-producing microorganism must be dominant in the mixed culture medium of the POME treatment plant to ensure the maximum production of hydrogen from the anaerobically digested POME. *Clostridium sp.* and *Enterobacter sp.* have been identified as hydrogen-producing bacteria [116]. However, the methods for enriching these species through manipulation of the operating parameters of the reactor have yet to be identified. Current methods of enriching these species of bacteria are costly and not feasible to be implemented at the industrial level.

Biohydrogen is a product of the acidogenesis process. In the acidogenesis process, the volatile fatty acids (VFAs) are produced at a higher proportion. These VFAs will cause the pH to decrease and consequently make the mixed liquor acidic. This phenomenon

is indeed an inhibitor for the production of biohydrogen [117]. Previous work has demonstrated that the optimum pH for biohydrogen production from wastewater is 5.5 [118]. To ensure optimum biohydrogen production, a balance between adequate VFA production and the pH of the mixed liquor must be found. VFA production could not be compromised merely for the sake of biohydrogen generation as VFA is important for the accumulation of PHA.

The generation of biohydrogen in a continuous mode is an another challenge that must be addressed. Ideally, continuous generation of biohydrogen is highly desired in a wastewater treatment plant to ensure the chain of energy supply for the treatment plant or trading purposes. However, few have reported on the continuous generation of biohydrogen in the wastewater treatment plant, let alone a POME treatment plant.

9. Future work

From this review, production of PHA in waste aerobic granules is evidently one method to transform a wastewater treatment plant into a revenue-generation platform. This transformation would ensure the release of cleaner wastewater and at the same time, the palm oil mill would be able to operate sustainably. As a first step, aerobic granules should be formed on a pilot plant scale SBR by using POME. Later, the wasted aerobic granules can be used as a medium to accumulate PHA inside the cells with fermented POME as the carbon source. PHA, POME and aerobic granules have previously been studied as separate entities, and promising results were obtained from those studies [20,93]. Later, a continuous mode reactor must be designed to ensure that PHA accumulation could occur at the same HRT of the wastewater. This exclusive design of the reactor setup requires extensive detailing before the process could be further upscaled. Comprehensive studies on the inhibition process, optimum conditions for PHA accumulation in waste aerobic granules and a sustainable PHA recovery process are critically needed to ensure the success of this treatment system. The inhibition may be caused by the presence of various non-accumulating microorganisms, the operating parameters of the reactor or the predators present in the POME. Therefore, the need to analyse the inhibitory mechanism and its effect on PHA accumulation is critically needed if this technology is to be transformed to the industrial scale. Efforts must be dedicated towards finding a higher yield of PHA production per unit of POME treated. These aforementioned steps are the fundamentals in producing the PHA on a large scale by using waste aerobic granules.

Biohydrogen production can be coupled with the PHA accumulation as the revenue-generating source of a POME treatment plant. Biohydrogen production lies in the pathway of PHA production, so the need for any additional processes for biohydrogen generation may be eliminated. It is therefore a win-win situation for the production of biohydrogen and PHA accumulation in bulk amounts. To make the palm oil mill sustainable, the opportunity of using the biohydrogen gas as a power source should not be ignored. The biohydrogen produced could power the palm oil mill, and at the same time, the waste of an energy source could be avoided. Moreover, using the biohydrogen gas as a power source will reduce the dependence on the national grid for electricity and if there is any surplus of electricity generated, surplus electricity could be sold to the national power grid or utility companies [119]. Another source of income could be tapped for maximum usage.

Modelling also plays an important role in the transformation of this technology from laboratory scale to pilot and industrial scale. Thus, the development of models for PHA production in an aerobic granular system is highly desired. Modelling could first be done for the pilot plant scale PHA production using waste aerobic granules. The main focus has to be given to the yield of the PHA accumulation inside the waste aerobic granules. Unforeseen circumstances such as presence of toxic material inside the wastewater should be considered during the modelling process. A complete model would aid the upscaling process significantly.

In depth cost analysis of the PHA and biohydrogen production using aerobic granules in SBR is critically needed. In depth cost analysis would provide an all-inclusive knowledge about the economic feasibility of PHA production using aerobic granules in an SBR treating POME. PHA produced in aerobic granules could be able to generate profit with the use of SBR technology. However, application of SBR technology to the POME treatment itself is very rare. The cost analysis for PHA production in an SBR treating POME on the industrial level is difficult (almost impossible). Thorough cost analysis using pilot plant scale SBR would help to estimate the overall operating cost and subsequently determine the return of investment. Envisaging the potential of the aerobic granules, the POME wastewater treatment plant could be a real 'gold mine' if this method could be implemented in the future.

10. Conclusion

By-products of the POME treatment plant, especially PHA and biohydrogen, have a major potential to be commercialised. Commercialisation of PHA and biohydrogen would offer a return of investment as well as zero discharge from the POME treatment plant. Methods to enhance the productivity must be developed and implemented. PHA production from a POME treatment plant could be given attention as PHA production from a POME treatment plant has high market value and is sustainable. This naturally forming PHA could be commercialised by proper mitigation of operating parameters and via comprehensive understanding of the entire accumulation mechanism. Biohydrogen production would also generate income for the treatment plant. In tandem with these findings, SBR technology using aerobic granules should be studied extensively because SBR technology using aerobic granules is able to produce both PHA and biohydrogen as the by-product while maintaining its primary role to treat POME efficiently. Adoption of this technology at the industrial level is expected to pique the interest of the mill operators to invest in new technologies for a wastewater treatment plant.

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References

[1] Rupani PF, Singh RP, Ibrahim MH, Esa N. Review of current palm oil mill effluent (POME) treatment methods: vermicomposting as a sustainable practice. World Applied Sciences Journal 2010;11:70–81.
[2] The Sustainable Scale Project; 2003. Available: ⟨http://www.sustainablescale.org/areasofconcern/population/populationandscale/quickfacts.aspx⟩ [accessed 20.03.2013].
[3] MPOC. Malaysian Palm Oil Industry; 2012. Available: ⟨http://www.mpoc.org.my/Malaysian_Palm_Oil_Industry.aspx⟩ [accessed 31.7.2012].
[4] Economic Contribution. Malaysian Palm Oil Council (MPOC); 2013. Available: ⟨http://theoilpalm.org/economic-contribution/⟩ [accessed 17.03.2013].
[5] MPOC. The Oil Palm Tree; 2009. Available: ⟨http://www.mpoc.org.my/The_Oil_Palm_Tree.aspx⟩ [accessed 15.5.2010].
[6] MPOC. Malaysia Palm Oil Industry, 2011. Available: ⟨http://www.mpoc.org.my/Malaysian_Palm_Oil_Industry.aspx⟩ [accessed 31.7.2012].
[7] Economic Planning Unit. Rancangan Malaysia ke 10. Prime Minister's Office, 2010. Available: ⟨http://www.pmo.gov.my/dokumenattached/RMK/RMK10_Mds.pdf⟩ [accessed 30.12.2012].

[8] Yeo A. Palm oil: environmental curse or a blessing?; 2010.
[9] Foo KY, Hameed BH. Insight into the applications of palm oil mill effluent: a renewable utilization of the industrial agricultural waste. Renewable & Sustainable Energy Reviews 2010;14:1445–52.
[10] Ma AN, Singh G, Lim KH, Leng T, David LK. Oil palm and the environment: a Malaysian perspective. Kuala Lumpur: Malaysia Oil Palm Growers' Council; 1999. p. 113–26.
[11] Report TH. Palm Oil Production Comparison, 2013. Available: ‹http://www.cmegroup.com/trading/agricultural/files/hz_charts/bo_w_mpalmprod.gif› [accessed 15.07.2013].
[12] Borja R, Banks CJ, Sanchez E. Anaerobic treatment of palm oil mill effluent in a two-stage up-flow anaerobic sludge blanket (UASB) system. Journal of Biotechnology 1996;45:125–35.
[13] Oswal N, Sarma PM, Zinjarde SS, Pant A. Palm oil mill effluent treatment by a tropical marine yeast. Bioresource Technology 2002;85:35–7.
[14] Ahmad AL, Ismail S, Bhatia S. Water recycling from palm oil mill effluent (POME) using membrane technology. Desalination 2003;157:87–95.
[15] Wu TY, Mohammad AW, Jahim JM, Anuar N. Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. Biochemical Engineering Journal 2007;35:309–17.
[16] Bhatia S, Othman Z, Ahmad AL. Pretreatment of palm oil mill effluent (POME) using Moringa oleifera seeds as natural coagulant. Journal of Hazardous Materials 2007;145:120–6.
[17] Zhang YJ, Yan L, Qiao XL, Chi L, Niu XJ, Mei ZJ, et al. Integration of biological method and membrane technology in treating palm oil mill effluent. Journal of Environmental Sciences 2008;20:558–64.
[18] Wong YS, Kadir M, Teng TT. Biological kinetics evaluation of anaerobic stabilization pond treatment of palm oil mill effluent. Bioresource Technology 2009;100:4969–75.
[19] Damayanti A, Ujang Z, Salim MR, Olsson G, Sulaiman AZ. Respirometric analysis of activated sludge models from palm oil mill effluent. Bioresource Technology 2010;101:144–9.
[20] Gobi K, Mashitah MD, Vadivelu VM. Development and utilization of aerobic granules for the palm oil mill (POM) wastewater treatment. Chemical Engineering Journal 2011;174:213–20.
[21] Chan YJ, Chong MF, Law CL. Biological treatment of anaerobically digested palm oil mill effluent (POME) using a Lab-Scale Sequencing Batch Reactor (SBR). Journal of Environmental Management 2010;91:1738–46.
[22] Chan KS, Choo CF. Ponding system for palm oil mill effluent treatment. In: Proceedings of the regional workshop on palm oil mill technology & effluent treatment; 1984. p. 185–92.
[23] Ahmad AL, Chong MF, Bhatia S, Ismail S. Drinking water reclamation from palm oil mill effluent (POME) using membrane technology. Desalination 2006;191:35–44.
[24] Cheng J, Zhu X, Ni J, Borthwick A. Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Bioresource Technology 2010;101:2729–34.
[25] Najafpour GD, Zinatizadeh AAL, Mohamed AR, Hasnain Isa M, Nasrollahzadeh H. High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochemistry 2006;41:370–9.
[26] Yacob S, Ali Hassan M, Shirai Y, Wakisaka M, Subash S. Baseline study of methane emission from anaerobic ponds of palm oil mill effluent treatment. Science of the Total Environment 2006;366:187–96.
[27] Metcalf Eddy I. Wastewater engineering treatment and reuse. 4th edMcGraw Hill; 2003.
[28] Gerardi MH. The microbiology of anaerobic digesters. New Jersey: Wiley-Interscience; 2003.
[29] Chin KK, Lee SW, Mohammad HH. A study of palm oil mill effluent treatment using a pond system. Water Science and Technology 1996;34:119–23.
[30] Borja R, Banks CJ. Anaerobic digestion of palm oil mill effluent using an up-flow anaerobic sludge blanket reactor. Biomass & Bioenergy 1994;6:381–9.
[31] Borja R, Banks CJ. Treatment of palm oil mill effluent by upflow anaerobic filtration. Journal of Chemical Technology & Biotechnology 1994;61:103–9.
[32] Vijayaraghavan K, Ahmad D, Aziz M. Aerobic treatment of palm oil mill effluent. Journal of Environmental Management 2007;82:24–31.
[33] Agamuthu P, Tan EL, Shaiful AA. Effect of aeration and soil inoculum on the composition of palm oil effluent (POME). Agricultural Wastes 1986;15:121–32.
[34] Pujan M, Werner U, Yuan Z. Effect of long term anaerobic and intermittent anaerobic/aerobic starvation on aerobic granules. Water Research 2009;43:3622–32.
[35] Ahmad AL, Sumathu S, Hameed BH. Coagulation of residue oil and suspended solid in palm oil mill effluent by chitosan, alum and PAC. Chemical Engineering Journal 2006;118:99–105.
[36] Ahmad AL, Bhatia S, Ibrahim N, Sumathi S. Adsorption of residual oil from palm oil mill effluent using rubber powder. Brazilian Journal of Chemical Engineering 2005;22:371–9.
[37] Ahmad AL, Ismail S, Ibrahim N, Bhatia S. Removal of suspended solids and residual oil from palm oil mill effluent. Journal of Chemical Technology & Biotechnology 2003;78:971–8.
[38] Ahmad AL, Chong MF, Bhatia S. A comparative study on the membrane based palm oil mill effluent (POME) treatment plant. Journal of Hazardous Materials 2009;171:166–74.
[39] Lam MK, Lee KT. Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win-win strategies toward better environmental protection. Biotechnology Advances 2011;29:124–41.
[40] Afonso MD, Yañez RB. Nanofiltration of wastewater from the fishmeal industry. Desalination 2001;139:429.
[41] Chuprapat S, Laklam T. Enhancing digestion efficiency of POME in anaerobic sequencing batch reactor with ozonation pretreatment and cycle time reduction. Bioresource Technology 2011;102:4061–8.
[42] Chan YJ, Chong MF, Law CL. An integrated anaerobic–aerobic bioreactor (IAAB) for the treatment of palm oil mill effluent (POME): start-up and steady state performance. Process Biochemistry 2012;47:485–95.
[43] Choi W-H, Shin C-H, Son S-M, Ghorpade PA, Kim J-J, Park J-Y. Anaerobic treatment of palm oil mill effluent using combined high-rate anaerobic reactors. Bioresource Technology 2013;141:138–44.
[44] Bhatia S, Othman Z, Ahmad AL. Palm oil mill effluent pretreatment using Moringa oleifera seeds as an environmentally friendly coagulant: laboratory and pilot plant studies. Journal of Chemical Technology & Biotechnology 2006;81:1852–8.
[45] Ahmad AL, Ismail S, Bhatia S. Optimization of coagulation–flocculation process for palm oil mill effluent using response surface methodology. Environmental Science and Technology 2005;39:2828–34.
[46] Argenent LT, Karim K, Al-Dahhan MH, Wrenn BA, Domiguez-Espinosa R. Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends in Biotechnology 2004;22:477–85.
[47] Gongyn City Xianke Water Supply Material Co., Ltd.;Ltd. 2009.Gongyn City Xianke Water Supply Material Co., Ltd.; 2009. Available ‹http://www.activatedcarbonuk.com/Product/Activated%20Carbon/Wood_Based_Powdered_Activated_Carbon_.html› [accessed 18.07.2013].
[48] .SEDA. Feed-in Tariff (FiT) rates for biogas; 2012. Available: ‹www.seda.gov.my› [accessed 18.07.2013].
[49] Guzman Dd. Bioplastic development increases with new applications; 2010. Available: ‹http://www.acs.com/Articles/2010/10/25/3402443/bioplastic+development+increases+with+new+applicanons.html› [accessed 18.07.2013].
[50] Arthur R, Glover K. Biomethane potennial of the POME generated in the palm oil industry in Ghana from 2002 to 2009. Bioresource Technology 2012;111:155–60.
[51] Gobi K, Mashitah MD, Vadivelu VM. Adsorptive removal of Methylene Blue using novel adsorbent from palm oil mill effluent waste activated sludge: equilibrium, thermodynamics and kinetic studies. Chemical Engineering Journal 2011;171:1246–52.
[52] Li W-H, Yue Q-Y, Gao B-Y, Wang X-J, Qi Y-F, Zhao Y-Q, et al. Preparation of sludge-based activated carbon made from paper mill sewage sludge by steam activation for dye wastewater treatment. Desalination 2011;278:179–85.
[53] Tsai J-H, Chiang H-M, Huang G-Y, Chiang H-L. Adsorption characteristics of acetone, chloroform and acetonitrile on sludge-derived adsorbent, commercial granular activated carbon and activated carbon fibers. Journal of Hazardous Materials 2008;154:1183–91.
[54] Gulnaz O, Kaya A, Dincer S. The reuse of dried activated sludge for adsorption of reactive dye. Journal of Hazardous Materials 2006;134:190–6.
[55] Anfruns A, Martin MJ, Montes-Morán MA. Removal of odourous VOCs using sludge-based adsorbents. Chemical Engineering Journal 2011;166:1022–31.
[56] Zou J, Dai Y, Wang X, Ren Z, Tian C, Pan K, et al. Structure and adsorption properties of sewage sludge-derived carbon with removal of inorganic impurities and high porosity. Bioresource Technology 2013;142:209–17.
[57] Polyhydroxyalkanoate market - PHA packaging, bio-medical, food services, agriculture application analysis & forecast 2018 in New Research Report at ReportsnReports.com; 2013. PRWEB. Available: ‹http://www.prweb.com/releases/polyhydroxyalkanoate-pha/market-forecast-2018/prweb10685585.htm› [accessed 18.07.2013].
[58] Chakravarty P, Mhaisalkar V, Chakrabarti T. Study on poly-hydroxyalkanoate (PHA) production in pilot scale continuous mode wastewater treatment system. Bioresource Technology 2010;101:2896–9.
[59] MPOB. Annual and forecast of crude palm oil production (tonnes) 2011 & 2012; 2013. Available: ‹http://bepi.mpob.gov.my/index.php/statistics/production/71-production-2012/296-annual-forecast-production-of-crude-palm-oil-2011-2012.html› [accessed 30.07.2013].
[60] MPOB. Downstream – mill effluent; 2012. Available: ‹http://www.mpob.gov.my/en/palm-info/environment/520-achievements› [accessed 31.07.2012].
[61] Liu Y, Tay JH. State of the art of biogranulation technology for wastewater treatment. Biotechnology Advances 2004;22:533–63.
[62] Song ZW, Ren NQ, Zhang K, Tong LY. Influence of temperature on the characteristics of aerobic granulation in sequencing batch airlift reactors. Journal of Environmental Sciences 2009;21:273–8.
[63] Muda K, Aris A, Salim MR, Ibrahim Z. Sequential anaerobic–aerobic phase strategy using microbial granular sludge for textile wastewater treatment. Biomass now – sustainable growth and use; 2013.
[64] Chakraborty P, Gibbons W, Muthukumarappan K. Conversion of volatile fatty acids into polyhydroxyalkanoate by Ralstonia eutropha. Journal of Applied Microbiology 2009;106:1996–2005.
[65] Wu C-Y, Peng Y-Z, Wang K-D, Zhou Y-X. Understanding the granulation process of activated sludge in a biological phosphorus removal sequencing batch reactor. Chemosphere 2012;86:767–73.
[66] Wu C-Y, Peng Y-Z, Wang S-Y, Ma Y. Enhanced biological phosphorus removal by granular sludge: from macro- to micro-scale. Water Research 2010;44:807–14.
[67] Abdullah N, Ujang Z, Yahya A. Aerobic granular sludge formation for high strength agro-based wastewater treatment. Bioresource Technology 2011;102:6778–81.
[68] Gao J-F, Zhang Q, Su K, Wang J-H. Competitive biosorption of Yellow 2G and Reactive Brilliant Red K-2G onto inactive aerobic granules: simultaneous

determination of two dyes by first-order derivative spectrophotometry and isotherm studies. Bioresource Technology 2010;101:5793–801.
[69] Pijuan M, Werner U, Yuan Z. Reducing the startup time of aerobic granular sludge reactors through seeding floccular sludge with crushed aerobic granules. Water Research 2011;45:5075–83.
[70] Wang D, Zheng G, Zhou L. Isolation and characterization of a nitrobenzene-degrading bacterium Klebsiella ornithinolytica NB1 from aerobic granular sludge. Bioresource Technology 2012;110:91–6.
[71] Hassan MA, Yee L-N, Yee PL, Ariffin H, Raha AR, Shirai Y, et al. Sustainable production of polyhydroxyalkanoates from renewable oil-palm biomass Biomass & Bioenergy 2013;50:1–9.
[72] Sudesh K, Abe H, Doi Y. Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Progress in Polymer Science 2000;25:1503–55.
[73] Albuquerque MGE, Torres CAV, Reis MAM. Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection. Water Research 2010;44:3419–33.
[74] Salehizadeh H, Van Loosdrecht MCM. Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance. Biotechnology Advances 2004;22:261–79.
[75] Castilho LR, Mitchell DA, Freire DMG. Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. Bioresource Technology 2009;100:5996–6009.
[76] Albuquerque MGE, Martino V, Pollet E, Avérous L, Reis MAM. Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: effect of substrate composition and feeding regime on PHA productivity, composition and properties. Journal of Biotechnology 2011;151:66–76.
[77] Venkateswar Reddy M, Venkata Mohan S. Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. Bioresource Technology 2012;103:313–21.
[78] Keshavarz T, Roy I. Polyhydroxyalkanoates: bioplastics with a green agenda. Current Opinion in Microbiology 2010;13:321–6.
[79] Mota R, Lemos PC. Biopolymers production from mixed cultures and pyrolysis by-products. Journal of Biotechnology 2012;157:578–83.
[80] Morgan-Sagastume F, Karlsson A, Johansson P, Pratt S, Boon N, Lant P, et al. Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus Water Research 2010,44:5196–211.
[81] Chang H-F, Chang W-C, Chuang S-H, Fang Y-L. Comparison of polyhydroxyalkanoates production by activated sludges from anaerobic and oxic zones of an enhanced biological phosphorus removal system: effect of sludge retention time. Bioresource Technology 2011;102:5473–8.
[82] Beccari M, Majone M, Massanisso P, Ramadori R. A bulking sludge with high storage response selected under intermittent feeding. Water Research 1998;32:3403–13.
[83] Gao X, Chen J-C, Wu Q, Chen G-Q. Polyhydroxyalkanoates as a source of chemicals, polymers, and biofuels. Current Opinion in Biotechnology 2011;22:768–74.
[84] Mumtaz T, Yahaya NA, Abd-Aziz S, Rahman NAA, Yee PL, Shirai Y, et al. Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent – a Malaysian perspective. Journal of Cleaner Production 2010;18:1393–402.
[85] Wang B, Sharma-Shivappa RR, Olson JW, Khan SA. Production of polyhydroxybutyrate (PHB) by Alcaligenes latus using sugarbeet juice. Industrial Crops and Products 2013;43:802–11.
[86] Johnson K, Jiang Y, Kleerebezem R, Muyzer G, van Loosdrecht MCM. Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. Biomacromolecules 2009;10:670–6.
[87] Johnson K, Kleerebezem R, van Loosdrecht MCM. Influence of ammonium on the accumulation of polyhydroxybutyrate (PHB) in aerobic open mixed cultures. Journal of Biotechnology 2010;147:73–9.
[88] Serafim LS, Lemos PC, Oliveira R, Reis MAM. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. Biotechnology and Bioengineering 2004;87:145–60.
[89] Liu Z, Wang Y, He N, Huang J, Zhu K, Shao W, et al. Optimization of polyhydroxybutyrate (PHB) production by excess activated sludge and microbial community analysis. Journal of Hazardous Materials 2011;185:8–16.
[90] Wen Q, Chen Z, Tian T, Chen W. Effects of phosphorus and nitrogen limitation on PHA production in activated sludge. Journal of Environmental Sciences 2010;22:1602–7.
[91] Waller JL, Green PG, Loge FJ. Mixed-culture polyhydroxyalkanoate production from olive oil mill pomace. Bioresource Technology 2012;120:285–9.
[92] Chen H, Meng H, Nie Z, Zhang M. Polyhydroxyalkanoate production from fermented volatile fatty acids: effect of pH and feeding regimes. Bioresource Technology 2013;128:533–8.
[93] Md. Din MF, Mohanadoss P, Ujang Z, van Loosdrecht M, Yunus SM, Chelliapan S, et al. Development of Bio-PORec n system for polyhydroxyalkanoates (PHA) production and its storage in mixed cultures of palm oil mill effluent (POME). Bioresource Technology 2012;124:208–16.
[94] Albuquerque MGE, Concas S, Bengtsson S, Reis MAM. Mixed culture polyhydroxyalkanoates production from sugar molasses: the use of a 2-stage CSTR system for culture selection. Bioresource Technology 2010;101:7112–22.
[95] Fang C, O-Thong S, Boe K, Angelidaki I. Comparison of UASB and EGSB reactors performance, for treatment of raw and desisled palm oil mill effluent (POME). Journal of Hazardous Materials 2011;189:229–34.
[96] Rasdi, Z. Mumtaz, T. Abdul N.A. Rahman Hassan, M.A. Kinetic analysis of biohydrogen production from anaerobically treated POME in bioreactor under optimized condition. International Journal of Hydrogen Energy 2012. 37, 17724–30.
[97] Bengtsson S, Pisco AR, Johansson P, Lemos PC, Reis MAM. Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures. Journal of Biotechnology 2010;147:172–9.
[98] Hassan MA, Shirai Y, Kusubayashi N, Karim MIA, Nakanishi K, Hashimoto K. The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by Rhodobacter sphaeroides. Journal of Fermentation and Bioengineering 1997;83:485–8.
[99] Alias Z, Tan IKP. Isolation of palm oil-utilising, polyhydroxyalkanoate (PHA)-producing bacteria by an enrichment technique. Bioresource Technology 2005;96:1229–34.
[100] Hassan MA, Shirai Y, Umeki H, Karim MIA, Nakanishi K, Hashimoto K. Acetic acid separation from anaerobically treated palm oil mill effluent for the production of polyhydroxyalkanoate by Alcaligenes eutrophus Bioscience, Biotechnology, and Biochemistry 1997;61:1465–8.
[101] Chong M-L, Rahim RA, Shirai Y, Hassan MA. Biohydrogen production by Clostridium butyricum EB6 from palm oil mill effluent. International Journal of Hydrogen Energy 2009;34:764–71.
[102] Atif AAY, Fakhru'l-Razi A, Ngan MA, Morimoto M, Iyuke SE, Veziroglu NT. Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. International Journal of Hydrogen Energy 2005,30.1393–7.
[103] Leaño EP, Babel S. The influence of enzyme and surfactant on biohydrogen production and electricity generation using palm oil mill effluent. Journal of Cleaner Production 2012,31:91–9.
[104] Chong M-L, Sabaratnam V, Shirai Y, Hassan MA. Biohydrogen production from biomass and industrial wastes by dark fermentation. International Journal of Hydrogen Energy 2009;34:3277–87.
[105] Manish S, Banerjee R. Comparison of biohydrogen production processes International Journal of Hydrogen Energy 2008;33:279–86.
[106] Vijayaraghavan K, Ahmad D. Biohydrogen generation from palm oil mill effluent using anaerobic contact filter International Journal of Hydrogen Energy 2006;31:1284–91.
[107] O-Thong S, Prasertsan P, Intrasungkha N, Dhamwichukorn S, Birkeland N-K. Improvement of biohydrogen production and treatment efficiency on palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. Enzyme and Microbial Technology 2007;41:583–90.
[108] Prasertsan P, O-Thong S, Birkeland N-K. Thermophilic biohydrogen production from palm oil mill effluent under batch and fed-batch cultivation with microbial community analysis. Journal of Biotechnology 2008;Supplement: SG2):136.
[109] Jamil Z, Mohamad Annuar MS, Ibrahim S, Vikineswary S. Optimization of phototrophic hydrogen production by Rhodopseudomonas palustris PBUM001 via statistical experimental design. International Journal of Hydrogen Energy 2009;34:7502–12.
[110] Mamimin C, Thongdumyu P, Human A, Prasertsan P, Imai T, O-Thong S. Simultaneous thermophilic hydrogen production and phenol removal from palm oil mill effluent by *Thermoanaerobacterium*-rich sludge. International Journal of Hydrogen Energy 2012.
[111] Badiei M, Jahim JM, Anuar N, Sheikh Abdullah SR. Effect of hydraulic retention time on biohydrogen production from palm oil mill effluent in anaerobic sequencing batch reactor. International Journal of Hydrogen Energy 2011;36:5912–9.
[112] Yossan S, O-Thong S, Prasertsan P. Effect of initial pH, nutrients and temperature on hydrogen production from palm oil mill effluent using thermotolerant consortia and corresponding microbial communities. International Journal of Hydrogen Energy 2012,
[113] Singh L, Siddiqui MF, Ahmad A, Rahim MHA, Sakinah M, Wahid ZA. Biohydrogen production from palm oil mill effluent using immobilized mixed culture. Journal of Industrial and Engineering Chemistry 2013;19:659–64,
[114] Ismail I, Hassan MA, Abdul Rahman NA, Soon CS. Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture. Biomass & Bioenergy 2010;34:42–7,
[115] Mohammadi M, Hassan MA, Phang L-Y, Shirai Y, Man HC, Ariffin H. Intracellular polyhydroxyalkanoates recovery by cleaner halogen-free methods towards zero emission in the palm oil mill. Journal of Cleaner Production 2012;37:353–60.
[116] Yokoi H, Tokushige T, Hirose J, Hayashi S, Takasaki Y. H2 production from starch by a mixed culture of Clostridium butyricum and Enterobacter aerogenes. Biotechnology Letters 1998;20:143–7,
[117] Chow MC, Ho CC. Surface active properties of palm oil with respect to the processing of palm oil. Journal of Oil Palm Research 2000;12 107–16.
[118] O-Thong S, Mamimin C, Prasertsan P. Effect of temperature and initial pH on biohydrogen production from palm oil mill effluent. long-term evaluation and microbial community analysis. Electronic Journal of Biotechnology 2011:14,
[119] PNMB. Law of Malaysia Act 725 – Renewable Energy Act 2011. Kuala Lumpur: Percetakan National Malaysia Berhad, 2011.