Chemical Engineering Journal 269 (2015) 288–297

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Strategy for the biotransformation of fermented palm oil mill effluent into biodegradable polyhydroxyalkanoates by activated sludge

Wee Shen Lee [a], Adeline Seak May Chua [a,*], Hak Koon Yeoh [a], Tadashi Nittami [b], Gek Cheng Ngoh [a]

[a] Department of Chemical Engineering, Faculty of Engineering, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia
[b] Division of Materials Science and Chemical Engineering, Faculty of Engineering, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

HIGHLIGHTS

- Effective production of valuable PHA from fermented POME by activated sludge.
- Successful enrichment of PHA producers via aerobic dynamic feeding process.
- PHA production is best conducted at pH 7.
- Highest PHA content achieved was 64 wt% PHA per sludge dry weight.

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 12 January 2015
Accepted 28 January 2015
Available online 4 February 2015

Keywords:
Aerobic dynamic feeding
Feast and famine
Palm oil mill effluent
Polyhydroxyalkanoates
Resource recovery
Volatile fatty acids

ABSTRACT

Management of wastewater by resource recovery approach allows the transformation of wastewater into valuable resources. This work examines the biotransformation of fermented palm oil mill effluent (POME) into biodegradable plastics – polyhydroxyalkanoates (PHA) – through cultivation and enrichment of PHA-accumulating organisms in activated sludge and the subsequent production of PHA by the cultivated sludge. Enrichment of PHA-accumulating organisms via aerobic dynamic feeding process was effective and had significantly enhanced the PHA storage capacity of the sludge (wt% PHA per sludge dry weight) from 4 wt% (seed sludge) to 40–64 wt% (sludge cultivated for 50 days and more). The cultivated sludge comprised of 42 ± 12% *Betaproteobacteria*, 35 ± 7% *Alphaproteobacteria*, and 13 ± 4% *Gammaproteobacteria*, as estimated by fluorescent *in situ* hybridization. The influence of pH (4.5 in the absence of pH control; and pH 7, 8 and 9) on the production of PHA by the cultivated sludge was subsequently investigated. Neutral pH was the most favorable for PHA production, resulting in a PHA content of 64 wt% in 8 h. The PHA produced was made up of 77 mol% 3-hydroxybutyrate and 23 mol% 3-hydroxyvalerate. These findings signify that the combination of fermented POME and activated sludge offers an alternative to the palm oil and the plastics industries for a more sustainable POME management and an economical PHA production route.

1. Introduction

Palm oil is a vegetable oil with numerous industrial applications. It has been used extensively in food, oleochemical and energy industries for the manufacturing of cooking oil, margarine, soap, biodiesel, etc. Palm oil is produced from oil palm fruit bunches through a series of processes namely sterilization, threshing, digestion, pressing and oil purification. Large volume of high strength wastewater – known as palm oil mill effluent (POME) – is generated from the processing of oil palm fruit bunches. It is estimated that one tonne of palm oil production could result in more than 2.5 tonnes of POME [1].

POME is commonly managed via treatment-oriented approach and most palm oil mills adopt the open ponding system to treat POME [2,3]. This system requires a large footprint due to a long retention time of 20–200 days [2]. Besides, the open emission of methane gas generated from the anaerobic pond contributes substantially to global warming. These drawbacks have prompted the development of better POME management system. One of the options is resource recovery which transforms the organic pollutants in POME into useful resources such as organic acids [4], hydrogen [5] and electricity [6]. Resource recovery management approach allows concurrent minimization of waste and generation of valuable products. We are interested to utilize

* Corresponding author. Tel.: +60 3 79675291; fax: +60 3 79675319.
E-mail address: adeline@um.edu.my (A.S.M. Chua).

http://dx.doi.org/10.1016/j.cej.2015.01.103
1385-8947/

List of abbreviations and symbols

3HB	3-hydroxybutyrate
3HV	3-hydroxyvalerate
ADF	aerobic dynamic feeding
Cy3	sulfoindocyanine dyes
DO	dissolved oxygen
FISH	fluorescence *in situ* hybridization
FLUOS	5(6)-carboxyfluorescein-N-hydroxysuccinimide ester
HRT	hydraulic retention time
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
P(3HB)	poly(3-hydroxybutyrate)
P(3HB-co-3HV)	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PHA	polyhydroxyalkanoates
$PHA_{initial}$	concentration of PHA measured at the beginning of batch PHA production
PHA_{final}	concentration of PHA measured at the end of batch PHA production
POME	palm oil mill effluent
q_{PHA}	specific PHA production rate
q_{VFA}	specific VFA consumption rate
q_{X}	specific growth rate
sCOD	soluble chemical oxygen demand
SRT	solids retention time
t	duration of batch PHA production
VFA	volatile fatty acids
$VFA_{initial}$	concentration of VFA measured at the beginning of batch PHA production
VFA_{final}	concentration of VFA measured at the end of batch PHA production
VSS	volatile suspended solids
vvm	gas volume flow per reactor working volume per minute
$X_{initial}$	concentration of cell measured at the beginning of batch PHA production
X_{final}	concentration of cell measured at the end of batch PHA production
$Y_{PHA/VFA}$	PHA yield on VFA
$Y_{X/VFA}$	biomass yield on VFA
$Y_{O_2/VFA}$	respiration yield on VFA

fermented POME rich in volatile fatty acids (VFA) for the production of polyhydroxyalkanoates (PHA).

PHA are biodegradable plastics with similar mechanical properties to polyethylene and polypropylene [7]. Commercial PHA production is achieved by pure microbial culture using pure carbon substrate [8]. This results in high PHA production cost which has reduced the competitiveness of PHA in commercial market. The market price of PHA is approximately $ 4.4–6.0 per kg [9] which is considerably higher than that of the conventional petrochemical-based plastics at around $ 1.5 per kg [10]. One of the ways to reduce PHA production cost is by replacing the pure microbial culture with mixed microbial culture such as activated sludge [11]. Such substitution eliminates the need of maintaining high-energy-demanding sterile condition in PHA production by pure microbial culture. The non-sterile condition also permits the use of waste-derived carbon substrate, thus the cost of PHA production can be further reduced as carbon substrate accounts for 31% of the total cost [12].

The production of PHA from wastes (e.g. paper mill effluent and sugar cane molasses [13–15]) by activated sludge is commonly accomplished in a three-step system, as illustrated in Fig. 1. In the first step, waste is converted into VFA – the preferred carbon substrate for PHA production – via acidogenic fermentation process conducted in an anaerobic reactor. Subsequently, a portion of the VFA-rich fermented waste is used in the cultivation and enrichment of PHA-accumulating organisms in activated sludge. The cultivation step aims to produce sludge of high PHA storage capacity. Finally, the cultivated sludge is employed for PHA production with VFA-rich fermented waste as the carbon substrate. Through such a system, Jiang et al. [13] obtained a PHA content of 77 wt% using paper mill effluent as feedstock.

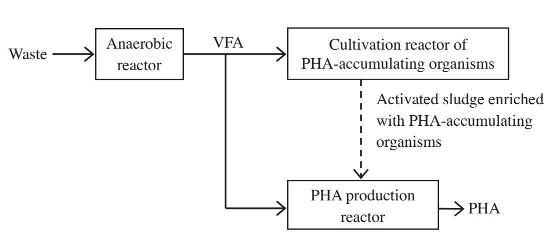

Fig. 1. Biotransformation of waste into VFA for the production of PHA by activated sludge.

In the literature, several studies [16–18] had examined the production of PHA from POME by activated sludge. Din et al. [16] investigated the effect of COD:N:P on PHA production. In their study, the highest PHA content of 44.5 wt% PHA per sludge dry weight was achieved at COD:N:P ratio of 180:0.7:1. On the other hand, Salmiati et al. [17] managed to attain a maximum PHA content of 40 wt% by doubling the loading of fermented POME supplied to the reactor. Din et al. [18] achieved a relatively high PHA content of 74 wt% through the application of microaerophilic condition, but the entire PHA production process was fairly long as it took about 40 h. Hence, there is a need for more studies on the strategy for efficient production of PHA from POME. A shorter PHA production process yielding higher PHA content is highly desirable.

This study adopts the above-mentioned three-step system for PHA production from POME. The first step of acidogenic fermentation of POME had been examined in our recent work [19]. It proved the viability of producing high concentration of VFA from POME. It was also recognized that the fermented POME having a high molar ratio of VFA-C:N:P is a suitable feedstock for PHA production. By using fermented POME as the sole carbon substrate, the present work investigates possible ways for enhancing the performance of PHA production by activated sludge. Two aspects are explored, i.e. enriching the activated sludge with PHA-accumulating organisms and fine-tuning the conditions of PHA production using the cultivated sludge as inoculum.

2. Materials and methods

2.1. Source and preparation of the fermented POME

Fermented POME was obtained from a lab-scale fed-batch anaerobic reactor operating at room temperature (~30 °C). The operational details of the anaerobic reactor could be found in Lee et al. [19]. The characteristics of fermented POME are listed in Table 1.

To maintain a reasonably uniform feed quality, the fermented POME was first filtered (MGC glass microfiber filter, Sartorius) to remove coarse solid particles, diluted with reverse osmosis water, and the pH adjusted (where needed) with 1 M NaOH solution before being fed into the reactors. Unless specified otherwise,