



## Characterization of aerobic granular sludge treating high strength agro-based wastewater at different volumetric loadings

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### HIGHLIGHTS

- ▶ Fractal dimension averaged at 1.90 indicating good compactness of granules.
- ▶ Significant microbial evolutionary shift was observed during aerobic granulation.
- ▶ Raup–Crick indices decreased upon formation of mature aerobic granular sludge.

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### ABSTRACT

Understanding the relationship between microbial community and mechanism of aerobic granulation could enable wider applications of granules for high-strength wastewater treatment. The majority of granulation studies principally determine the engineering aspects of granules formation with little emphasis on the microbial diversity. In this study, three identical reactors namely R1, R2 and R3 were operated using POME at volumetric loadings of 1.5, 2.5 and 3.5 kg COD m<sup>-3</sup> d<sup>-1</sup>, respectively. Aeration was provided at a volumetric flow rate of 2.5 cm s<sup>-1</sup>. Aerobic granules were successfully developed in R2 and R3 while bioflocs dominated R1 until the end of experiments. Fractal dimension ( $D_f$ ) averaged at 1.90 suggesting good compactness of granules. The PCR–DGGE results indicated microbial evolutionary shift throughout granulation despite different operating OLRs based on decreased Raup and Crick similarity indices upon mature granule formation. The characteristics of aerobic granules treating high strength agro-based wastewater are determined at different volumetric loadings.

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### 1. Introduction

Palm oil production has gained significant attention in recent years due to its many competitive advantages over other competing oils i.e. olive, vegetable and sunflower oils, for having low cost production, high yield and being free from trans-fatty acids. The utilization of palm oil has also increased rapidly owing to its multiple uses in both food and non-food industries contributing to greater demand and higher prices for palm oil production. The global aspiration to substitute fossil fuel with renewable fuel has given rise to increased demand for palm oil which is one of the sources for biofuel. The risks of pollution generated from the indus-

try have been escalating following the rapid expansion of palm oil industry worldwide. The production of palm oil generates a large amount of solid and liquid wastes in the form of empty fruit bunch (EFB) and palm oil mill effluent (POME), respectively. Malaysia's palm oil industry produced almost 80 million dry tonnes of solid biomass per annum (Agensi Inovasi Malaysia, 2011). This volume is projected to increase to 85–110 million dry tonnes by 2020. Similarly, the current POME volumes are expected to increase from 60 million tonnes to 70–110 million tonnes by 2020. The untreated POME is to comply with legislation limits of BOD<sub>5</sub> of 20 mg L<sup>-1</sup> for Standard A as outlined in the Fifth Schedule Paragraph 11(1) (a) Environmental Quality (Industrial Effluents) Regulations 2009 (Federal Subsidiary Legislation, 1974). The new regulations also outlined the effluent discharge standard to comply with color discharge of 100 ADML. Therefore, color removal is fast becoming an important research parameter in relation to agro-based industrial wastewater treatments. Additionally, in recent years, the significance of technological improvements in handling of res-

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idues from palm oil production compared to conventional practices are also being increasingly addressed through application of life cycle assessment (LCA) tools (Hansen et al., 2012).

Ponding systems which are commonly used for POME treatment have certain drawbacks including: methane emissions, long retention times, large area requirements and excessive sludge accumulation (Chan et al., 2010). Novel POME treatment methods have been proposed including anaerobic treatment methods and high-rate anaerobic digesters (Poh and Chong, 2009). Conventional anaerobic digesters require large reactors and long retention times to ensure satisfactory digestion of the treated effluent. High-rate anaerobic bioreactors have been used to overcome the problem of long retention time when treating POME (Zinatizadeh et al., 2007).

POME in its untreated form is classified as a high strength agro-based wastewater with COD and BOD concentrations ranging from 50,000 to 90,000 mg L<sup>-1</sup>. Recently, a respirometric study on POME found the Activated Sludge Model (ASM) heterotrophic yield coefficients and some COD fractionations of POME which could be used as basis for design and optimization of a POME treatment process (Damayanti et al., 2010). The typical characteristics of POME and ASM model coefficients are given in Table 1.

Aerobic granular sludge is a novel, compact consortium of self-immobilized bacteria with high-rate biological wastewater treatability. Aerobic granular sludge characteristically contains higher biomass concentrations within the same reactor than floccular sludge systems (Beun et al., 1999). Other main advantages of the aerobic granular sludge are high biomass retention in the reactor, good settling properties and the capacity to withstand high OLR, which all contribute to the very small footprint of this technology in comparison to conventional activated sludge based systems. Aerobic granulation has been observed in an SBR fed with various organic substrates including industrial wastewaters and landfill leachates (Cassidy et al., 2005; Kishida et al., 2009). To date, aerobic granular sludge has been successfully developed using POME at OLR 2.5 kg COD m<sup>-3</sup> d<sup>-1</sup> (Abdullah et al., 2011). Within the laboratory scale granulation SBR, numerous operational parameters may be manipulated to actively select for stable aerobic granular sludge formation including settling time, aeration intensity, feeding regime, substrate composition and organic loading rate. Relatively little is known about the microbial ecology of granular sludge system and the little we do know is derived from the systems fed with synthetic wastewater. Thus, this study sought to investigate the

relationship between the microbial community structure and aerobic granular sludge formation using real wastewater such as POME.

## 2. Methods

### 2.1. Reactor set-up

The schematic design of the reactor setup is given in Fig. 1. Three identical reactors with internal diameter of 50 mm and effective height to diameter (H/D) ratio of 17 were setup with several modifications to accommodate a working volume of 3 L. The reactor was constructed using Borosilicate glass due to its chemical resistance, inert characteristics and transparency, allowing visual monitoring of the mixed liquor and reactor contents. Each reactor was equipped with an internal down-comer tube located at the bottom of the reactor for influent feeding, an outlet port placed at mid-height of the reactor yielding a volumetric exchange rate (VER) of 50% and two sampling ports for mixed liquor and aerobic granular sludge samplings. Aeration was provided inside each reactor by means of air bubble diffusers at a volumetric flow rate of 3 L h<sup>-1</sup>, which is equivalent to a superficial air velocity of 2.5 cm s<sup>-1</sup>. The reactors were operated at room temperature (27 °C) in successive cycles of 3 h, which comprised of influent feeding (5 min), aeration (110 min), anaerobic reactions (45 min), settling (15 min) and finally effluent withdrawal (2 min).

### 2.2. Wastewater and seed sludge preparation

Both POME seed sludge and raw POME were kept at 4 °C to minimize degradation. Prior to feeding, the raw POME was centrifuged

**Table 1**  
Characteristics of POME and ASM estimated model parameters.

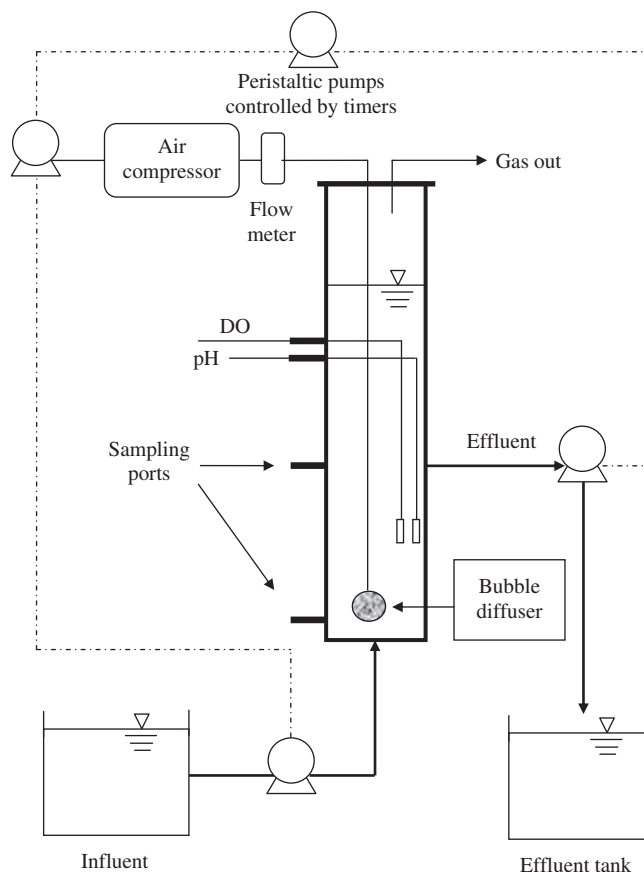
Parameters <sup>a</sup>	POME values (this study)	POME range <sup>c</sup>	ASM heterotrophic yield coefficients <sup>d</sup>	
			ASM model coefficient	Values
Chemical oxygen demand (COD)	69,500	15,000–100,000	Total COD	45,500
Biochemical oxygen demand (BOD) <sup>b</sup>	25,000	10,300–44,000	$S_s$	50
pH	3.8	3.4–5.2	$S_i$	16,600
Total solid	55,000	11,500–79,000	$X_s$	25,500
Suspended solid	33,600	5,000–54,000	$X_i$	2800
Volatile suspended solid	24,000	9,000–72,000	$Y_H$	0.44
Total nitrogen	800	80–1,400	Cell COD	14,100
Ammoniacal nitrogen	45	4–80	$\mu_A$	0.76
Total phosphorus	38	–	$\mu_H$	0.78
PO <sub>4</sub> <sup>3-</sup>	6	–	$K_s$	100
Oil and grease	Not measured	150–18,000	$b_H$	0.33

<sup>a</sup> All parameters unit in mgL<sup>-1</sup> except pH

<sup>b</sup> Sample is incubated for 3 days at 30 °C

<sup>c</sup> Source: Ujang et al., 2010

<sup>d</sup> Adapted from Damayanti et al., 2011



**Fig. 1.** Schematic diagram of operational reactor setup.

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