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Reactivation of Preserved Aerobic Granular Sludge Using Palm Oil Mill Effluent.

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ABSTRACT

Generation of wastes in the form of palm oil mill effluent (POME) is unavoidable due to increase in oil palm development worldwide. Direct discharge of POME into receiving water bodies is strongly discouraged due to high chemical and biochemical oxygen demand concentrations, respectively. Aerobic granulation technology features microbial aggregates generated following immobilization and cell-to-cell adhesion of multiple bacteria. Primary observation has shown that bacterial aggregates may survive for a long period of time at 4°C and possess the capability to recover their metabolic activities upon reactivation. In this study, preserved aerobic granular sludge previously developed using POME was reactivated in modified laboratory scale sequencing batch reactors (SBRs) termed as R1 and R2 which were operated at fixed OLR of 2.5 kgCOD $m^{-3}d^{-1}$. R1 was operated with the preserved granules while R2 was the control reactor. Microscopic examinations have shown good microstructure of aggregates in R1 as compared to R2. Good chemical oxygen demand (COD) removal of 75% was achieved in R1 as compared to 68% in R2 indicating successful reactivation of aerobic granules despite 3 months preservation period.
Keywords: reactivation; aerobic granular sludge; POME; SBR; preservation

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INTRODUCTION

The conventional activated sludge system is used as the basis for developing aerobic granular sludge using POME. While conventional activated sludge system is commonly associated with poor settling properties, the bioflocs should be easily separated from treated wastewater in settling tank. It is presumed that changes in reactor configuration or operating strategies may influence the characteristics of sludge developed in this system. However, for POME, the formation of bioflocs with good settling properties is difficult due to the complex characteristics of raw POME containing colloidal biomass wastewater with high suspended solids content. Therefore, the production of aerobic granular sludge or granular biomass is an alternative to promote better biomass-liquid separation and biomass retention in the activated sludge system. In addition, since POME contains high BOD and COD concentrations, aerobic granulation in a sequencing batch reactor (SBR) are useful for simultaneous nutrient removal.

Aerobic granules are microbial aggregates developed following active immobilization and cell-to-cell adhesion of various communities of bacteria [1]. These granules have shown significantly high settling velocities leading to good solid-liquid separation, high biomass retention, high bioactivity, higher tolerance against shock loading rates and resistance to toxic materials [1, 2]. In addition, simultaneous phosphorus and nitrogen removals have been successfully performed in SBR by using aerobic granular sludge [3]. Moreover, studies on different kinds of synthetic and non-synthetic wastewaters such as acetate molasses, sucrose, ethanol and POME have been performed in aerobic treatment system [4, 5].

In palm oil processing, high variation of flow and concentration of wastewater is common phenomenon. As a result, wastewater i.e. POME was intermittently or seasonally generated. There may be periods during which wastewater treatment plant has to be temporarily shut down due to decreased oil palm harvest or weather changes. The SBR technology has been applied in coping with fluctuating wastewater flows [6]. In addition, SBR could handle prolonged out-of-service periods [7]. Compared to activated sludge bioflocs, aerobic granules possess several unique characteristics such as dense and strong microbial structure and good settling ability making it viable for polishing the intermittent or seasonal wastewater from treatment plants.

Aerobic granules were developed in an SBR at OLR 2.5 kgCOD $m^{-3}d^{-1}$ using POME as the main substrate, and preserved over a period of 3 months. Then, the preserved aerobic granules were reactivated in the SBR at similar cultivation OLR. This study investigates the reactivation characteristics and COD removal efficiency of the preserved aerobic granules for POME treatment.

MATERIALS AND METHODS

Reactor set-up and operation

Aerobic granules were previously cultivated in an open, cylindrical column typed SBR with a total volume of 3L and a working volume of 1L following [8]. Influent was fed from a 5L storage canister at an organic loading rate (OLR) of 2.5 kgCOD $m^{-3}d^{-1}$ and introduced through a feeding point at the bottom of the reactor while effluent was discharged through an outlet point placed at medium height of the reactor resulting in fixed volumetric exchange ratio of 50%. Aeration was provided at a superficial air velocity of 2.5 cms^{-1}. The reactor was operated in successive cycles of 3h comprehended a feeding period of 5 min, a reaction period consisting an anaerobic and aerobic condition of 45 and 110 min, respectively, a settling period of 15 min, an effluent withdrawal period of 3 min and an idle period of 2 min. After 3 months of idle period following discontinuation of the feeding, aeration and decanting processes, aerobic granules were reactivated in two identical SBRs at similar operational conditions as applied previously. The first SBR was termed R1 containing the preserved granules while the second SBR was termed R2 which was operated without the preserved granules as the control reactor.

POME and seed sludge preparations

Raw undiluted POME was collected from a local mill at Felda Bukit Besar Palm Oil Mill, Kulai, Malaysia. The raw POME was centrifuged at 15,000 rpm for 40 min to eliminate suspended solids and debris which can cause clogging to the influent tubes. A suitable amount of tap water was added to the POME according to the desired OLR. The POME wastewaters had alkalinities of around 90 and 15–25 mg L^{-1} as CaCO3, respectively [8, 9]. Prior to feeding the pH of the mixed liquor was adjusted to a level of between 6.5 and 7.0 using 2M NaOH resulting in alkalinity of mixed liquor in the reactor of between 1000 and 2000 mg L^{-1} as CaCO3. A suitable amount of NH_4Cl and K_2HPO_4 were supplemented to ensure a feed COD:N:P ratio of 100:5:1 based on Metcalf and Eddy [10]. The sludge was sieved with a mesh of 1.0 mm twice to remove large debris such as dry leaves and palm fruit fibers before inoculation. The reactor was inoculated with 500 mL of seed sludge resulting in an initial MLSS concentration of 3000 mg L^{-1} in the reactor.

General Analytical Procedures

Parameters such as MLSS and COD were carried out according to Standard Methods for the Examination of Water and Wastewater [11],[12]. The morphological and structural observations of granular sludge were conducted by using a stereo microscope equipped with digital image analyzer (PAX-ITv6, ARC PAx-CAM). The microstructure compositions within the granule were observed with scanning electron microscope (FESEM-Zeiss Supra 35 VPFESEM).

EXPERIMENTAL RESULTS AND DISCUSSION

Structure and appearance of granules

Aerobic granular sludge was successfully developed at OLR 2.5 kgCOD $m^{-3}d^{-1}$ [8]. The biomass content was dominated by bioflocs during the reactor start-up. Bioflocs are most commonly associated with conventional activated sludge process. As hydrophobic binding is prime for cell to cell interactions in aerobic granular sludge formation, it is thought that an increase in the hydrophobicity of the cell surface was one of the factors contributing to the dominance of bioflocs during start-up of the reactor. Hence, bioflocs are unable to withstand the compression subjected by hydrodynamic shear force from the aeration intensity during start-up. However, this phenomenon improved towards the end of the experiment. When aerobic granular sludge was formed, the number of co-existing bioflocs in reactors visually decreased to less than 35% compared to about 48% during reactor start-up.

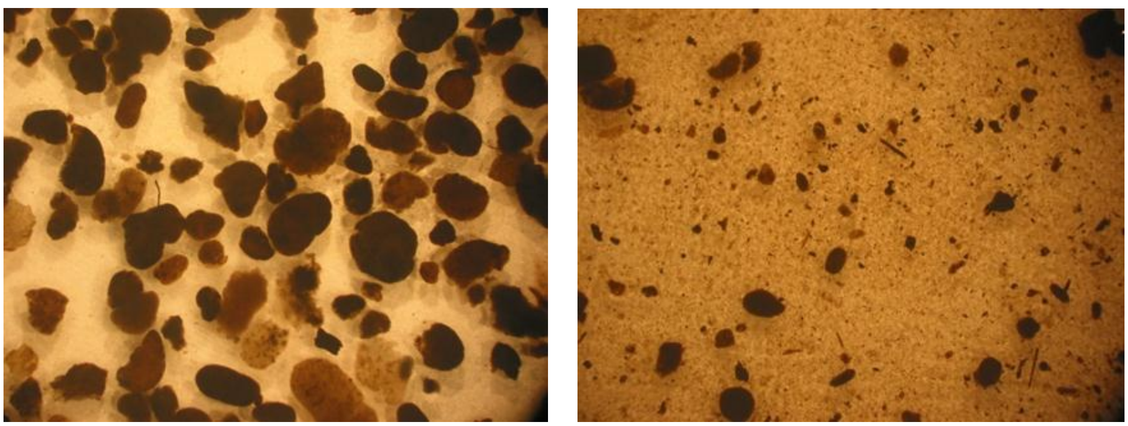

Figure 1:Microscopic photographs of the seed sludge and mature granules in the SBR (a) activated seed sludge on day-0; (b) aerobic granular sludge in R1 after day-40 of experiment (Scale bar represents 1 mm)

Figure 1(a) shows the actual activated seed sludge sourced from POME at the beginning of the experiment. The dispersed seed sludge gradually changes into bioflocs before being fully transformed into mature aerobic granules in the SBR as shown in Figure 1(b). In process start-up and initiation of aerobic granulation, the seeding sludge featured a distinct dispersed bioparticles of sand-like sizes. However, on day-40 of operation, both reactors exhibited a significant change in the morphology of these bioparticles via microscopic observations. Upon mature granulation, the initial smooth granules increased in size from 2.0 mm to 5.0 mm with a clearly defined outline boundary.

The morphology and microstructure of the granules were observed in more detail by using Scanning Electron Microscopy (SEM). SEM examinations of the cultivated aerobic granules in POME indicated that the microstructure of the sludge to be completely different from the seed sludge used as an inoculum. SEM analyses of the sludge used as an inoculum indicated a typical flocculent activated sludge with a comparatively feathery structure surrounded by fluffy edges and were comprised of seed-like sludge particles. The formation of these granules may be initiated by the microbial self-adhesion of mycelial pellets as explained by Beun *et al.* [13].

The structure appearance of reactivated aerobic granules is presented in Figures 2(a) and 2(b). The porous structure of the aerobic granules as indicated in an SEM examined sample shown in Figure 2(a) enables the facilitation of oxygen and substrate transfer as well as the release of metabolic products within the aerobic granular sludge. Previous observations by Li and Logan demonstrated that fractal aggregates possess a heterogeneous mass distribution, a structure resulting from the coagulation of small and densely packed clusters into larger and less dense aggregates. Moreover, the macropores formed between these clusters within a fractal aggregate also demonstrated that fractal aggregates i.e. aerobic granular sludge, are highly permeable. The macropores will enhance the mass transport rate through fractal aggregates such as aerobic granular sludge, resulting in a higher settling velocity [14]. As indicated in Figure 2(a), most of the aerobic granules were visually in good spherical shape after 3 months of preservation period with no significant changes as compared to when it was first cultivated. Figure 2(b) featured structural morphology of irregularly formed microbial communities of the seed sludge sampled from R2 which was attributed to poor settling of seed sludge in the SBR. Successful reactivation was achieved in R1 which was operated using preserved aerobic granules cultivated using POME.

a. R1 on day-40

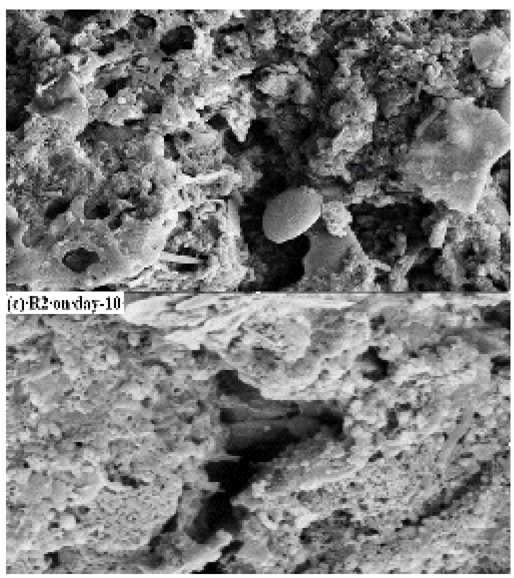

Figure 2: SEM examinations of reactivated aerobic granules. (a) R1 indicates active immobilization of microorganisms on granules surface with EPS binding; (b) R2 indicates absence of aerobic granules formation

The substantial amount of EPS produced by the indigenous microbes in R1 enhances granule formation with cavities that resembles channel like structure which accommodates very few bacteria. The EPS is essential in tolerating shock loading by acting as a protective functional part against the toxicity in high strength wastewater. According to SEM examinations as represented in Figure 2(a) almost all the microbial consortia on the granular surface have been embedded within the EPS matrix either as a single cell or dense cluster to form a more compact structure. The polysaccharide units of EPS served as backbone of the granule and help the microbial cells by connecting the cellular networks for granule stability [15].

Reactor Performance

Figure 3 gives the COD removal efficiencies of the SBR throughout reactivation of aerobic granules. COD removal in R1 increased higher to 75% on day-40 as compared to 68% in R2 presumably due to the addition of reactivated aerobic granules in R1. With addition of the preserved aerobic granules, the influent concentration was gradually improved. This suggests the ability of bio-degrading microbes retained within the reactor to efficiently utilize the organic waste to generate energy and nutrients for cellular proliferation. More than 80% of POME influent was oxidized at latter stage of the reactor operation signifying increased viability of the degrading microbes to establish stable and efficient substrate utilization. In a previous study performed by Liu *et al*. (2011)[16], the COD removal efficiency of reactor cultivated with granules which was designed to treat mixed wastewater with high toxicity in organic matters achieved 80% which is similar to the findings in this study. Based on this parameter, the performance of the granulation system clearly states that POME utilization as the main substrate for microbial viability in the SBR under sequential aerobic-anoxic phases was efficient. Furthermore, aerobic granules could withstand high organic loading rate without compromising granule integrity [17].

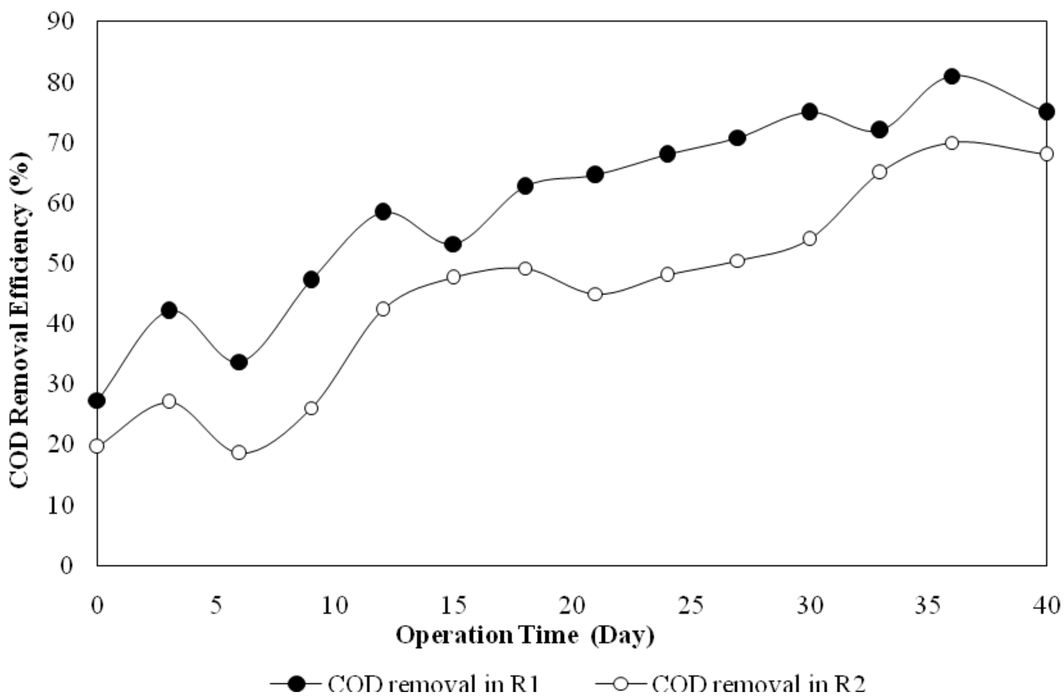

Figure 3:COD removal efficiency for R1 (containing reactivated aerobic granules) and R2 (control reactor operated without preserved aerobic granules)

Observation in R2 indicates that COD removal efficiency was slightly lower at 68% at the end of the experiments as compared to 75% in R1. Despite not being enriched with reactivated aerobic granules, COD removal in R2 in indicative of good SBR performance for treatment of agro-industrial wastewater such as POME. With aerobic granulation, efficient removals of COD and ammonia of above 85% were observed in the treatment of real wastewaters including POME [8], textile wastewater [18], livestock wastewater [3] and brewery wastewater [19].

Biomass Concentration in SBR

In this study, activated sludge taken from a local palm oil mill was seeded directly into the reactors without any acclimation due to fungal growth and foaming conditions associated with POME. At the end of the reactor operation on day-40, samples of flocs and/or granules were collected from the reactors and the reactor effluents and analyzed for MLSS concentrations.

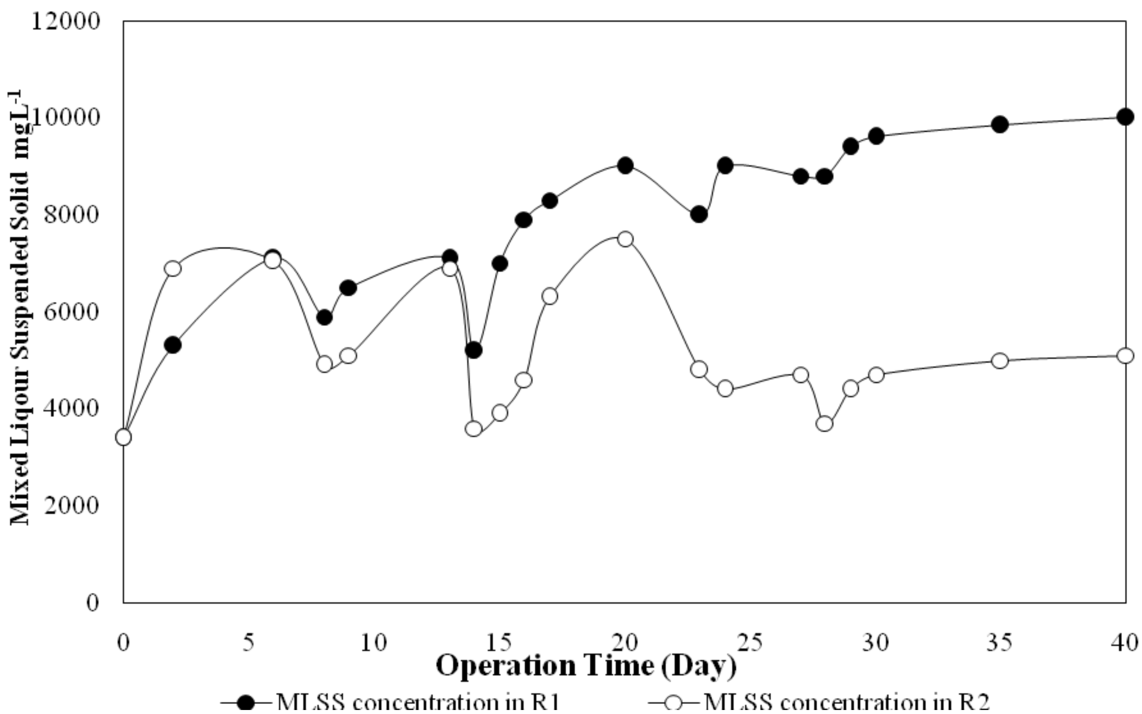

Figure 4:Biomass concentrations in both R1 and R2

Previous study has shown that aerobic granules was successfully developed using POME after 60 days of SBR operation [9]. Compact structured granules were not detected in R2 on day-40 of experiments which was operated as control reactor. Instead, the biomass content in R2 was dominated by bioflocs with average MLSS concentrations of 5100 mgL^{-1} while R1 illustrates much higher average MLSS concentrations of above 8000 mgL^{-1} indicating a gradual increase of sludge concentration in the reactor. A significant fluctuations of reactor biomass was also observed in R1 following unstable biomass concentrations and domination of bioflocs in the reactor throughout the experiments course. The settling ability of aerobic granules determined the solid-liquid separation that was important for the efficient treatment of agro-industrial wastewater such as POME. Visual observations have shown disintegration of granules at the beginning of reactivation process. Based on Figure 4, the MLSS concentrations gradually increased with time. However, MLSS fluctuated until about day-14 of operation due to disintegration of granules. When the granules disintegrate, a great quantity of bioflocs was discharged with the effluent, leading to decreased MLSS as shown in Figure 4. Better settling of biomass was then observed in R1 which was operated with reactivated aerobic granules whilst R2 has shown significantly lower biomass retainable in the SBR towards the end of the experiments. Mixed liquor in R1 also shown better settling ability based on proper solid-liquid separation which was visually observed in the SBR.

CONCLUSION

Successful reactivation of aerobic granules was observed in R1 which was operated with granules that have been preserved for 3 months. SEM examinations illustrated no significant changes in granules morphology sampled from R1 following reactivation process. Good reactor performance was observed in R1 with 80% COD removal while R2 recorded lower COD removal at only 68%. R1 has shown good solid-liquid separation based on stable MLSS concentrations despite disintegration of granules at the beginning of the reactivation process. This study indicated that aerobic granules could be preserved and re-activated which would be useful for solving the intermittent or seasonal wastewater from plants such as palm oil mills.

ACKNOWLEDGEMENTS

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