

DEVELOPMENT AND MICROBIAL CHARACTERIZATION OF AEROBIC
GRANULATION USING PALM OIL MILL EFFLUENT (POME)

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...to my lovely sons Adam and Harris
husband Dr. Muhamad Ali Muhammad Yuzir
and our future newborn
with lots of love and shiny sparkles...

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ABSTRACT

Palm oil is widely used for food and non-food manufacturing industries and as a biofuel. 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. As POME contributes to a high pollution process waste, the need to find an efficient and practical approach to preserve the environment is essential. Novel aerobic granular sludge is a compact consortium of self-immobilized bacteria with high rate biological wastewater treatability. This study is aimed at investigating aerobic granular sludge formation in lab-scale sequencing batch reactor (SBR) using POME as substrate. The efficiency of aerobic granular sludge developed for the treatment of POME in corresponds to the structure of bacterial population is monitored. Aerobic granular sludge was developed at volumetric exchange rate (VER) of 50% and cycle duration of 3 hours at flow rate of 3 Lm⁻¹ in reactors R1, R2 and R3 operated at OLR of 1.5, 2.5 and 3.5 kgCOD m⁻³d⁻¹, respectively. Shock load conditions were introduced by increasing the OLR in R2 from 2.5 to 19 kgCOD m⁻³d⁻¹. Aerobic granular sludge was successfully formed at an OLR of 2.5 and 3.5 kgCOD m⁻³d⁻¹, respectively while bioflocs remained dominant in R1. Under shock loading rate, aerobic granular sludge was partially disintegrated due to mass transfer limitation within the granule. The average sizes of granules were between 1.0 mm to 4.0 mm. Upon steady state, COD removal efficiency of greater than 80% was recorded for all reactors. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and fluorescence *in situ* hybridisation (FISH) techniques were used to show that the bacterial population changes during aerobic granular sludge formation at different OLRs. The average Raup and Crick similarity indices obtained during the acclimation/granulation and the maturation phases were 0.95 ± 0.02 and 0.72 ± 0.06, respectively. Mature granules maintained good settling property and dominant granular morphology as evidenced by low SVI of 19.9 mL g⁻¹ SS at 3.5 kgCOD m⁻³d⁻¹. Visible bands from the DGGE profiles indicated the shift of bacterial population during the aerobic granulation process. *Comamonadaceae* sp., *Leadbetterella* sp. and *Runella* sp. are dominant bacteria that consistently present throughout the granulation process. Distribution of AOB in mature granule was confirmed by FISH technique thus contributing to efficient removal of ammonia in POME of > 80%. Despite the different OLRs, aerobic granular sludge formation was successfully achieved for the treatment of high strength wastewater such as POME.

ABSTRAK

Minyak sawit digunakan secara meluas di dalam industri pembuatan bahan makanan dan bukan makanan dan juga sebagai bio-bahan api. Penghasilan minyak sawit menghasilkan sejumlah besar sisa pejal dan cecair di dalam bentuk tandan kelapa sawit kosong (EFB) dan air sisa minyak sawit atau ringkasnya *palm oil mill effluent* (POME). Memandangkan POME menyumbang kepada sisa cemar pemprosesan yang tinggi, keperluan untuk mendapatkan pendekatan olahan yang praktikal untuk melindungi alam sekitar adalah penting. Butiran enapcemar aerobik merupakan konsortium yang padat dengan bakteria yang mempunyai kadar olahan air sisa biologi yang tinggi. Kajian ini menjurus kepada pembentukan butiran enapcemar aerobik dengan menggunakan POME sebagai substrat di dalam *sequencing batch reactor* (SBR). Keberkesanan butiran enapcemar aerobik yang terbentuk untuk olahan POME bersesuaian dengan struktur populasi bakteria telah di pantau. Butiran enapcemar aerobik terbentuk pada nisbah pertukaran volumetrik (VER) sebanyak 50% dan kitaran selama 3 jam dan kadar alir sebanyak 3 Lm^{-1} di dalam tiga reaktor iaitu R1, R2 dan R3 yang beroperasi pada kadar suapan organik sebanyak 1.5, 2.5 dan $3.5 \text{ kgCOD m}^{-3}\text{d}^{-1}$. Keadaan beban kejutan dikenakan secara meningkatkan kadar suapan organik di dalam R2 daripada 2.5 ke $19 \text{ kgCOD m}^{-3}\text{d}^{-1}$. Butiran enapcemar aerobik berjaya dibentuk pada kadar suapan organik sebanyak 2.5 dan $3.5 \text{ kgCOD m}^{-3}\text{d}^{-1}$, walaubagaimanapun bioflok kekal mendominasi R1. Pada kadar masukan tinggi, butiran enapcemar aerobik berintegrasi disebabkan had pemindahan jisim di dalam butiran. Saiz purata butiran adalah di antara 1.0 mm hingga 4.0 mm. Pada paras stabil, permintaan oksigen biokimia (COD) adalah melebihi 80% bagi kesemua reaktor walaupun pada kadar masukan yang berbeza. Teknik molekular piawai seperti *polymerase chain reaction-denaturing gradient gel electrophoresis* (PCR-DGGE) dan *fluorescence in situ hybridisation* (FISH) telah digunakan bagi menyelidiki struktur microbial butiran enapcemar aerobik. Purata kebersamaan *Raup dan Crick* yang didapati semasa proses aklimasi/pembutiran dan pematangan butiran enapcemar aerobik adalah 0.95 ± 0.02 dan 0.72 ± 0.06 . Butiran yang matang mempunyai kemampuan enapcemar dan morfologi butiran yang baik berdasarkan paras SVI $19.9 \text{ mL g}^{-1} \text{ SS}$ pada kadar masukan $3.5 \text{ kgCOD m}^{-3}\text{d}^{-1}$. Profil DGGE menunjukkan struktur microbial berubah secara signifikan semasa proses pembutiran. *Comamonadaceae* sp., *Leadbetterella* sp. dan *Runella* sp. telah diperolehi sepanjang proses pembutiran. Pembahagian spatial AOB di dalam butiran enapcemar aerobik juga terbukti melalui FISH yang menyumbang kepada penyingkiran kandungan ammonia di dalam POME yang melebihi 80%. Walaupun pada kadar masukan berbeza, butiran enapcemar aerobik telah berjaya dibentuk untuk perawatan air sisa berkadaran tinggi seperti POME.