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Research article

Bioaugmentation and enhanced formation of biogranules for degradation of oil and grease: Start-up, kinetic and mass transfer studies

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ABSTRACT

Biogranulation technology is an emerging biological process in treating various wastewater. However, the development of biogranules requires an extended period of time when treating wastewaters with high oil and grease (O&G) content. A study was therefore conducted to assess the formation of biogranules through bioaugmentation with the *Serratia marcescens* SA30 strain, in treating real anaerobically digested palm oil mill effluent (AD-POME), with O&G of about 4600 mg/L. The biogranules were developed in a lab-scale sequencing batch reactor (SBR) system under alternating anaerobic and aerobic conditions. The experimental data were assessed using the modified mass transfer factor (MMTF) models to understand the mechanisms of biosorption of O&G on the biogranules. The system was run with variable organic loading rates (OLR) of 0.69–9.90 kg/m^{3}d and superficial air velocity (SAV) of 2 cm/s. After 60 days of being bioaugmented with the *Serratia marcescens* SA30 strain, the flocculent biomass transformed into biogranules with excellent settleability with improved treatment efficiency. The biogranules showed a compact structure and good settling ability with an average diameter of about 2 mm, a sludge volume index at 5 min (SVI_5) of 43 mL/g, and a settling velocity (SV) of 81 m/h after 256 days of operation. The average removal efficiencies of O&G increased from 6 to 99.92%, respectively. The application of the MMTF model verified that the resistance to O&G biosorption is controlled via film mass transfer. This research indicates successful bioaugmentation of biogranules using the *Serratia marcescens* SA30 strain for enhanced biodegradation of O&G and is capable to treat real AD-POME.

1. Introduction

The Malaysian palm oil industry has achieved exceptional distinction, constituting the agricultural sector's backbone. However, the main back draw of the industry is the discharge of palm oil mill effluent (POME) that is rich in solid, O&G and high in organic matter (Mohammad et al., 2021). The discharge of POME from the oil palm sector causes both economic and technical issues for wastewater managers and may cause environmental ecosystem damage. The conventional treatment of POME which remains a challenge for environmental scientists, has several drawbacks which include a long hydraulic retention time, large land requirement, sensitivity of microorganisms to variations in environmental conditions, foul smell and greenhouse gas emissions (Soo et al., 2022; Udaiyappan et al., 2020). The increase of environmental awareness and the toughening of government policies has made it necessary to develop a new environmentally friendly, effective and low-cost treatment system to treat POME.

Recent research indicates that biogranulation technology has grown in popularity in wastewater treatment systems due to its advantageous properties, which have resulted in a significant decrease in energy demand and land footprint while simultaneously improving treatment effectiveness (Purba et al., 2022). Moreover, it has been reported that biogranules possess excellent compact structure, a variety of microbial species, excellent settling abilities, the ability to shock loading resistance, high tolerance to challenging conditions of the environment and high organic removal efficiency, making them promising for sustainable

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wastewater treatment (Cydzik-Kwiatkowska et al., 2022; Han et al., 2022a,b). Hitherto biogranulation technology, aerobic biogranules have been widely researched to treat various types of real wastewater, including domestic wastewater (We et al., 2021; Sun et al., 2021; Sguanci et al., 2019; Derlon et al., 2016), swine wastewater (Mourão et al., 2021), industrial wastewater containing ethylene glycol (EG) (Qi et al., 2020), petrochemical shipboard slops wastewater (Campo and Bella, 2019), textile wastewater (Bashiri et al., 2018; Krishnen et al., 2017; Muda et al., 2010), livestock wastewater (Othman et al., 2013), and rubber wastewater (Rosman et al., 2013). However, further process optimisation is required to allow for broader industrial application.

Several treatment technologies, including biological, physical and chemical are commonly applied to treat oily wastewater (Adetunji and Olaniran, 2021). In recent decades, biogranulation technology has been studied for industrial O&G-bearing wastewaters (Wang et al., 2022a). Numerous studies have looked into the treatment of real and synthetic wastewater containing O&G, such as oil refinery wastewater (Wang et al., 2022a), industrial shipboard slop wastewater (Campo et al., 2021), refinery and brewery wastewater (Ghosh and Chakraborty, 2019), and petroleum wastewater (Chen et al., 2019). Most of these studies revealed an extended granulation development time, unstable biogranules, and poor removal efficiency (Chen et al., 2019). The formation of a lipid coat around floc, filamentous bacteria, and sludge bulking in AD-POME decreased the rate of cell-aqueous phase transfer due to high O&G concentration, thus hindering the development of biogranules (Ahmad et al., 2020; Cammarota and Freire, 2006).

Bioaugmentation of pre-screened bacteria with specific functions effectively improve wastewater treatment efficiency (Han et al., 2022a, b; Nzila et al., 2016). This is carried out by adding specific single strains or microbial consortia into the bioreactor system, thereby improving the original design's microbial community diversity and the biodegradation of target pollutants (Ma et al., 2022). Liu et al. (2015) successfully cultivated biogranules using *Rhizobium* sp. NJUST18 in a SBR. After 120 days of cultivation, the biogranules had a diameter between 0.5 and 1 mm, an SV of 37.2 ± 2.7 m/h, and an SVI of 25.6 ± 3.6 mL/g. Hailei et al. (2011) reported that fungal *chlamydospores* were added to the biogranular system during phenol wastewater treatment. Table 1 shows the various types of wastewaters containing O&G that were treated using bioaugmentation through the application of biogranulation technology.

Culturing biogranules capable of degrading O&G compounds is challenging as compared to degrading specific nutrients and organic pollutants. Typically, the cultivation of biogranules requires a few months or more. However, developing biogranules using bioaugmentation to treat real oily wastewater requires additional study. Research on the mass transfer kinetics throughout the O&G biosorption process by biogranules could provide a new understanding of optimising the performance of the biogranular system process based on the behaviour of O&G removal. Even though Fulazzaky et al. (2019, 2018, & 2017) has reported the biosorption of nutrients and organic matter through biogranulation technology by determining the mass transfer resistance, the mass transfer kinetics for the removal of O&G in biogranulation technology is yet to be fully comprehended.

To the best of our knowledge, no work has been reported on removing O&G from real AD-POME with bioaugmentation. Therefore, this paper presents the work on treating real AD-POME with a specific focus on O&G removal under the influence of *Serratia marcescens* SA30. *Serratia marcescens* SA30 is a negative gram bacterium widely found in soil, water, and food, and has been found to degrade different types of refractory organics, including O&G (Lokhande et al., 2023; Affandi et al., 2014). The effects of the *Serratia marcescens* SA30 strain addition on the O&G removal performance of the biogranulation system are discussed. Additionally, the kinetics and mechanisms of mass transfer on the biosorption of O&G using the MMTF model are presented.

2. Materials and methods

2.1. Wastewater and sludge

The real AD-POME samples were taken from anaerobic pond no. 2 of a palm oil mill located in Kahang, Johor (2.171377 N, 103.480879 E). The anaerobic pond no. 2 received the anaerobically digested POME which occurred in anaerobic pond no. 1. The AD-POME is more suitable to be treated with the aerobic biogranulation process as it has a much lower organic content as compared to raw POME with BOD_5 up to 25,000 mg/L (Isa et al., 2022; Mahmod et al., 2022). The samples were filtered using a muslin cloth to separate suspended materials. The samples were then collected and kept in polyethylene containers, closed tightly, in a cooler box, and quickly transported to the laboratory. In order to prevent the real AD-POME from degrading biologically due to

Table 1
Comparative study on bioaugmentation of biogranules obtained in this study and other studies to treat oily wastewater.

Type of wastewater	Initial inoculum	Biogranules characteristics	Removal performances	References
Synthetic oily wastewater	Activated sludge + *Brevibacterium paucivorans, Micrococcus aloeverae* and *Staphylococcus hominis*	Size: 3.71 mm; SV: 23.21 m/h; SVI: 39.80 mL/g	COD: 99%; NH_4^+ − N: 99.9%; Oil: 61.34%	Ghosh and Chakraborty (2019)
Synthetic petroleum wastewater	*Propioniciclava, Micropruina, Alphaproteobacteria, Flavobacterium,* and *Sulfuritalea*	Size: 0.46–0.9 mm	COD: 95%; NH_4^+ − N:100%; TN: 100%	Chen et al. (2019)
Synthetic hypersaline wastewater	Autochthonous seawater-born microbes and *Stappiaceae* and	Size: 1.9–2.2 mm; SV: 37–69.5 m/h; SVI_5: 8.3–19.6 mL/g	TOC: 93%; TN: 82–99%; TP: 95–96%	Sarvajith and Nancharaiah (2020)
Synthetic saline refinery wastewater	Seed sludge + *Brevibacterium paucivorans, Staphylococcus hominis* and *Micrococcus aloeverae*	Size: 0.9–2.7 mm; MLVSS: 3–8 g/L; SVI_{30}: 23–45 mL/g; SV: 31–43 m/h; IC: 12–29%	COD: 71–92%; TPH: 72–93%; Phenol: 70–100%; Cresol: 97–100%; NH_4^+- N: 89–96%	Ghosh and Chakraborty (2021)
Synthetic oil refinery wastewater	Sewage + Rhodococcus	Size: >0.45 mm	COD: 97.9%; O&G: 97.4%; NH_4^+ − N: 97.2%; TN: 90.2%	Wang et al. (2022b)
Synthetic petroleum wastewater	*Pseudomonas mendocina* K0, *Brucella* sp. K1, *Pseudomonas putida* T4 and *Paracoccus* sp. T9	Size: >2.0 mm	NH_4^+ − N: 92.4%; TN: 79.8%	Wang et al. (2022a)

TOC: Total organic carbon, TN: Total nitrogen, TP:Total phosphorus, TPH: Total phenolic hydrocarbon,: NH_4^+ − N: Ammonium nitogen, IC: Integrity coefficient, SVI_{30}: Sludge volume index at 30 min.

microscopic organism action, it was maintained at a temperature below 4 °C but above the freezing point. It was then centrifuged for 40 min at 15,000 rpm to remove any remaining particles. The physicochemical characteristics of the real AD-POME and other literature based on oily wastewater are presented in Table 2.

The sludge seed for the inoculation was obtained from a sewage treatment plant (STP) aeration tank in Skudai, Johor (1.526410 N, 103.651358 E), and from anaerobic pond no. 2 of the same palm oil mill. All the sampling procedures were carried out according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

2.2. Serratia marcescens SA30 strain

As mentioned earlier, the *Serratia marcescens* SA30 strain was used in this study for O&G biodegradation. The strain was previously isolated and applied for the degradation of O&G (Affandi et al., 2014). The *Serratia marcescens* SA30 strain was inoculated using a nutrient agar (NA) (Merck KGaA, Darmstadt, Germany). The NA plates medium was prepared by dissolving 20 g of NA powder in 1 L of distilled water (Favorit, PTL Scientific Sdn. Bhd., Selangor, Malaysia) and sterilising it in an autoclave (HVE-50, Hirayama, Saitama, Japan) at 12 °C and 103 kPa for 20 min. The NA was cooled to 50 °C and poured aseptically into a sterile Petri dish. The Petri dish was then incubated (LOM-65, Digital shaker incubator, MUNRO Scientific, Harlow, UK) at 30 °C for 24 h to allow solidification. The Petri dish was sealed with parafilm for long-term nutrient agar medium storage to prevent contamination.

2.3. Bioreactor operating conditions

Fig. 1 is a schematic illustration of the bioreactor configuration. The bioreactor consists of a double-jacketed plexiglass column manufactured from acrylic material (height = 100 cm, inner diameter = 8 cm) with an effective working volume of 2 L. It was also equipped with a 5-L storage tank, feeding inlet, peristaltic pump (Cole-Palmer, Masterflex L/S 6–600 rpm, Minnesota, US), recycling pipe system, aerator (LP-100 Silent red oxygen pump, 100W, Chaozhou, China), outlet pipe and timer controller (Masterclear, Sum MFG (M) Sdn. Bhd., Johor, Malaysia). The temperature was controlled by adjusting the water circulating within the double-jacketed reactor at 30 ± 2 °C.

The real AD-POME was added into the bioreactor from the bottom. A diffuser of the fine air bubble at the base of the bioreactor column supplied air to the reactor. The bioreactor airflow rate of 6 L/min was chosen to provide the necessary dissolved oxygen (DO). Three peristaltic pumps were utilised to feed the real AD-POME into the bioreactor, circulate the real AD-POME within the bioreactor, and withdraw the treated real AD-POME from the bioreactor. The volumetric exchange ratio was 50% withdrawing the supernatant through the outlet pipe. A pH/DO meter (Istek®, Korean Model 125 PD, Seoul, Korea) was utilised to monitor the real AD-POME reactor's pH and DO levels. Fig. S1 (supplementary material) shows the laboratory scale of the bioreactor used in this study.

The biogranular system was operated with 6 h cycles. This cycle agrees with other cycles typically used in treating various type of wastewater (Rollemberg et al., 2018; Khan et al., 2013). Each cycle has filling, reaction, settling, and decanting phases. The cycle was initiated by the addition of influent (5 min) followed by the reaction (340 min), settling (5 min), decanting (5 min), and idle phase (5 min). The reaction phase started with a 40 min anaerobic phase, followed by a 130 min aerobic phase, another 40 min of the second anaerobic phase, and 130 min for the second aerobic phase. The experimental period was divided into two phases: before the addition of the *Serratia marcescens* SA30 strain (1–177 days) and after the addition of the *Serratia marcescens* SA30 strain (178–256 days). During the first phase, the real AD-POME was added into the biogranular system without the addition of the *Serratia marcescens* SA30 strain. In this phase, the biogranular system experienced the growth of filamentous bacteria, the formation of scum and stable foam, and the washout of slow-settling biomass, which was attributed to the high concentration of O&G in the real AD-POME. The biogranules were still not observed during the first phase in the reactor. To overcome this problem, the biogranular system was then added with the *Serratia marcescens* SA30 strain pure culture for the degradation of O&G and fed with the real AD-POME. 25 mL of the *Serratia marcescens* SA30 strain was added every week (for four weeks, totalling 100 mL) into the reactor.

2.4. Analytical methods

The O&G concentration in the wastewater was determined before and after the treatment. The analysis was carried out as described in Method 5520 B (APHA, 2012). The O&G was extracted using n-hexane and hydrochloric acid (HCl) in an acidic medium (pH < 2) (Affandi et al., 2014; Jeganathan et al., 2006). The oil was dissolved in n-hexane, which forms the organic layer. The n-hexane extract was collected into a pre-weighed beaker bypassing the organic layer through a funnel that

Table 2
Physico-chemical characteristics of oily wastewater derived from the present study and other sources.

Oily wastewater source	Real AD-POME	Synthetic Refinery wastewater	Refinery wastewater	Petrochemical industry wastewater	Synthetic oily wastewater	Synthetic oily wastewater
Parameters[a]						
pH	7.2	7.33	8.2	6.5–9.1	7	–
Temperature[b]	28	–	26.3	18.3–22.4	–	–
Color[c]	5470	–	–	–	–	–
O&G	4637	234	1025	600–2200	10–320	70–80
BOD[d]	800	–	145.3	36.1–650	–	–
COD	4600	1300–1350	166.7	–	1540–1200	600
TN	450	–	–	1200–2736	–	–
NH_4^+ – N	–	51	–	–	5–50	–
PO_4^{-3}	–	250	–	–	–	–
TSS	2900	–	–	–	–	–
TOC	3120	–	–	–	–	–
VSS	2200	–	–	–	–	–
References	(This study)	Ghosh and Chakraborty (2021)	Ezeonuegbu et al. (2021)	Wei et al. (2020)	Ghosh and Chakraborty (2019)	Wang et al. (2017)

[a] All parameters in mg/L except pH.
[b] Temperature °C.
[c] Colour in ADMI.
[d] BOD_5 = BOD at 5 days at 20 °C, PO_4^{-3} = Phosphate, VSS =Volatile Suspended Solids.

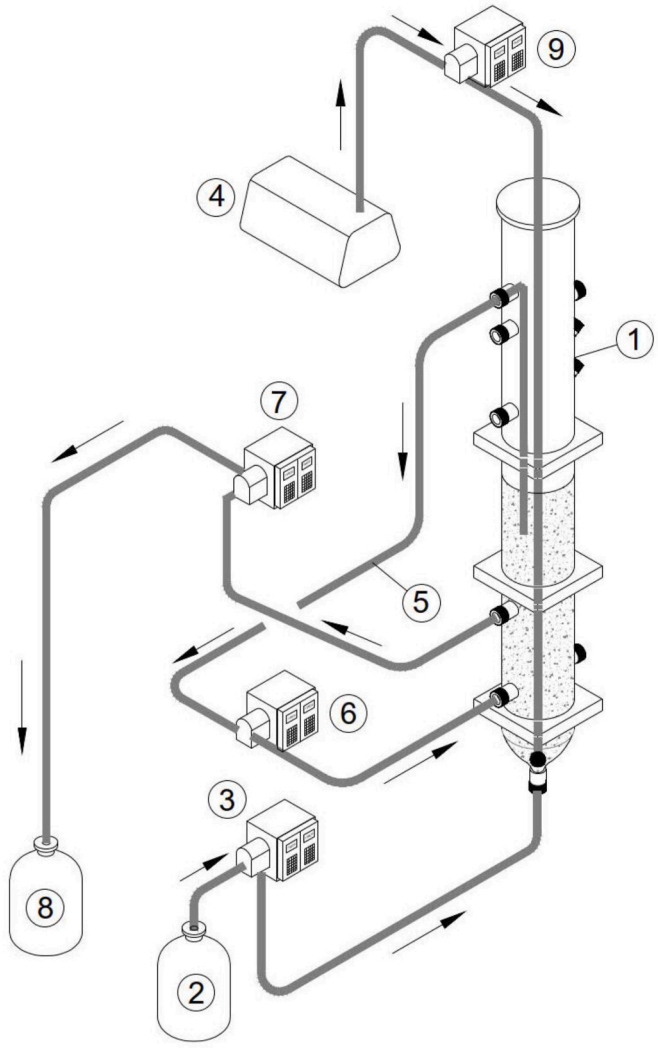

Fig. 1. Schematic diagram of laboratory-scale bioreactor. (1) Reactor, (2) Raw POME storage tank, (3) Timer controller for regulating the POME feeding, (4) Aerator, (5) Recirculation flow tubing, (6) Timer controller for the circulating of POME, (7) Timer controller for regulating the POME discharged, (8) Treated POME storage tank, (9) Timer controller for aerator.

contains anhydrous sodium sulphate. Hydrochloric acid, n-hexane and anhydrous sodium sulphate was obtained from Merck KGaA, Darmstadt, Germany. All the reagents purchased were of ACS grade and used as received. The filtrate was warmed at 85 °C to expel the n-hexane; the residue was then dried, cooled, and weighed until a constant weight was achieved, which provided the concentration of O&G in the sample. All tests were conducted at an ambient temperature of 26–30 °C. The mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and SVI_5 were carried out according to Method 2540 D, Method 2540 E, and Method 2710 D, respectively (APHA, 2012).

The diameter of the biogranules was measured using a stereo microscope equipped with digital image management and analysis software (PAX-ITv6, ARC PAX-CAM, Carlsbad, California). The microscope was connected to an image management tool, i.e., PAX-IT® equipped with PAX-cam (digital camera). By using PAX-IT®, the development process of biogranules was determined in terms of shape, diameter, and colour. Advantageously, this tool provides online and offline analysis as well as systematic data management.

The SV of the biogranules was determined by randomly taking individual biogranules from the bioreactor to settle inside a 1 L graduated cylinder under quiescent conditions. The SV was determined by dividing the distance travelled by the biogranules by their average time to settle (Ji et al., 2010).

The strength determination of the biogranules followed the method ascribed by Ghangrekar et al. (2005). The weight of residual biogranular sludge was also determined, and the results were expressed in terms of IC, which is defined as the ratio of solids in the supernatant to the total weight of the biogranular sludge. The IC is interpreted as an indirect index of the strength of the biogranules; the lower the IC value, the stronger the biogranules. The formula for calculating IC is given in Eq. (1).

$$\mathrm{IC} = \left(\frac{\mathrm{RG}}{\mathrm{SG} + \mathrm{RG}}\right) \text{ x } 100\% \tag{1}$$

whereby,

RG = Residual granules (mg)

SG = Settled granules (mg)

2.5. Mass transfer kinetic model

The removal efficiency (E) of the O&G by the biogranulation bioreactor system was determined using Eq. (2),

$$\mathrm{E} = \frac{C_0 - C_s}{C_0} \text{ x } 100\% \tag{2}$$

whereby E represents the removal performance of O&G in the biogranular system (%), C_0 is the beginning O&G concentration (mg/L) and C_s is the discharge O&G concentration at time t (mg/L).

The amount of O&G accumulated onto the biogranules when using a feed O&G concentration with its OLR was calculated using Eq. (3) (Öztürk and Kavak, 2005),

$$q = \int_0^V \frac{(C_o - C_s)\, dV}{m} \tag{3}$$

where q is the cumulative amount of O&G entered into the biogranules (mg/g), V is the adequate volume of real AD-POME treated in the biogranular system (L), and m is the dried mass of the biogranules (g).

The modified mass transfer factor model is based on the extracellular precipitation, cell surface sorption, and intracellular accumulation modes of O&G biosorption by the biogranules (Jena et al., 2022). The initial part of extracellular precipitation is associated with external mass transfer (EMT). In contrast, the second and third parts of cell surface sorption and intracellular accumulation are associated with internal mass transfer (IMT). In order to investigate the kinetics of mass transfer during the biosorption of O&G by the biogranules, it is necessary to implement the MMTF model:

$$ln\left(\frac{C_0}{C_s}\right) = [k_L a]_g \times e^{-\beta \times ln(q)} \times t \tag{4}$$

where $[k_L a]_g$ represents the global mass transfer (GMT) factor (1/h), β represents the absorbate-adsorbent affinity (g h/mg), and t is the experimental run time (h).

A mathematical deduction from Eq. (4) yields the following linear equation as follows (Fulazzaky et al., 2013):

$$\ln(q) = \frac{1}{\beta} \times \ln(t) + B \tag{5}$$

with

$$B = \frac{ln[k_L a]_g - ln\left(\frac{C_o}{C_s}\right)}{\beta} \tag{6}$$

where B is the potential mass transfer index relating to the mass transfer's driving force (mg/g).

The correlation between the EMT and GMT factor can be expressed

as follows (Fulazzaky et al., 2013):

$$[k_La]_f = [k_La]_g \times e^{-\beta \times \ln[q]} \quad (7)$$

where $[k_La]_f$ is the EMT factor (1/h).

The variation of variables $[k_La]_g$ and $[k_La]_f$ concerning the time of the experimental run to follow the q value can be determined using Eqs. (6) and (7), respectively. As shown in Eq. (5), the values of β and B were determined by plotting $\ln(q)$ versus $\ln(t)$ on a straight line.

It is recognised that the IMT factor is the difference between the GMT and EMT factors; therefore, it can be and can be expressed by Eq. (8) (Fulazzaky et al., 2013),

$$[k_La]_d = [k_La]_g - [k_La]_f \quad (8)$$

where $[k_La]_d$ is the IMT factor (1/h).

The time-dependent variations of $[k_La]_d$ can be determined using Eq. (8), while the time-dependent variations of $[k_La]_g$ and $[k_La]_f$ were determined using Eqs. (6) and (7), respectively.

3. Results and discussion

3.1. Biomass characteristics

3.1.1. Biogranules' sizes

The inoculating biomass had a loose and irregular morphology. Before adding the *Serratia marcescens* SA30 strain to the biogranular system, no biogranules were observed. After the addition of the *Serratia marcescens* SA30 strain, small biogranules were formed, and their diameters gradually increased over the entire development period. The diameter range of the biogranules observed in this study was between 2.09 and 2.19 mm, similar to those cultivated with synthetic petroleum wastewater (Wang et al., 2022a), synthetic saline refinery wastewater (Ghosh and Chakraborty, 2021) and synthetic hypersaline wastewater (Sarvajith and Nancharaiah, 2020).

However, the size was bigger as compared to biogranules fed with synthetic oil refinery wastewater (Wang et al., 2022b), and synthetic petroleum wastewater (Chen et al., 2019). The main reason for the relatively bigger size could be due to the high organic content in the real AD-POME (OLR ranging from 0.69 to 9.90 kg/m^{3}d). The biogranules' sizes decreased with increasing oil concentration in real/synthetic wastewater. This indicated that the degradation of O&G was somewhat inhibited because some components in oily wastewater reduced the activity of microorganisms. Ghosh and Chakraborty (2019) reported that increasing diesel concentration caused partial biogranules rapture, and bigger biogranules formed due to lower HRT and the type of oily wastewater used.

3.1.2. Biomass profile

The development of biomass during the biogranulation process is presented in Fig. 2. As observed, the profile of the biomass concentration fluctuated during the first 176 days, believed to be caused by the high concentration of O&G in real AD-POME, which ranged from 3100 to 5800 mg/L. This was possibly due to the formation of a lipid coating around the biological flocs, resulting in the biogranules being unable to form (Cammarota and Freire, 2006). In addition, the development of filamentous bacteria and floating flocs with undesirable physical properties increased the washout of slow-settling biomass. Ahmad et al. (2020) reported that poor activity due to excessive O&G in the wastewater inhibits sedimentation and results in biomass losses through the reactor's outflow. Several studies report a similar phenomenon in a laboratory-scale reactor (Song et al., 2022; Ganidi et al., 2009; Liu et al., 2004).

Throughout the first 8 days of operation, the MLSS in the reactor increased from 7660 to 8700 mg/L. However, most of the biomass was washed out in 15 days, causing the biomass concentration to decline to

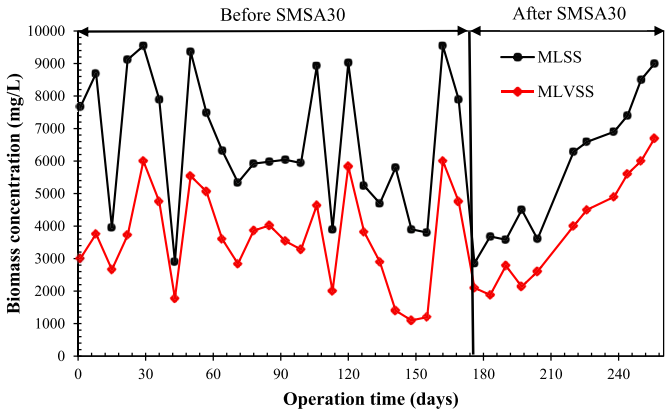

Fig. 2. The biomass concentration in the reactor.

3960 mg/L. This was mainly due to the short settling time of 5 min and the flotation of sludge generated by the foaming condition associated with real AD-POME. The biomass concentration fluctuated in the following weeks until the 176th day in the 2860 to 9540 mg/L range. To overcome the problem caused by the high concentration of O&G, 25 mL of the *Serratia marcescens* SA30 strain was added every week (for four weeks, totalling 100 mL) into the reactor, as mentioned earlier. As the *Serratia marcescens* SA30 strain has high biosurfactant production and bacterial adherence to the hydrocarbon (Affandi et al., 2014), the strain was added to degrade the O&G in the real AD-POME.

As shown in Fig. 2, after the addition of the *Serratia marcescens* SA30 strain, the biomass started to increase gradually. The biomass concentration in the reactor slightly fluctuated in the following weeks until the 204th day in the range of 2860–4500 mg/L, apparently due to the *Serratia marcescens* SA30 strain acclimatising with the wastewater. Over the subsequent nine weeks, the biomass flocs transformed gradually into biogranules. The small biogranules proliferated in the following weeks while more floc-like sludge washed out and biogranules accumulated. With the gradual granulation of the sludge, the biomass accumulation eventually took place, and the MLSS increased to 6280 mg/L after 220 days.

Most floc biomass was washed out, and visible biogranules increasingly appeared in the following days. Increased particle size improved the settleability of the sludge, preventing the biogranules from being washed out in the effluent. Biogranules that had reached maturity were maintained in the system, and an MLSS concentration of approximately 9000 mg/L was obtained at the end of the experiment. The MLVSS/MLSS ratio increased significantly during the granulation process from 0.39 to 0.74. This agrees with the MLVSS/MLSS ratio values for biogranules reported by Rosman et al. (2014) and Thi et al. (2018), which were between 0.60 and 0.85 and 0.75 and 0.80, respectively.

3.1.3. Strength of biogranules

Fig. 3 shows the strength enhancement of biogranules during the development period shown by IC. Typically, a lower value of IC indicates a more excellent stability of biogranules (Chen et al., 2021). The IC was reduced as the biogranules were developed. Before the addition of the *Serratia marcescens* SA30 strain in the biogranular system, the values of IC ranged from 98.4 to 92.3, implying that the high concentration of O&G had a powerfully negative effect on the strength of biogranules with the growth of fast-growing filamentous bacteria. During this time, no biogranules were observed in the biogranular system, as mentioned previously.

After the addition of the *Serratia marcescens* SA30 strain, the IC reduced slowly as the biogranules started to develop. As shown in Fig. 3, within 12 days after the addition of the *Serratia marcescens* SA30 strain, the strength of the biogranules obtained was stable. High strength of biogranules was indicated by the smaller IC of biogranules, and stronger

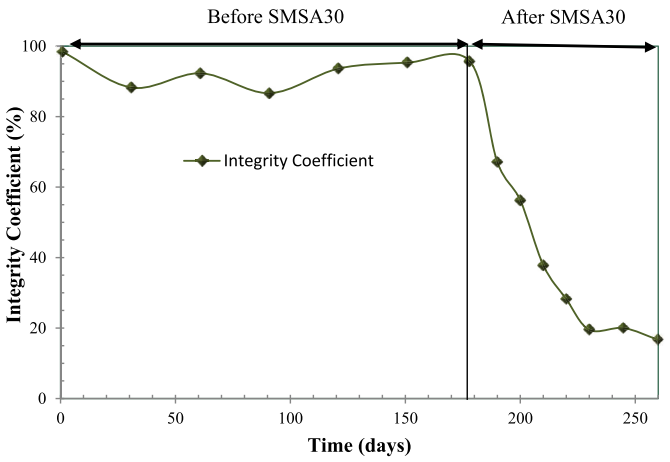

Fig. 3. The profile of IC represents the strength of the biogranules.

bonds were formed within the biogranules (Ghosh and Chakraborty, 2020; Ghangrekar et al., 2005). However, its strength improved predicated by time, as shown by the reduction of IC value from 67.1 to 37.8% after 210 days. After that, the biogranules strength slightly increased, and finally, the IC value reached 16.7% on the last day of the study. Similar results demonstrating a low value of IC signify the effectiveness of using single strains of *Brevibacterium paucivorans* and *Staphylococcus hominis* biogranules to achieve faster, more robust, and more stable biogranules while treating oily wastewater (Ghosh and Chakraborty, 2021).

3.1.4. Settling profile

The SVI is an important parameter to evaluate the settling ability of the biomass. Fig. 4 depicts the biomass SVI profile over the study's entire duration. Before adding the *Serratia marcescens* SA30 strain, the biomass's SVI was unstable and fluctuated. During this phase, the SVI increased from 80.9 mL/g to 127 mL/g in the first 15 days before decreasing to 50.9 mL/g on day 22. Subsequently, the SVI rocketed from 46.1 to 216.7 mL/g due to the proliferation of filamentous organisms from day 36–43 and on several other days as well, as indicated by the circles in the figure. The proliferation of filamentous microorganisms has been studied by numerous researchers in biogranules with various types of influxes and varying operational conditions (Adetunji and Olaniran, 2021; Meunier et al., 2016; Muszyński and Miłobędzka, 2015; Li et al., 2014; Liu and Liu, 2006). Muszyński and Miłobędzka (2015) reported on the deterioration of the settling properties in an acetate-fed biogranular system, caused by the growth of filamentous bacteria when the ratio of COD to TP was increased to 100:1. While the presence of low

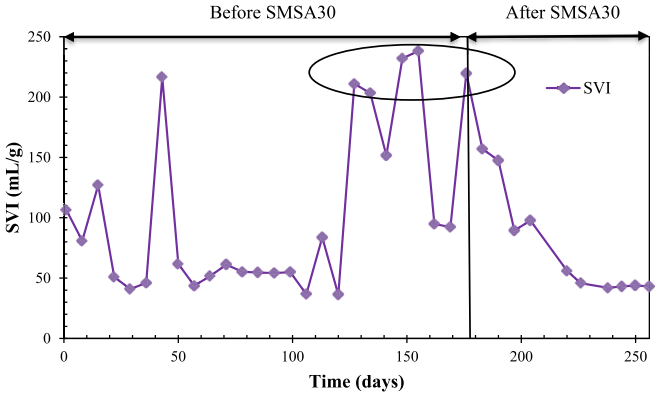

Fig. 4. Profile of the biogranular system sludge SVI during the operation period.

or moderate level of filamentous bacteria in biogranules served as a backbone of biogranules (Liu and Tay, 2004), their outgrowth caused a poor settling ability and subsequent sludge washout, as indicated by a significant drop in the biomass concentration, reported by Liu and Liu (2006) and Meunier et al. (2016).

After the addition of the *Serratia marcescens* SA30 strain, the SVI showed a progressively downward trend over time and was stabilised at 46 mL/g on the 226th day. A minimum SVI of 43 mL/g was obtained at the end of the study. This agrees with the SVI values (in the range of 8–45 mL/g) obtained by the biogranulation process in treating oily wastewater, as reported in previous studies (Ghosh and Chakraborty, 2019, 2021; Sarvajith and Nancharaiah, 2020) as shown in Table 1. The SVI value obtained in the present work on real AD-POME after the addition of the *Serratia marcescens* SA30 strain is comparable with other research works on oily wastewater treatment using biogranulation technology.

At the end of the study (after the addition of the *Serratia marcescens* SA30 strain), the SV of the biogranules obtained in this study was 81 m/h. This value is almost similar to the SV of biogranules reported by Corsino et al. (2015) but is higher than those reported by Sarvajith and Nancharaiah (2020) and Ghosh and Chakraborty (2021).

Settling velocity is related to the size and structure of biogranules (Tomar and Chakraborty, 2020). This might be one of the possible reasons for getting a higher SV value after the addition of the *Serratia marcescens* SA30 strain in the biogranular system as a result of the considerable size of the biogranules and better structure prior to the addition of the *Serratia marcescens* SA30 strain. This study's shear force imposed on developing biogranules regarding superficial air velocity (SAV) was 2.0 cm/s. High SAV is required in this study as compared to other studies (Han et al., 2022b) for the purpose of detaching filamentous bacteria, thereby improving both the density and stability of biogranules (Beun et al., 1999).

According to the findings, an increase in the size of biogranules causes an increase in the SVI value, resulting in a lower density and poor settling ability of the biogranules (Dahalan et al., 2015; Gobi and Vadivelu, 2015). Therefore, stable biogranules depend on multiple parameters, which select slow-growing microorganisms and wash out fast-growing microorganisms. Earlier studies have demonstrated that a high SAV is necessary for the formation and stability of biogranules. However, although the formation of biogranules is possible even at low SAV, other parameters are equally crucial in the formation of biogranules.

The complexity, toxicity, and non-biodegradable nature of oily wastewater can result in the slower growth of microorganisms, which inhibits biogranulation technology. In addition, some dispersed oil may cover the surface of the biogranules, preventing oxygen and nutrient utilisation, thereby causing microbial autolysis and death, and the disruption of biogranules. In the present treatment study for real AD-POME, the biogranules are believed to have been formed 80 days after the addition of the *Serratia marcescens* SA30 strain into the biogranular system and had an average size of 2.09–2.19 mm. These findings show the suitability of biogranulation technology for oily wastewater treatment, provided appropriate microbial strain is added.

3.2. Oil and grease removal

Fig. 5 shows the removal efficiencies of O&G in the biogranular system before and after the addition of the *Serratia marcescens* SA30 strain from the beginning until the end of the experiment. Throughout the experiment, the concentration of anaerobically digested O&G ranged from 3100 to 5800 mg/L with an average of 4565 ± 629 mg/L. The removal performance was low before the addition of the *Serratia marcescens* SA30 strain, ranging from 2 to 9%, with an average of 6% ± 3%.

After the addition of the *Serratia marcescens* SA30 strain, the removal efficiencies of O&G significantly increased, ranging between 78.7 and

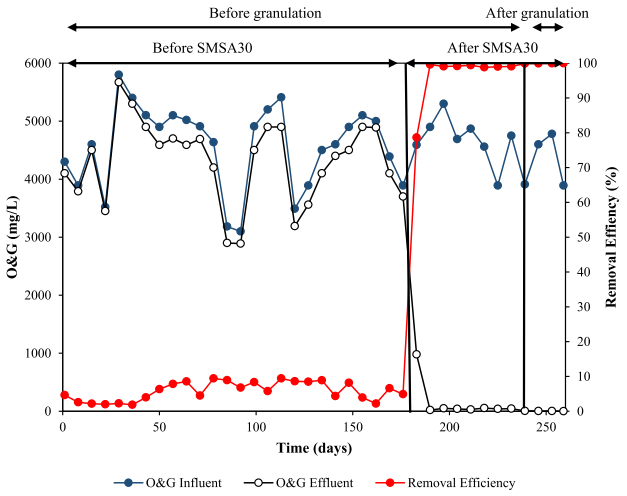

Fig. 5. Variations of O&G and removal efficiencies according to time for the experiments to monitor the influent and effluent of O&G concentrations and O&G removal efficiencies.

99.6% (average of 96.6% ± 6.8) at the initial stage of development of the biogranules. After 60 days of the *Serratia marcescens* SA30 strain addition, upon the biogranules formation, the removal efficiencies of O&G increased, reaching up to 99.9%. The bioaugmentation process improved the oil degradation and thus, accelerated the biogranulation process.

The performance of bioaugmented biogranules in treating O&G with the addition of SMSA 30 is preferable to other biological processes, such as microbial fuel cells (MFCs) (Abu-Reesh et al., 2022; Mohanakrishna et al., 2020). The results are also similar to those reported by Prasad and Manjunath (2011). They reported lipase-producing bacteria such *as Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens, Serratia marcescens, Pseudomonas aeruginosa,* and *Staphylococcus aureus* are potential agents for lipid degradation from O&G-containing wastewaters derived directly from palm oil mill, dairy, slaughterhouse, and soap industries. In addition to the enhancement of O&G removal that complied with the Department of Environment Regulation (50 mg/L, Lokman et al., 2021) for standard discharge limits of POME were established, the *Serratia marcescens* SA30 strain also minimised the foaming and scum formation problems in the biogranular system. Affandi et al. (2014) reported that the *Serratia marcescens* SA30 strain has the highest biosurfactant production and bacterial adherence to hydrocarbon values and showed a maximum O&G degradation ability of about 91%.

Abdel-Shafy et al. (2014) also found that the O&G removal efficiency was increased with the addition of effective microorganisms (EM) from 63.5% to 97.6%, respectively, to treat greywater in a hybrid integrated system. Bioaugmentation of desirable and selected or mixed cultures into a bioreactor can degrade and break down large molecules of O&G by secretion of the lipases and esterases enzymes and convert O&G into harmless products by secretion of appropriate metabolites (Adetunji and Olaniran, 2021; Nzila et al., 2017; Alkhatib et al., 2015). Consequently, the microorganisms and lipolytic enzymes secreted from them can significantly influence O&G removal efficiency from real AD-POME. The availability of O&G in the wastewater resulted in the formation of lipid coating surrounding biological flocs (Cammarota and Freire, 2006). Therefore, after adding selected bacteria to degrade O&G, it could be more efficient in the attached growth phase (Nzila et al., 2017).

3.3. Kinetic study

3.3.1. Linear regression analysis of O&G

In order to better understand the mechanism and kinetic mass transfer step in the overall biosorption process, the regression analysis was conducted for the experimental data of O&G by the MMTF model. Fig. S2 (supplementary material) shows the typical plots of MMTF model expressions before and after adding the *Serratia marcescens* SA30 strain into the bioreactor for the O&G biosorption. The values of $1/\beta$ and B are derived from the slope, and the y-intercept of the plot of ln (q), respectively ln (t), and the correlation coefficients are provided in Table 3. A straight-line graph with a high correlation coefficient ($R^2 >$ 0.99) validated the linear regression analysis, indicating an excellent correlation using the experimental findings. The experimental results showed that the MMTF model perfectly linked the experimental data and reasonably described the biosorption of biogranules to oil degradation.

The values of B and β could be helpful to describe the mass transfer kinetics for the biosorption of O&G from real AD-POME before and after the addition of the *Serratia marcescens* SA30 strain to influence the formation of biogranules. Biosorption of O&G by biogranules depends on the characteristics of O&G passing through three successive points of extracellular accumulation outside the biomass, cell surface-sorption at the interfacial water-biomass interface, and intracellular accumulation located within the biomass (Gaur et al., 2014). Thus, various environmental conditions before or after the addition of the *Serratia marcescens* SA30 strain in the bioreactor can influence the mass transfer kinetics of O&G during the formation of biogranules and the O&G removal performance. Before the addition of the *Serratia marcescens* SA30 strain, a high O&G concentration in real AD-POME increased the driving force for O&G biosorption and the formation of lipid coat on the surface of the sludge, which inhibited the formation of biogranules.

Experimental data verification demonstrates that the value of$B$ declined from 2.234 g h/mg to 0.804 g h/mg before and after the addition of *Serratia marcescens* SA30, respectively. Likewise, the value of βalso declined from 0.563 g h/mg to −1.978 g h/mg after the addition of *Serratia marcescens* SA30 in the bioreactor. The reduction in the β and B values after adding the *Serratia marcescens* SA30 strain is due to the degradation of the O&G. After removing the O&G from real AD-POME, the negatively charged microbial cells were able to bind with metal ions to form microbial nuclei, enhance the sludge's flocculation, and promote granulation. According to these results, the high removal of O&G in the real AD-POME improved the biogranulation process in the bioreactor.

3.3.2. Global, external and internal kinetics of mass transfer

The numerical simulation of the MMTF model enables the integration of information regarding the kinetics of global, external and internal mass transfer within biogranules Fig. 6 shows the $[k_La]$ versus time before and after the addition of the *Serratia marcescens* SA30 strain, respectively. This provides the conformational variations of $[k_La]_g$, $[k_La]_f$, and $[k_La]_d$ based on the time the experiment was conducted. The various values of OLRs are directly related to mass transfer and kinetic rate processes. The trend in the value of $[k_La]_d$, $[k_La]_f$, and $[k_La]_g$ can be used to evaluate the mass transfer kinetics of GMT, EMT, and IMT for the biosorption of O&G by biogranules before and after the addition of the *Serratia marcescens* SA30 strain in the bioreactor.

A zigzag pattern of $[k_La]_g$ and $[k_La]_d$ values are remarkably similar and stand very high as compared to the values of $[k_La]_f$. The factor influencing a zigzag pattern is the concentration of real AD-POME entering the bioreactor. According to empirical evidence, the values of $[k_La]_f$ along the curves in Fig. 6 are negligible compared to those of $[k_La]_d$, leading to a conclusion that the resistance mass transfer for O&G

Table 3
The values of β and B derived from the slope and interception, respectively, of plotting ln (q) and ln (t) for two experimental conditions.

Experimental circumstance	β (g h/mg)	B (mg/g)	R^2
Before *Serratia marcescens* SA30	0.5628	2.2342	0.9818
After *Serratia marcescens* SA30	−1.9781	0.8041	0.9931

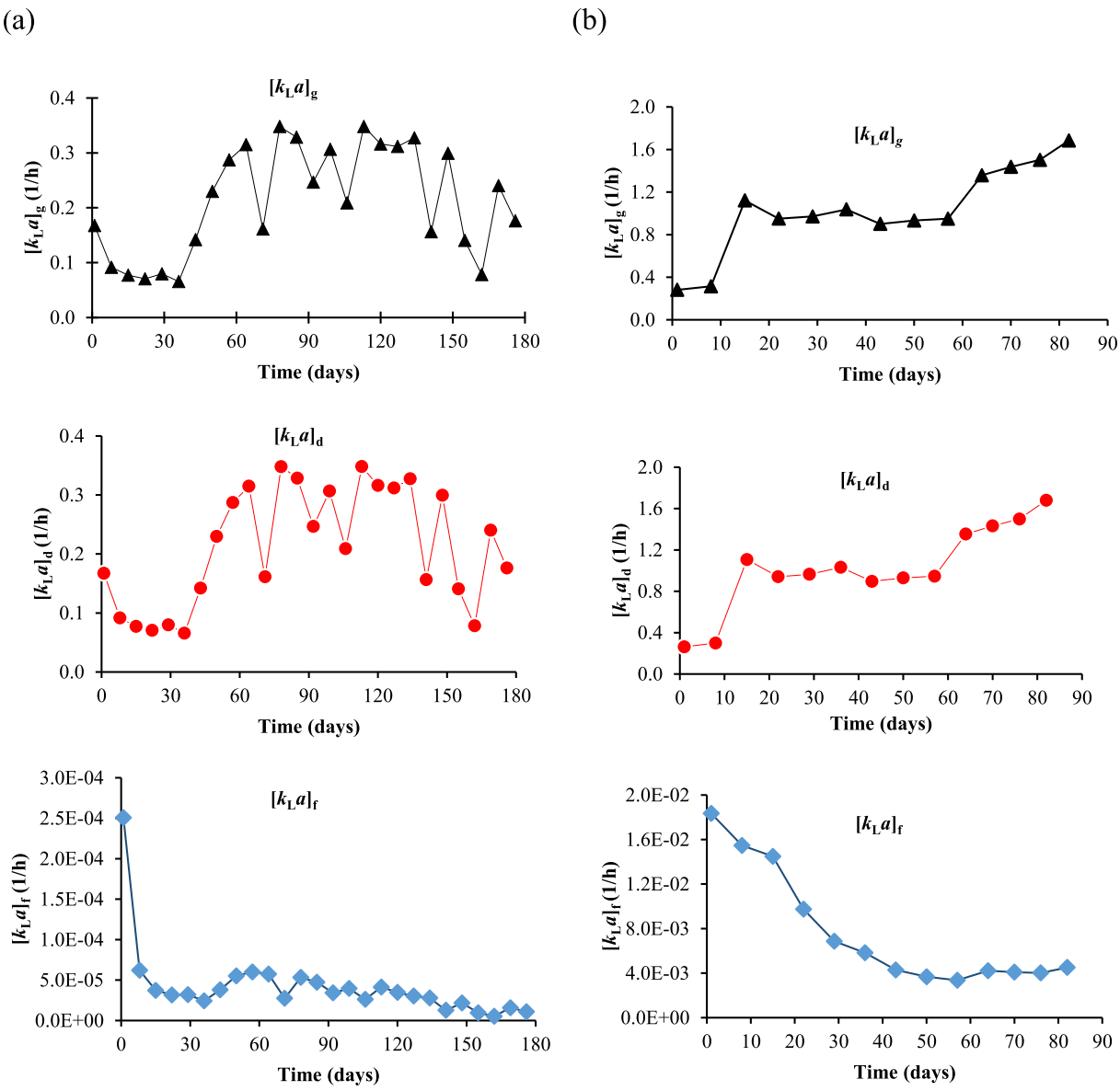

Fig. 6. Curve of plotting $[k_La]$ versus time for the experiments (a) before the addition of *Serratia marcescens* SA30 and (b) after the addition of *Serratia marcescens* SA30 where $[k_La]_g$ represents global mass transfer, $[k_La]_d$ represents internal mass transfer, and $[k_La]_f$ represents external mass transfer.

biosorption depends on extracellular precipitation on the surface of biogranules and is controlled by EMT. Mass transfer resistance before a breakthrough depends on the film mass transfer, as transporting solute molecules from the bulk of liquid to the film zone is tricky.

As shown in Fig. 6a, the values of $[k_La]_d$ before the addition of the *Serratia marcescens* SA30 strain are in the range of 0.066–0.348/h with an average of 0.212 ± 0.01/h. These are much lower than those after the addition of *Serratia marcescens* SA30 (Fig. 6b), which range from 0.263 to 1.680/h (average 1.026 ± 0.42/h). The exponential growth of the *Serratia marcescens* SA30 strain and emulsification on the biogranules enhanced the mass transfer driving force. It reduced the resistance of the transport of O&G molecules to intracellular accumulation at the cell surface.

The structure of the biomass before the addition of the *Serratia marcescens* SA30 strain was loose with the presence of many holes. According to Song et al. (2022), these holes may significantly affect the transportation of substrate, oxygen, and metabolic products into and leaving the biomass. The holes in the biogranules may reduce their strength; regrettably, the biogranules' compact structure can ensure their stability during long-term operation (We et al., 2021). The growth of dense and compact biomass after the addition of the *Serratia marcescens* SA30 strain enhanced the transportation process. Various metabolic processes could coincide within the biomass because the biogranules are densely compacted with heterotrophic and autotrophic bacteria (Cydzik-Kwiatkowska, 2015). Furthermore, as reported by Affandi et al. (2014), the biosurfactant activity caused by *Serratia marcescens* SA30 produced a greater hydrophobic water-insoluble substrate surface area, thus, enhancing the overall O&G degradation and the bioavailability of hydrophobic compounds. This remarkably increased the kinetic rate of IMT.

4. Conclusions

This research demonstrates the successful development of biogranules in a laboratory-scale bioreactor for treating real AD-POME by bioaugmentation with the O&G-degrading *Serratia marcescens* SA30 strain. Subsequently, mature biogranules with a compact structure, diameter size in the 2.09–2.19 mm range, excellent settling velocity of 81.0 m/h, and an average SVI value of 43.0 mL/g were achieved at the end of the research. The average removal efficiencies of O&G increased

after the addition of the *Serratia marcescens* SA30 strain from 6 to 99.66%. Kinetic data showed that the values of $[k_La]_g$ and $[k_La]_d$ are very similar and significantly greater than those of the values of $[k_La]_f$, presumably as a result of the diverse characteristics of real AD-POME entering the bioreactor. The results of the MMTF model show that the O&G biosorption by biogranules in the biogranular system is controlled by the resistance of EMT. The findings provide helpful information for using biogranules to treat oily wastewater.

Credit author statement

Maria Nuid: Writing-Original draft preparation, Visualization, Investigation.**Azmi Aris:** Supervision, Writing-Reviewing and Editing, Funding Acquisition.**Shakila Abdullah:** Resources. **Mohamad Ali Fulazzaky:** Validation, Formal Analysis. **Khalida Muda:** Conceptualization, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Azmi Aris reports financial support was provided by Malaysia Ministry of Higher Education. Maria Nuid reports financial support was provided by Universiti Technologi Malaysia.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118032.

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