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BIODEGRADATION OF OIL AND GREASE FROM AGRO-FOOD INDUSTRY BY IMMOBILISED *Serratia marcescens* SA30

(Biodegradasi Minyak dan Gris dari Industri Agro-Makanan oleh *Serratia marcescens* SA30 yang Dipegunkan)

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Abstract

The agro-food industrial wastewater (AFIW) contains high concentrations of oils and grease (O&G), which are significant threats to aquatic environments. In the context of the removal of contaminants from wastewater, the capability of *Serratia marcescens* SA30 immobilized in a packed-bed column reactor (PBCR) of O&G removal from AFIW needs to be verified. This study analyses the *Serratia marcescens* SA30 immobilized on oil palm frond (OPF) in PBCR in order to elucidate its removal ability of O&G from AFIW. The physicochemical parameters of the AFIW samples collected from the agro-food industry were analyzed according to Standard Methods for Examination of Water and Wastewater. The PBCR treatment system was set up using immobilized *Serratia marcescens* SA30 onto OPF for the removal of O&G from AFIW. The AFIW samples were collected at the inlet and outlet of the PBCR, and the respective concentrations of O&G were determined. These values assert that the parameters does not comply the production limit set in Environmental Environment Quality B (Industrial Effluent Regulations, 2009). The performance of the PBCR realized 100% efficiency, with the population ranging from 10^8 – 10^7 with the immobilized *Serratia marcescens* SA30 acting as a biosurfactant-producing bacteria, which was achieved by experiments ran at a volumetric flow rate of 3 mL/min during treatment using concentrations of O&G at 100% v/v after 144 hours operation in the PBCR. The data obtained would provide a green and sustainable pathway for the removal of O&G from water.

Keywords: Agro-food industrial wastewater, packed-bed column reactor, *Serratia marcescens* SA30, oil and grease, immobilized

Abstrak

Sisa air perindustrian agro-makanan (AFIW) mengandungi kepekatan minyak dan gris (O&G) yang boleh memberikan ancaman kepada persekitaran akuatik. Dalam konteks penyingkiran bahan bukan organik dan organik dari air sisa, keupayaan *Serratia marcescens* SA30 yang dipegunkan dalam reaktor turus terpadat tunggal (PBCR) perlu disahkan. Kajian ini menganalisis *Serratia marcescens* SA30 yang dipegunkan di pelepah kelapa sawit (OPF) dalam PBCR untuk menjelaskan kemampuan penyingkiran O&G dari AFIW. Parameter fizikokimia sampel AFIW yang diambil dari industri agro-makanan dianalisis berdasarkan Kaedah Piawai untuk Pemeriksaan Air dan Air Sisa. Sistem rawatan PBCR dibentuk menggunakan *Serratia marcescens* SA30 yang dipegunkan ke OPF untuk penyingkiran O&G dari AFIW. Sampel AFIW dikumpulkan di saluran masuk dan keluar PBCR, dan

kepekatan O&G masing-masing ditentukan. Nilai-nilai tersebut menegaskan bahawa parameter tersebut tidak mencapai limit pengeluaran yang telah ditetapkan dalam Kualiti Persekitaran Lingkungan B (Peraturan Efluen Industri, 2009). Prestasi PBCR mencapai kecekapan 100% dengan populasi antara 10^8-10^7 dengan *Serratia marcescens* SA30 yang dipegunkan bertindak sebagai bakteria penghasil biosurfaktan, yang dicapai dengan eksperimen yang dijalankan pada kadar aliran volumetric 3 mL/min semasa rawatan menggunakan kepekatan O&G pada 100% v/v setelah 144 jam beroperasi dalam PBCR. Kajian ini akan memberikan laluan hijau dan lestari untuk menyingkirkan O&G dari air.

Kata kunci: Sisa air perindustrian agro-makanan, reaktor turus terpadat tunggal, *Serratia marcescens* SA30, minyak dan gris, dipegunkan

Introduction

Agro-food industries are one of the significant contributors to environmental pollution. The rapid expansion of small and medium industries (SMEs) in Malaysia, particularly in rural areas, led to water body pollution due to most of these industries discharging a large amount of untreated wastewater into the environment [1]. Factors such as O&G, chemical oxygen demands (COD)~~,~~ and total suspended solids (TSS) can be minimized *via* proper storage, cleaning, slicing, washing, frying, salting, and coating and packing [2]. However, the lack of prior treatment of wastewater results in pollution, which can be harmful to humans. The current physicochemical and biological processes for the removal of O&G from industrial wastewaters include electrocoagulation, advanced oxidation process, and membrane technologies [3, 4, 5]. However, the processes mentioned above are costly undertakings and result in byproducts, such as excessive gases and sludges. Due to increased environmental awareness and stringent public policies, it is becoming necessary to compel industries to limit their respective discharges [6].

In response to this, industries begin to utilize sustainable technologies for wastewater management. To improve the microbial remediation of an O&G the bacterial cells must be immobilized in a suitable carrier or used of biosurfactant as a biocatalyst has been investigated compared with the free cells in various biotechnological processes. The use of immobilized cells in biodegradation processes enhances the O&G removal rates and provides increased tolerance ability to unfavorable conditions [7]. Parthipan et al. [8] found that biosurfactant producing strain of *Bacillus subtilis* was able to degrade oil about 87% within a short period of time (7 days). A study by Shen et al. [9] elucidated the ability of immobilized microbial consortia in the degradation of different types of petroleum hydrocarbons. A recent study showed the importance of *Bacillus pumilus* isolated from *Chelidonium majus* L. exhibit potential for hydrocarbons degradation and biosurfactant production [10]. The success of biotechnological solutions for bioremediation applications depends on the identification of the indigenous microbial communities having the ability to degrade complex O&G and enhancing their biodegradation potential.

Therefore, the main objective of this present work was to elucidate the ability of immobilized *Serratia marcescens* SA30 onto OPF for the removal of O&G effluent from the agro-food processing industry in a column reactor. The optimal conditions for O&G degradation under the influence of immobilized *Serratia marcescens* SA30 onto OPF, such as inert supporting material, initial concentrations and times, and flow rates were established. The biodegradation potential was determined by evaluating the O&G removal rates and the presence of *Serratia marcescens* SA30 in the column reactor pre and post-treatment. This proposed approach could be an effective and environmentally-friendly biological treatment process that fulfills environmental regulations.

Materials and Methods

Characterization of agro-food industrial wastewater

The agro-food industrial wastewater (AFIW) samples were obtained from the final effluents discharged from an industrial food factory located in Batu Pahat, Johor. The AFIWs served as nutrients to the bacteria. The AFIWs' pH, microbiological count, temperature, color,

chemical oxygen demand (COD), and oil and grease (O&G) concentrations were determined. The samplings and characterizations were performed as per the Standard Methods for Examination of Water and Wastewater [11].

Bacteria

Serratia marcescens SA30 was isolated from the collected AFIW [12]. *Serratia marcescens* SA30 was cultivated in an NB at 30 ºC and 200 rpm for 24 hours. The strain was identified using the PCR-mediated amplification of the 16S rRNA gene sequencing analysis performed by Vivantis (M) Sdn. Bhd. The genes that were 100% similar to *Serratia marcescens* were obtained from the 1398 nucleotide sequence. The nucleotide sequence was deposited into the GenBank and marked with an accession number KF686740. A strain of *Serratia marcescens* SA30 is capable of producing biosurfactants [13].

Laboratory-scale column reactor

The experimental setup for the O&G biodegradation system consists of a tank, peristaltic pump, and a column (reactor), and is shown in Figure 1. The acrylic column reactor has a capacity of 5 L, and is 50 cm in height and has an inner diameter of (i.d) 4.6 cm. An acrylic column, divided into four sections measuring 2 cm each were filled with inert stones with a granular size of ~1-2 cm at the top and the bottom of the column. The stones were utilized to ensure excellent flow distribution within the column while retaining the column's content. The inert stones were soaked in pure water for ~24 hours to remove impurities, then rinsed with distilled water and dried in an incubator (Memmert IN 110) for 24 hours. 35 cm of the column was filled with 90g of inert OPF, while 1-2 cm length range was used as inert supporting materials for bacterial cell immobilization during treatment. A headspace of 0.46 L was retained to ensure the consistent availability of oxygen supply.

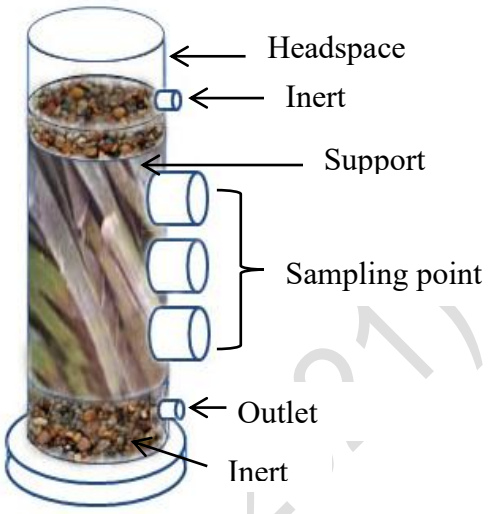

Figure 1. Schematic diagram of laboratory packed-bed column of degradation of oil and grease

Immobilization of *Serratia marcescens* SA30 onto oil palm frond

Serratia marcescens SA30 was used during the immobilization of cells onto OPF packed within the column. OPF was obtained from the oil palm tree in Kulai, Johor, Malaysia, and selected using the coning and quartering method. Fresh OPF was cut into irregular lengths of ~1-2 cm. The OPF was rinsed with deionized water at a constant flow rate of ~0.18L h^{-1} to prevent clogging of the column and allow for the electrostatic charge and hydrophobic interactions necessary for the bacterial adhesion to the supporting material. The *Serratia marcescens* SA30 was pumped at a continuous circulation at similar flow rates to allow initial bacterial attachment for 3 days, followed by 5 L of NB and AFIW to ensure the bacteria used the NB and AFIW as its sole carbon source.

Determination of biodegradation

The microbial degradation of O&G was performed in a column reactor. The samples were analyzed daily for 32 days. To optimize for immobilization and the degradation of O&G, the effect of initial concentrations (25%, 50%, 75%, and 100% (v/v)) and flow rates (1, 3, and 5 mL/min) were determined. At the end of the experiment, the O&G concentration and cell concentration (CFU/mL) were obtained.

Analytical method

The O&G was measured using the O&G determination method APHA 5520B (APHA, 2005) with n-hexane as its oil extraction solvent. The O&G content was determined for each sample pre and post-experiment. The experimental results of the analyses were expressed as mean ± standard deviation from the duplicate. The pH was determined using a pH meter (Eu-Tech), while the microbiological count was determined using the dislodging method via the spread-plate technique. ~1 g of OPF-attached bacteria samples were placed in a Bijou bottle containing 10 mL of sterilized DI and vortexed for 1 minute at high speed. For the quantification of bacteria, an aliquot (1 mL) of the suspension was diluted and spread onto the NA plates. The NA plates were then incubated at 30 ^{o}C for 24 hours in the incubator (Memmert IN110). The actual number of bacteria in the sample were then calculated and enumerated.

Results and Discussion

Characteristics of agro-food industrial wastewater

The results from the characterization of AFIW as shown in Table 1. Most of the parameters did not comply with the regulated discharge limit outlined in Standard B Environmental Quality (Industrial Effluent) Regulation 2009 [14], except for temperature and pH. The presence of high levels of O&G and other contaminants in the AFIW were assumed to be dependent on a series of industrial processes encompassing raw material storing, cleaning, shelling, slicing, washing, frying, salting, picking, coating, and packing due to the runoff water from cleaning of equipment or from fruit-washing, where trace concentrations of contaminants could leach from soil particles [12]. The wastewater was acidic due to the presence of fatty acids in the AFIW. The presence of 10 bacterial colonies indicated the possibility of biosurfactant producing bacteria that used the fatty acid in AFIW as nutrients for bacterial survival and growth [15].

Effect of concentration and time

The amount of O&G degradation at different concentrations onto OPF is shown in Figure 2. It can be seen that the O&G removal rate increases after 144 hours. The quickest O&G removal is observed at higher-level concentrations of 100% (v/v) AFIW, with complete removal being realized, followed by 75% (v/v), at 98% O&G removal. However, when decreasing the O&G concentrations from 50% (v/v) to 25% (v/v), the reactors demonstrated similar removal rate performance of complete degradation, corresponding to values of ~78% and ~72%, respectively. The degradation efficiency of O&G by immobilized cells increases with increasing O&G concentrations, even at a high O&G concentration of AFIW due to the growth of the biomass attached to a support material, rendering it possible for the O&G to be adsorbed on it while functioning as a protective shield against the toxicity of the O&G [16, 17]. Higher concentrations of AFIW encouraged the formation of biofilm while preventing the toxic effects of O&G towards the bacterial cells, which is due to the availability of reaction sites around or within the surface of *Serratia marcescens* SA30. This can be explained by the fact that higher concentrations result in faster transport prompted by the increased mass transfer coefficient of adsorption [18]. The rate of O&G can dictate the rate of O&G uptake transported from the exterior to the interior sites of the adsorbent particles and via the membrane of *Serratia marcescens* SA30 post-adsorption.

The bacteria demonstrated a faster degradation performance due to the adaptation of the isolates to the AFIW. The O&G environment is excellent for the lipolytic microorganisms due to the unrecovered O&G being present in the effluent, which induces the enzymes and biosurfactants to degrade O&G [19]. The yield of the enzyme lipase and biosurfactant increased alongside the concentration, which saturated the uptake system, as observed by the absence of O&G and free fatty acids in the medium. On the other hand, the free fatty acid, released into the medium, supported growth, which is indicated by the absence of the FFA from the growth medium or treated oily wastewater [20].The initial pH of the immobilized cells increased alongside time and became alkaline due to the absorption of the fatty acid present in the AFIW via the alteration to the charges of bacterial cell membranes and pH of ~7-9. The pH also affects the metabolism of microorganisms and its corresponding acceleration, which results in improved biodegradation of O&G [21].

Effect of flow rate

The effect of flow rate on O&G adsorption by AFIE was determined by setting different flow rates on the inlet solution (1, 3, 5, 7, and 9 mL/min) shown in Figure 3, resulting in a >70% of the O&G being removed, with complete reduction achieved after 144 hours of reactor operations. It should be pointed out that a flow rate of 3 mL/min resulted in the effective removal of O&G (100%), followed by ~84% (5 mL/min), ~83% (1 mL/min), ~72% (9 mL/min), and ~70% (7 mL/min). The increases in flow rate significantly affected the removal of O&G due to poor mass transfer, diffusional limitations, and short residence time [22]. Shorter times indicated by the increase flow rates resulted in the complexity of the AFIW, which extended the time it takes to facilitate the uptake process of the complex nutrient into the immobilized cell. When the influent flow rate was increased from 5 to 9 mL/min, the adsorption capacities were lower due to the insufficient residence time of the solute in the column and diffusion of the solute into the pores of the adsorbent, which results in the solute leaving the column before equilibrium [23]. The sorption capacity was smaller when the flow rate was high. It was generally assumed that at high flow rates, the residence period of O&G in the column was transitory, which cannot lead to the equilibrium of the sorption process [24]. The dominating roles of the biodegradation of O&G by the microbes attached to the bio-layer were formed on the surface of the adsorbent, making it one of the factors constituting increased removal rates.

The presence of *Serratia marcescens* SA30 in different parts of the column

The initial bacteria attachment shows a high cell concentration of *Serratia marcescens* SA30 value of $2.95 \times 10^9 \pm 7.07 \times 10^7$ (OD600 = 1.833 ± 0.018), which refers to the growth of *Serratia marcescens* SA30 in a rich medium NB. The cell concentration of *Serratia marcescens* SA30 in the column containing OPF gradually decrease to $3.95 \times 10^8 \pm 4.07 \times 10^7$ (OD600 = 1.377 ± 0.037) after 72 hours of continuous immobilization process. These values show that the *Serratia marcescens* SA30 has been attached to the support materials post-immobilization due to the decrease in cell concentrations.

In this study, the population of *Serratia marcescens* SA30 declined to a certain extent in the community after 32 days of operations at different parts of the column (top, middle, bottom, and effluent), ranging from 10^8 – 10^7 throughout the degradation at different concentrations and flow rates was shown in Figure 4 and Figure 5 respectively. The number of *Serratia marcescens* SA30 on OPF significantly decreased alongside decreasing bacterial concentration in AFIW [25], which could be due to the accumulation of nutrients and the activity of the cells varying from point-to-point along the individual pore channels, which suggests that competitive carbon sources and the exhaustion of nutrients commonly forces bacteria to remain in stationary or death phases, making it easily degradable in the AFIW.

The analysis confirmed that bacterial diversity increased gradually as the reactor is operating. The PBCR was not sterile, which results in the breeding of various microorganisms that could utilize O&G as a carbon source [26]. External microorganisms from the air or the influent could also grow on the OPF in the PBCR. However, the isolated bacteria from viable cell counts revealed that *Serratia marcescens* SA30 was the predominant microorganism on OPF in PBCR throughout the entire operation due to the high levels of colonization in the top, middle, and bottom of the column. The dominant *Serratia marcescens* SA30 in the PBCR is likely to be initiated by the adaptation of the bacteria to its proliferation in the different parts of the column, and the inoculation of the *Serratia marcescens* SA30, which reduces the toxic effects of the AFIW [27]. *Serratia marcescens* SA30 demonstrated a comparative advantage when competing with other microorganisms for habitat due to the strain possessing the highest oil-degrading activity.

Table 1. Characteristics of food industrial wastewater and total bacteria isolated

Parameters	Unit	Values	Standard B
Temperature	°C	28.9 - 34.3	40
pH	-	4.99 - 5.69	5.5 - 9.0
COD	mg/L	8750 - 34000	200
O&G	mg/L	8312 - 128007	10
Color	mg/L	3000 - 17125	200
Total no of bacteria isolated	-	10	-

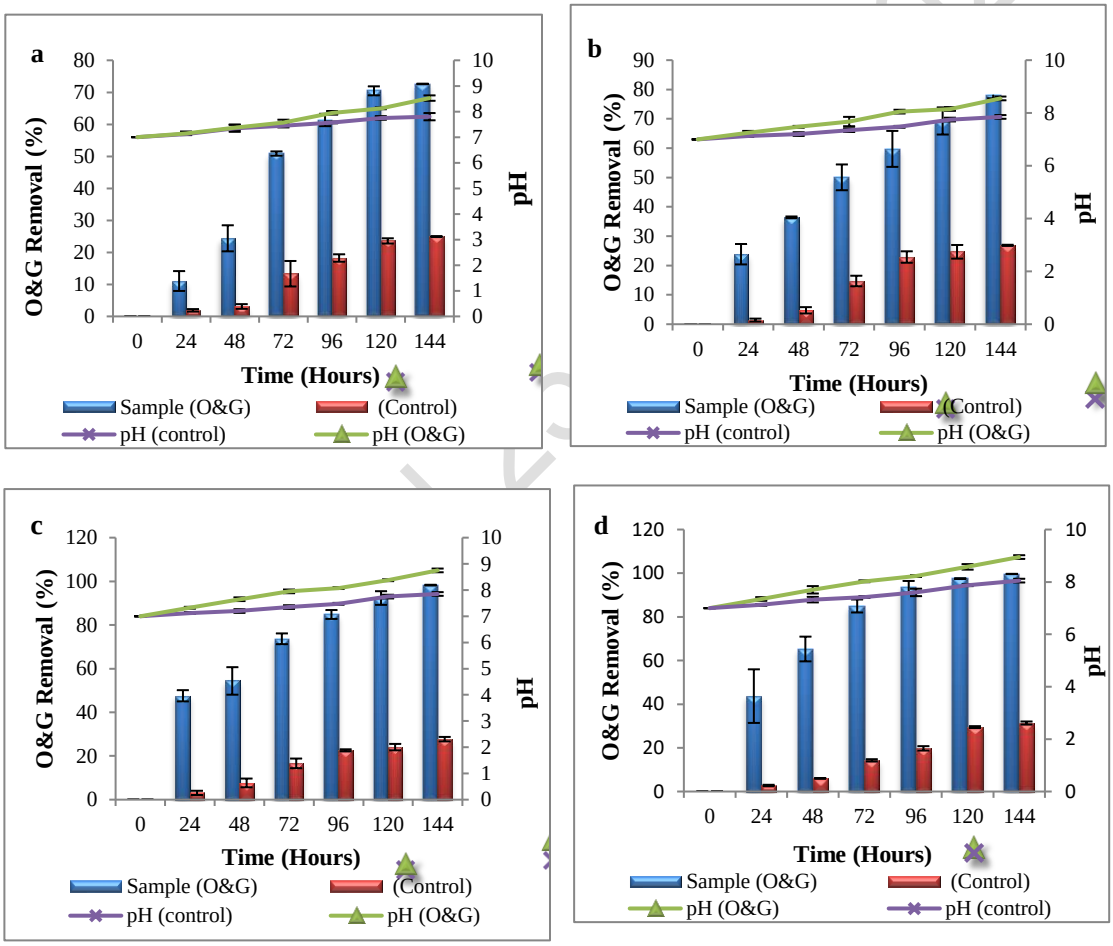

Figure 2. Percentage removal of O&G during biodegradation of AFIW by immobilized *Serratia marcescens* SA30 at different concentration and time intervals:(a) 25% (v/v) (b) 50% (v/v) (c) 75% (v/v) and (d) 100% (v/v)

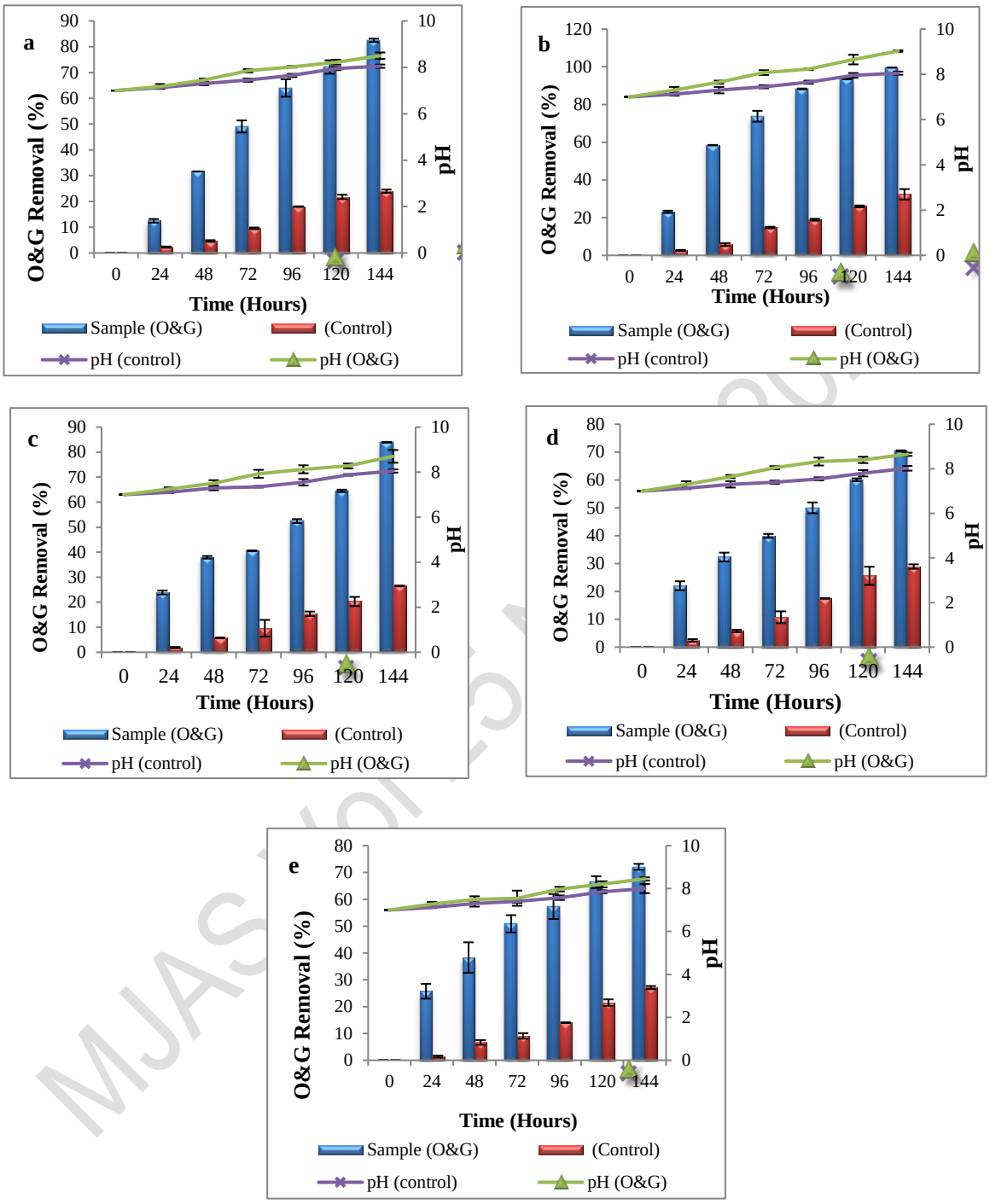

Figure 3. Percentage removal of O&G during biodegradation of AFIE by immobilized *Serratia marcescens* SA30 at different flow rate and time intervals: (a) 1mL/min (b) 3 mL/min (c) 5 mL/min (d) 7 mL/min and (e) 9 mL/m

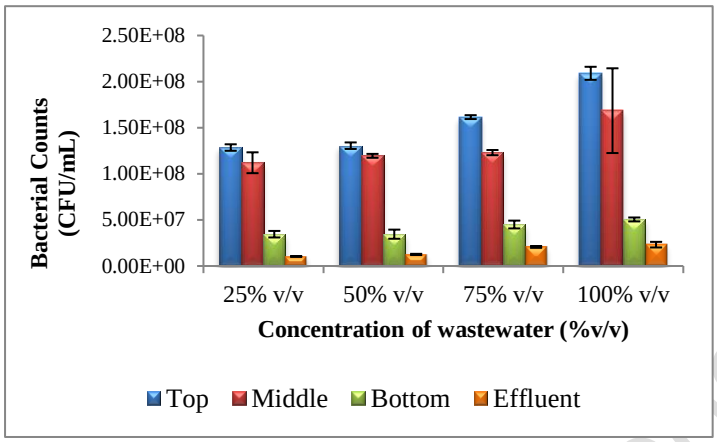

Figure 4. Distribution of *Serratia marcescens* SA30 cells at top, middle and bottom of the column (a) 25%, 50%, 75% and 100% (v/v)

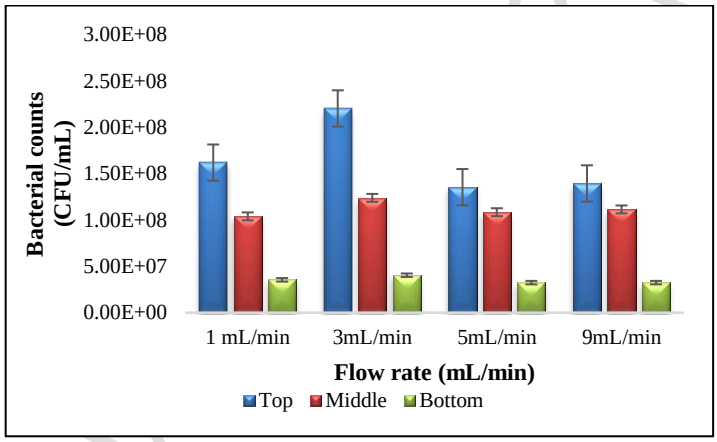

Figure 5. Distribution of *Serratia marcescens* SA30 cells at top, middle and bottom of the column 1, 3, 5 and 9 mL/min

Conclusion

This study assessed the capability of immobilized *Serratia marcescens* SA30 onto OPF to predict the optimum concentrations and flow rates for the removal of O&G from AFIW. It was found that the optimum operating of PBCR treated system realized 100% efficiency condition of 100% v/v concentration, a flow rate of 3 mL/min at a pH 7 after 144 hours operation. The use of *Serratia marcescens* SA30 strain immobilized onto the OPF removed O&G from AFIW, which contributes to the high efficiency of an environmentally-friendly method for treating wastewaters and advanced biotechnology studies.

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