Bioremediation of hydrocarbon-rich wastewater by aerobic granules of oil degrading bacterial strains in salinity influence

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ABSTRACT

Granulation of sewage and brewery sludge isolated oil degrader *Brevibacterium* and *Staphylococcus* were investigated in hydrocarbon-rich wastewater with 280 mg/L of hydrocarbon at 10-25 g/L NaCl salinity influence. *Brevibacterium* and *Staphylococcus* cultures were inoculated in aerobic granular reactors (AGRs) R1 and R2 which were operated with 24 h cycle time and 2 L/min air flow rate. Yellowish matured granules appeared within 15 days. R1 granules achieved stability till 15 g/L NaCl concentration but faced disintegration between 15-20 mg/L NaCl exposure which reduced granule size and hydrocarbon removal from 2.15-1.7 mm and 78-73%. R2 granules were more salt tolerant providing 2.5±0.5 mm granule size with 4±1 g/L volatile suspended solids (VSS) and 201±1 mg/g VSS extracellular polymeric substances (EPS) content achieving 81±0.7% hydrocarbon removal in 30 days. High granule stability and biomass concentration ensured less biomass washout from reactors. Granule settling velocity (GSV) in R1 and R2 reached 20±1 and 32±0.8 m/h which corresponded with granule size profiles. Kinetics analysis showed that at steady state, R1 and R2 were capable of 72 and 91% phenol removals in 30 and 24 h, respectively. Hence, the study provided salt tolerant oil degrading granules for refinery wastewater treatment.

Keywords: Aerobic granules, Freshwater microbes, Extracellular polymeric substances, Hydrocarbon removal, Saline wastewater.

1 Introduction

Aerobic granulation technology has become a popular bioremediation process for its microbial diversity, small footprint, compact granular structure and simultaneous C, N, P removal capacities (Zhang et al., 2016). Aerobic granulation has been used in treating several recalcitrant industrial wastewaters like phenolic wastewater (He et al., 2021), pulp and paper mill wastewater (Vashi et al., 2018), heavy metal contaminated wastewater (Wu et al., 2019), pharmaceutical wastewater (Amorim et al., 2018), petrochemical wastewater (Ghosh and Chakraborty, 2020) etc. by bio-adsorption of pollutants in granule EPS matrix or biodegradation of contaminants through several catabolic pathways in pollutant degrading microorganisms (Corsino et al., 2015).

Petrochemicals sectors are producers of large amount of oily wastewater containing diverse recalcitrant like oil, phenol, ammonia, heavy metals (like Fe, Pb, Cr etc.). Hydrocarbons are produced above 200 mg/L concentrations which are highly recalcitrant in nature and slowly biodegradable (Khaing et al., 2010). Crude oil distillation process is the major source of high salinity in petroleum refinery wastewater which is a barrier to achieve a stable biological treatment. Membrane separation, electrocoagulation, ion-exchange are the conventional methods for both oil and salinity removals from wastewaters (Jain et al., 2020). However, due to economical and environmental limitations biological processes are in research focus of the environment scientists.

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Aerobic granulation method was proven to be effective in oil and organics removal by changing initial sludge inoculum, AGR operational strategies or by employing co-substrates like glucose, sucrose. Petrochemical, coal gasification and saline petroleum slop wastewater was successfully treated in AGR systems achieving about 80-90% COD removal (Zhang et al., 2011; Milia et al., 2016; Campo and Bella, 2019). In saline wastewater treatment also microbial profiling of granules revealed the influence of halophilic bacteria in salinity stress resistance. Increasing salinity was the major cause to synthesize more EPS on granule surfaces to protect them from osmotic pressure. In the previous studies hydrocarbon load was very less (below 200 mg/L) in the oily wastewater (Chen et al., 2019; Campo and Bella, 2019). Moreover, studies on simultaneous salinity tolerance and oil removal in freshwater microbes are not well addressed.

Hence, the aim of this paper is to granulate sewage and brewery sludge originated *Brevibacterium paucivorans* and *Staphylococcus hominis* (Ghosh and Chakraborty, 2019) in synthetic refinery wastewater contaminated with phenol and oil, characterize granule size, VSS, stability and organics removal performances in a small scale AGR.

2 Material and methods

2.1 Source inoculum

About 5 g/L of overnight grown culture of *Brevibacterium* and *Staphylococcus* were inoculated in two reactors R1 and R2 for granule formation. The initial inoculum VSS and GSV values were 1.04 g/L and 3.5 m/h, respectively. EPS concentration for the inoculum culture ranged between 34-37 mg/g VSS.

2.2 Rector configuration and operating principles

Two number of 500 mL volume measuring cylinder were employed (R1, R2) as AGRs for granulation. Schematic of fabricated AGR is given in Figure 1. About 2 L/m of continuous aeration was provided by air-pumps and volume exchange ratio (VER) was maintained as 50%. AGRs were operated with 1 cycle per day. Initially 7 min settling time was provided which gradually reduced to 3 min after matured granulation. About 10 min of feeding time was maintained in the AGRs throughout the study. COD and hydrocarbon removal efficiency was measured by eqn.(1).

$$\text{Removal (\%)} = \frac{(S_0 - S_e)x100}{S_0} \quad (1)$$

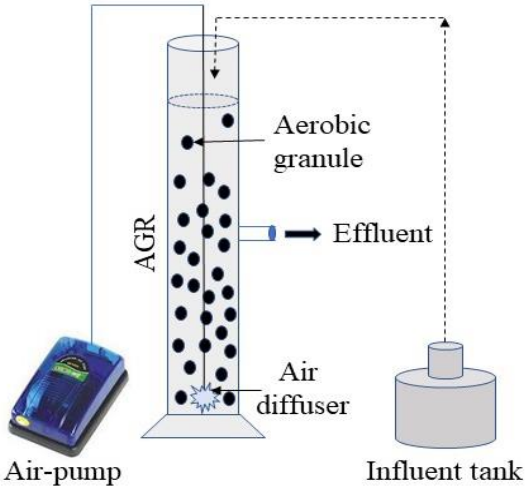

Figure 1. Schematic diagram of aerobic granular reactor (AGR)

2.3 Wastewater composition

A mixture of emulsified diesel (emulsifier: nonylphenol ethoxylate) and phenol were used as sole carbon source in the AGRs. In total 30 days of the study, 250 mg/L of diesel and 30 mg/L of phenol were fed to the AGRs which contributed to 1075 mg/L of influent COD concentration. About 180 mg/L NH_4Cl was used as nitrogen source of 50 mg/L of NH_4^+-N. Phosphate buffer (KH_2PO_4+K_2HPO_4) maintained reactor pH at 7±0.2. Trace metal solutions of 7000 mg/L $MgSO_4.7H_2O$, 10,000 mg/L $CaCl_2.2H_2O$, 7000 mg/L $FeCl_3.6H_2O$ and 1000 mg/L $CuCl_2$ was used as the mineral source for bacterial growth. NaCl concentration increased from 10 to 25 g/L in 25 days in every 5 days interval.

2.4 Analytical methods

Granule size was analyzed by using ImageJ software, version 1.47 (Tijani et al., 2015). Granule VSS and GSV were measured according to standard methods (APHA, 2005). To determine EPS concentration, conductivity of granular sludge sample was adjusted with 0.05% NaCl and after centrifugation of the sample, EPS was heat-extracted from aerobic granules (Li and Yang, 2007). The influent and effluent COD concentration was measured by following closed reflux titrimetric method and total phenolic hydrocarbon (TPH) was measured by gravimetric method after extracting in n-hexane (APHA, 2005). Phenol concentration was also determined by standard methods (APHA, 2005).

3 Results and discussion

3.1 Granulation

Figure 2 provides photographs of aerobic granules formed in R1 and R2 at 25 g/L NaCl exposure. Yellowish aerobic granules appeared after 5 days of inoculation. In R1, *Brevibacterium* strain was capable of producing matured granules till day 15 in 20 g/L of NaCl concentration. Granule VSS profile is described in Figure 3. R1 achieved 2.34 g/L of VSS with increasing 1.2-1.7 mm diameter confirming more organic matter and density of granules. But after 20 g/L of salinity, R1 faced partial disintegration as sewage sludge originated *Brevibacterium* could not withstand salinity shock. *Staphylococcus* granules faced less biomass washout from R2 and achieved 2.5±0.5 mm diameter and 4±1 g/L VSS. However gradual salt adaptation helped both R1 and R2 granules to achieve a stable VSS and granule size of 1.82±0.1, 4±1 g/L and 2.04±0.23, 2.5±0.5 mm, respectively (Figure 2).

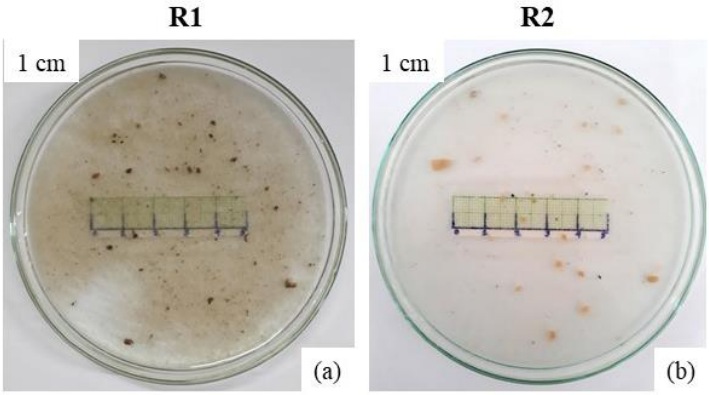

Figure 2. Matured aerobic granules produced in R1 (a) and R2 (b) on day 25

Wu et al. (2020) similarly observed that in saline wastewater granules faced less flocculent biomass washout from AGRs compared to floccular sludge which is supporting our results. R1 and R2 developed aerobic granules are given in Figure 2.

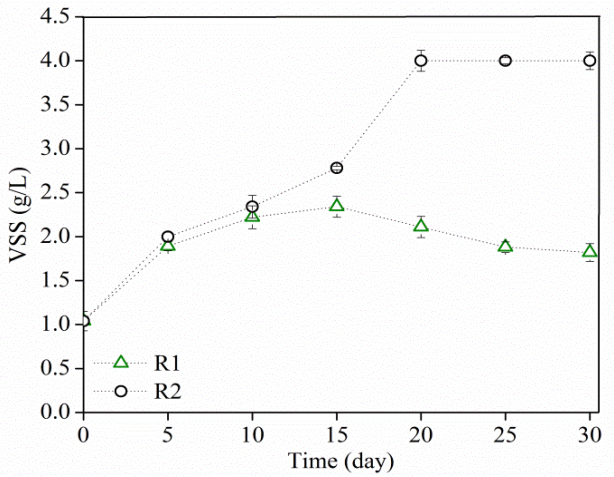

Figure 3. VSS profiles in R1 and R2 AGRs

3.2 Granule characterization

3.2.1 Granule settling behaviour

Figure 4 provides the GSV profiles in the AGRs. With increasing granule size granule settleability was increased providing GSV between 3.5-28 m/h in R1. However due to biomass washout from reactor, GSV value in R1 decreased till 20 m/h. On other hand, as R2 granules were comparatively stable in hydrocarbon recalcitrance and salinity shock providing stable at GSV 32±0.8 m/h in presence of 25 g/L salinity influence. Similarly, Corsino et al. (2015) achieved average 30±1 m/h GSV in aerobic granules in hypersaline oily wastewater which is supporting our study.

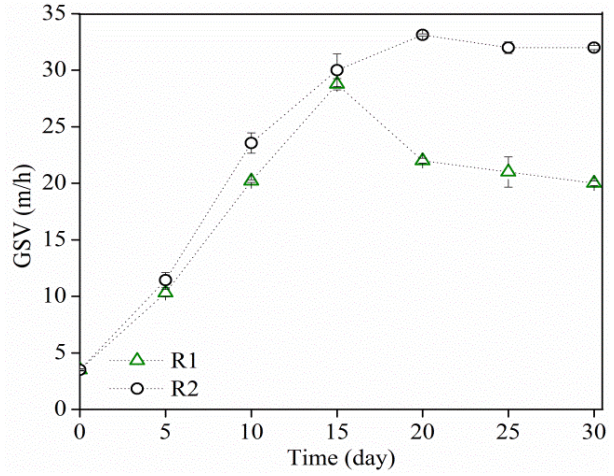

Figure 4 Changes in GSV profiles in R1 and R2

3.2.2 Variation in EPS

Changes in EPS concentration are described in Figure 5. With increasing salinity and hydrocarbon recalcitrance, aerobic granules experienced operational stress which helped in rapid synthesis of EPS. Hence with increasing granule biomass concentration EPS value reached about 115 and 200 mg/g VSS in R1 and R2. However, R1

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faced decreased EPS values due to granule rupture after 20 g/L NaCl exposure which further achieved stability at 98 mg/g VSS between day 20-30 days. Literature suggested that triggered EPS production with increased protein synthesis in salinity stress was responsible to enhance granule stability in AGR system which is corresponding with our results (Corsino et al., 2017).

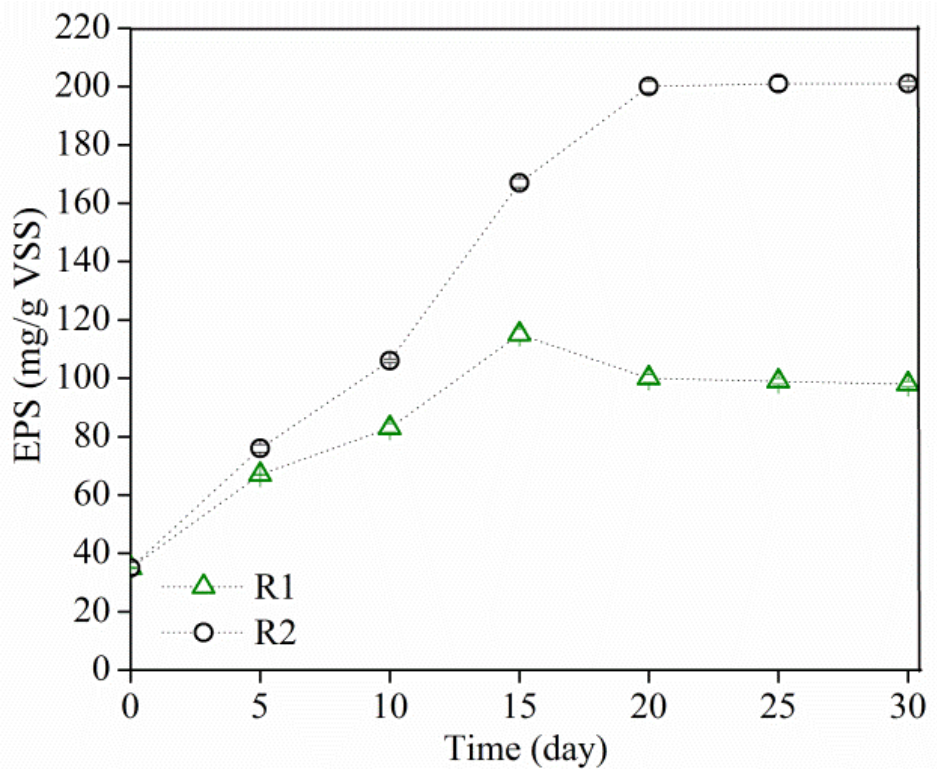

Figure 5 Changes in EPS concentrations in R1 and R2

3.3 Organics removal and kinetics analysis

Figure 6 describes COD and TPH removal patterns in R1 and R2. The influent COD and TPH concentrations were stable at 1075 and 280 mg/L throughout the 30 days of the study. Due to granule disintegration and reduced VSS values microbial activity was hampered in R1. So, R1 faced deterioration in organics removal. Between day 15 to 20, COD removal decreased between 78 to 73% in R1. But in R2, COD removal increased from 74 to 80% as *Staphylococcus* granules had maximum stability with 201 mg/g VSS EPS and above 4 g/L VSS concentration.

Incidents of enhanced hydrocarbon bio-adsorption with increased EPS concentration are reported in previous literature (Corsino et al., 2015). After hydrocarbon and salt adaptation in 25 g/L salinity and 280 mg/L TPH exposure both R1 and R2 achieved stability and provided maximum 72±0.2 and 81±1% TPH removal efficiencies, respectively (Figure 6b).

To determine the emulsified oil and phenol removal kinetics in the AGR we have collected effluent samples after day 20 when the AGRs had steady-state granules. We have checked the pollutant removal efficiency in every 6 h interval till 30 h of AGR cycle time and results obtained in triplicates. Pollutant removal kinetics results are summarized in Table 1.

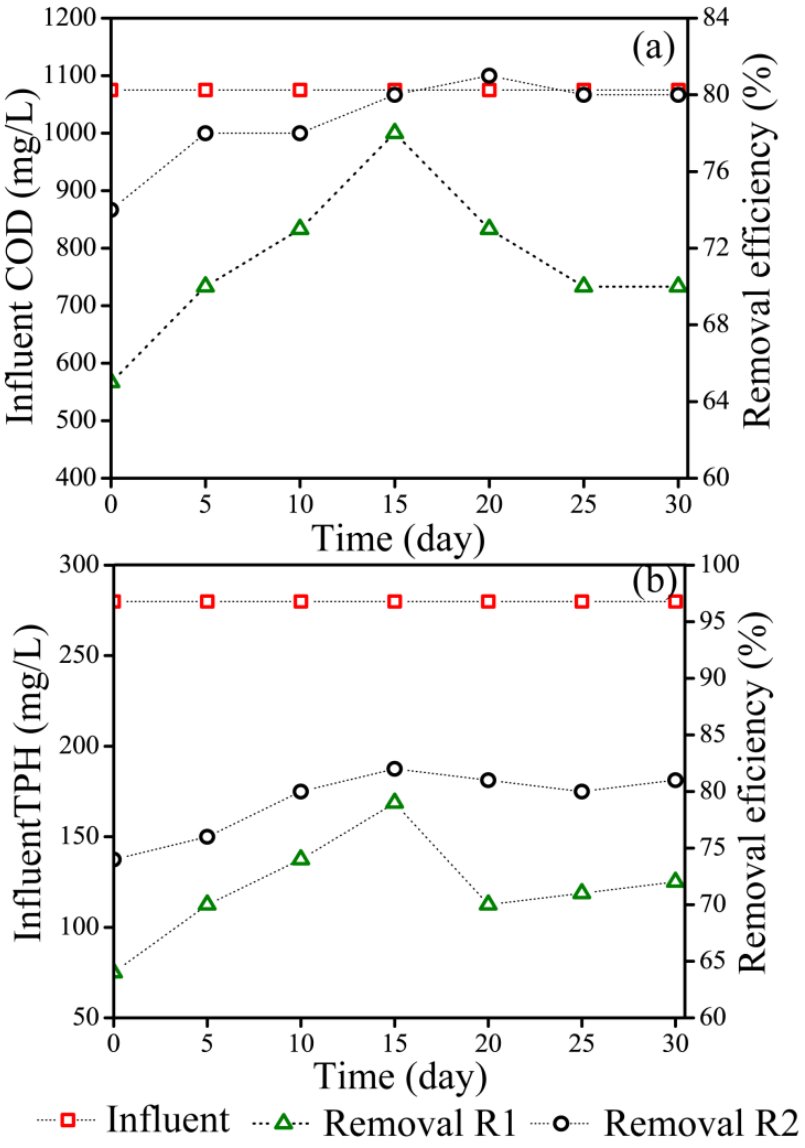

Figure 6. COD and TPH removal profiles in R1 and R2 AGR

Table 1. Diesel and phenol removal kinetics in R1 and R2 in 30 h of AGR reaction time

	R1		R2	
Time (h)	**Emulsified diesel (mg/L)**	**Phenol (mg/L)**	**Emulsified diesel (mg/L)**	**Phenol (mg/L)**
0	250	30	250	30
6	204.3±2	18.3±1.5	176.1±3	10.2±2
12	159.12±1	12.1±0.23	106.6±1.6	5.2±1
18	102±2	9.2±1	79.6±1	4.5±1
24	83.11±1	8.3±1.5	51.2±0.66	2.7±0.5
30	83±1	8.3±1	51.2±0.11	2.6±0.3

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The study confirmed that R1 granules achieved about 59% diesel removal in 18 h and reached maximum 67% removal in next 12 h. R2 granules achieved maximum 81% oil removal within 24 h which remained stable till 30 h. R1 and R2 had 83.11±1 and 51.2±0.66 mg/L effluent diesel concentration on day 30 of the study. In phenol removal, R1 and R2 had maximum 72 and 91% removals in 24 h of cycle time which contributed 8.3±1.5 and 2.7±0.5 mg/L of effluent phenol concentrations, respectively. Hence, the study proved that maximum stability and EPS content in R2 helped to achieve maximum 80% hydrocarbon removal in minimum time of 24 h.

4 Conclusions

Aerobic granulation was achieved in saline hydrocarbon-rich wastewater by using fresh water bacteria *Brevibacterium paucivorans* and *Staphylococcus hominis*. *Brevibacterium* achieved granule stability after facing initial granule rupture in combined salinity and hydrocarbon recalcitrance shock between 15-20 g/L NaCl concentration. *Staphylococcus* inoculated granules achieved rapidly increasing granule size till 2.5 mm and biomass concentration of 4 g/L. Salinity shock further enhanced EPS synthesis which also helped in hydrocarbon bio-adsorption in granules. Both *Brevibacterium* and *Staphylococcus* had salinity adaptation and achieved maximum 72 and 81% TPH removals, respectively. *Staphylococcus* granules were capable to achieve maximum 81% emulsified diesel and 91% phenol removals within minimum 24 h of reactor cycle time.

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