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# Studies on Bio-denitrification of Wastewater Using Immobilized GAC in Draft Tube Spouted Bed Reactor

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***ABSTRACT***

***Introduction:*** The draft tube-spouted bed bioreactor with GAC particles immobilized with Pseudomonas syringae is being evaluated to study the effect of suspended biomass and biofilm thickness on the rate of denitrification. Though the biofilm thickness is not directly controlled in wastewater treatment by the diffusion limitation and consequent substrate penetration in the biofilm, biofilm thickness will probably have a significant impact on bacteriological activity.

***Materials and Methods:*** The reactor studies were accomplished to study the result of dilution rate on attached biomass, suspended biomass, and biofilm thickness with nitrate reduction under steady-state conditions. A spouted bed reactor with the growth media prepared was used to study the bio-denitrification using Pseudomonas syringae.

***Results:*** The study of the attached biomass on nitrate reduction indicated that, as the attached biomass increased from 0.35 g/g to 0.54 g/g at a 0.166/h dilution rate, the nitrate reduction percentage decreased from 98.18% to 88.2%. During the study, it was observed that the biomass and biofilm thickness increased and decreased, respectively, with a rise in influent nitrate concentration and dilution rate. The rise in dilution rate as well as influent nitrate concentration throughout the study increased the rate of suspended biomass.

***Conclusion:*** The nitrate reduction rate was high with higher rates of loading in a draft tube spouted bed bioreactor, due to well-organized recirculation of the solids inside the reactor. The formation of biofilm thickness on solids is a significant character as it increases the nitrate reduction rate to meet the effluent standards.

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## Introduction

Protecting the aquatic environment has grown to be a significant global concern because of population growth and industrialization[1]. High nitrate and ammonia concentration with lower DO levels have an impact on aquatic life, human life and drinking water issues. These are the few negative consequences that can result from the build-up of different types of nitrogen and its different forms in the different water bodies. Electrodialysis, Ozonation, Reverse osmosis, are the physical and chemical processes utilized to reduce nitrate[2, 3]. However, the high capital and energy costs limit the application of these treatments[4]. Biological nitrate removal is the most economical strategy and enhanced option to use because it does not produce any by-products[5].

Owing to their increased efficiency per unit

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biomass, bioreactors with immobilized bacteria are perfect for laboratories and small firms with limited area for a traditional treatment site[6]. According to study investigations, immobilized attached cells on a diverse supporting medium can effectively remove nitrate[7,8,9,10]. The biological approach, which uses nitrate as a final electron acceptor in anoxic environments through microbial respiration, is thought to be the most practical and successful. Thus, biological treatment is more effective due to its potential to degrade almost all the pollutants while producing innocuous end products, reduce capital and operating costs, and maintain pollutant concentrations below the limit in the treated effluent. Denitrification can occur in the presence of oxygen in certain species too. Most of the organisms known to denitrify belong to bacterial genera belonging to *Pseudomonas, Rhizobium, Halobacterium, Halomona, Hyphomicrobium, Janthinobacterium, Neisseria, Paracoccus* (formerly *Micrococcus), Achromobacter, Acinetobacter, Aeromonas, Gallionella, Propionibacterium, Rhodobacter* (formerly *Rhodopseudomonas), Thiobacillus, Thiosphaera, Vibrio, and Xanthomonas* [11, 12, 13].

The reactors as MBR, SBR, packed bed reactors, and pulse plate reactors used for treating wastewater include non-ideality (dead/stagnant zones, channeling), uneven solid-liquid-gaseous phase mixing, increased energy needs, and higher fixed and recurrent costs [14]. The rate of oxygen and mass transport is subsequently impacted by these restrictions. Fluidized bed reactors for denitrification have been extensively studied by various researchers [15, 16, 17], and have shown some limitations like maximum bed expansion and retention time. The more nitrate is utilized at a lowest aeration frequency, with anoxic conditions in a draft tube being achieved in the annular part of the reactor [18].

The aerobic biological treatment plant of industrial wastewaters is effectively being used with gas-liquid-solid spouted bed bioreactor[19]. The advantages of the spouted bed bioreactor are higher interfacial velocities, good mass transfer, and a higher biofilm liquid interfacial area. The extreme increase of biomass on the media leads the washout of bioparticles from a reactor[20]. The high flow rates in the spouted bed with the least jetting velocity lower the bed pressure drop and hence reduce the cost of operation [21]. Thus, a spouted bed reactor has been tested for its performance in the denitrification process in the present study using immobilized cells of *Pseudomonas syringae as* an isolate used for nitrate biodegradation.

## Material and Methods

### *Microorganism*

A strain of *Pseudomonas syringae* was stored at 4°C, which was isolated from the wastewater plant of a fertilizer industry. It was sub-cultured once in 15 days on a nutrient agar plate and used for further studies[22].

### *Growth media composition*

The growth media composition, which included nitrate-rich media and trace element solution, was listed in Table 1[23, 24] along with the composition considered for 1 liter of water. The growth medium prepared was used in the experimental studies.

**Table 1: Growth media composition**

| Nutrient rich media | Composition |
|---|---|
| $NH_4Cl$ | 0.3 gm/L |
| $KH_2PO_4$ | 1.5 gm/L |
| $Na_2HPO_4.7H_2O$ | 7.9 gm/L |
| $KNO_3$ | 2 gm/L |
| $C_4H_4O_4Na_2.6H_2O$ | 27 gm/L |
| $MgSO_4.7H_2O$ | 20 gm/L |
| **Trace element solution** | **Composition** |
| EDTA | 50.0 gm/L |
| $ZnSO_4$ | 2.2 gm/L |
| $CaCl_2$ | 5.5 gm/L |
| $MnCl_2.4H_2O$ | 5.06 gm/L |
| $FeSO_4.7H_2O$ | 5.0 gm/L |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ | 1.1 gm/L |
| $CuSO_4.5H_2O$ | 1.57 gm/L |
| $CoCl_2.6H_2O$ | 1.61 gm/L |

### *Experimental bioreactor*

The detailed representation of the draft tube-spouted bed reactor was as shown in Figure 1. The medium, all connecting tubes and other accessories were sterilized. Synthetic and growth media were loaded in the reactor along with immobilized granular activated carbon with *P. syringae.* To

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have the controlled rate of proper supply of oxygen to a microorganism with spouting of solid, the compressed air was introduced through the filter. The occupied volume of the reactor was 1.2 liters with draft tube length 45 cm and diameter 1.2 cm.

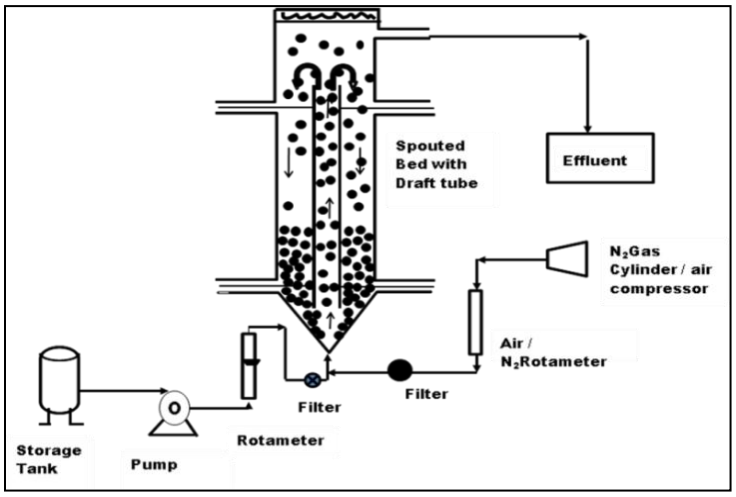



**Figure 1:** Schematic diagram of experimental setup.

### *Analytical methods*

The sampling was done from the draft tube reactor for every hour to study the characteristics of the sample and hence the performance of the reactor. The sample was centrifuged (REMI, India) at a speed of 10000 rpm for a duration of 10 min, and the supernatant obtained was used for the analysis of nitrate and nitrite. A UV spectrophotometer was used to analyse both nitrate and nitrite. The solids were washed with 0.25 N NaOH solution and were dried at 105 $^{o}$C for 24 h for analysing the attached biomass. The scanning electron microscope (SEM) was used to measure the biofilm thickness [22].

### *Granular activated load (GAC)*

GAC was procured from NICE Chemicals, Cochin, India. The GAC are screened for the desired size. The particles retaining on a 2 mm screen (average size = 2.4 mm) were taken for the study. The GAC was washed repeatedly using hot distilled water to remove all dirt, dust, and loose particles. Then, it was dried in an oven for two days at 105°C. This dried GAC was then used for immobilization. The properties of GAC used in the study are given in Table 2.

**Table 2:** Properties of granular activated carbon used in the study

| Sl. No | Properties | Value |
|---|---|---|
| 1 | Average size (mm) | 2.4 |
| 2 | Bulk density (kg/m$^3$) | 610 |
| 3 | True density (kg/m$^3$) | 1278 |
| 4 | Specific surface area (m$^2$/g) | 680 |
| 5 | Average pore radius (µm) | 12 |
| 6 | Pore volume (cm$^3$/g) | 0.45 |

## Results

### *Study of nitrate removal percentage during start-up of the reactor with varying dilution rate.*

It is evident from Figure 2 that, given an inlet nitrate concentration feed of 300 mg/L, the reactor's effluent nitrate concentration decreased continuously during the first phase of operation before slowing down for all dilution rates. The steady state was reached when the dilution rate was 0.166/h at 40$^{th}$ h, 0.333/h at 45$^{th}$ h, and 0.5/h at 50$^{th}$ h.

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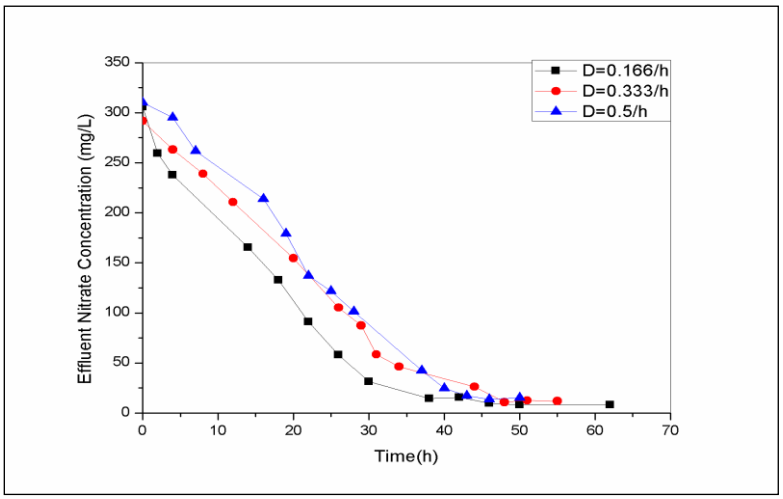



**Figure 2:** Study of nitrate removal percentage during start-up of the reactor with varying dilution rate with GAC loading of 40 g and nitrate concentration of 300 mg/L.

***Study of attached biomass on percent nitrate removal for different dilution rate***

It is evident from Figure 3 that the percentage of nitrate removal decreased as the amount of connected biomass increased. When the attached biomass was increased to 0.54 g/g with a dilution rate of 0.166/h, the percentage of nitrate removal dropped to 88.2% from 98.18% at 0.35 g/g (Figure 3). The nitrate removal was 99% with a dilution rate of 0.166/h and an attached biomass of 0.35 g/g GAC, 94% for a dilution rate of 0.5/h, and the same attached biomass quantity.

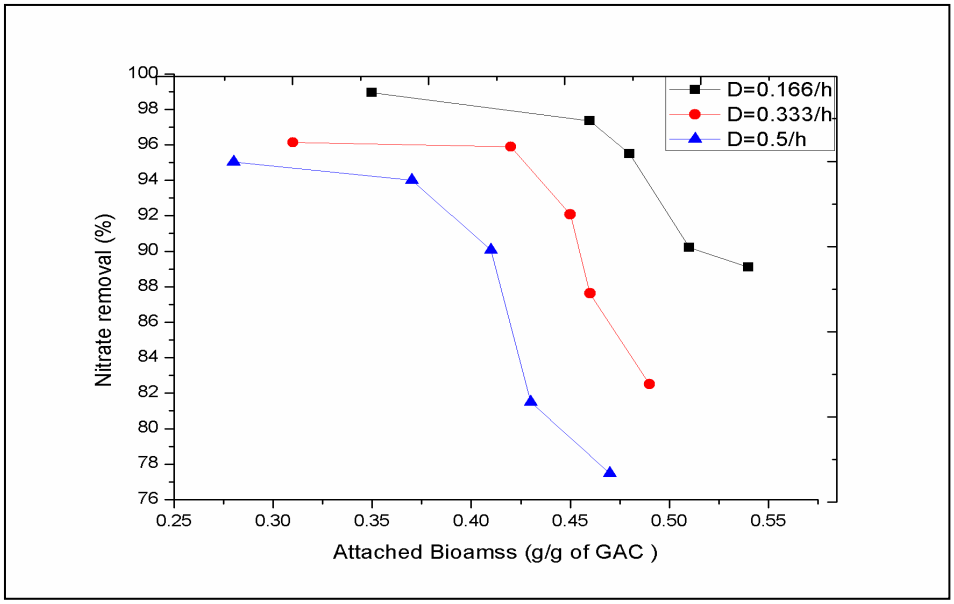



**Figure 3:** Study of attached biomass on percent nitrate removal at 40 g/g GAC loading.

***Study of attached biomass on dilution rate and influent nitrate concentration.***

It is observed from Figure 4 that the attached biomass quantity reduced as the dilution rate was increased with the initial concentration being lowered. The attached biomass weight was 0.35 g for the initial nitrate concentration of 100 mg/L and dilution rate of 0.166/h. When the dilution rate was increased to 0.5/h, the attached biomass weight dropped to 0.27 g for the same initial nitrate

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concentration. As the initial nitrate concentration rises, so does the attached biomass weight. The attached biomass weight was 0.35 g for the 100 mg/L initial nitrate concentration at the dilution rate of 0.166/h, and it rose to 0.55 g for the starting nitrate concentration of 1200 mg/L at the same dilution rate.

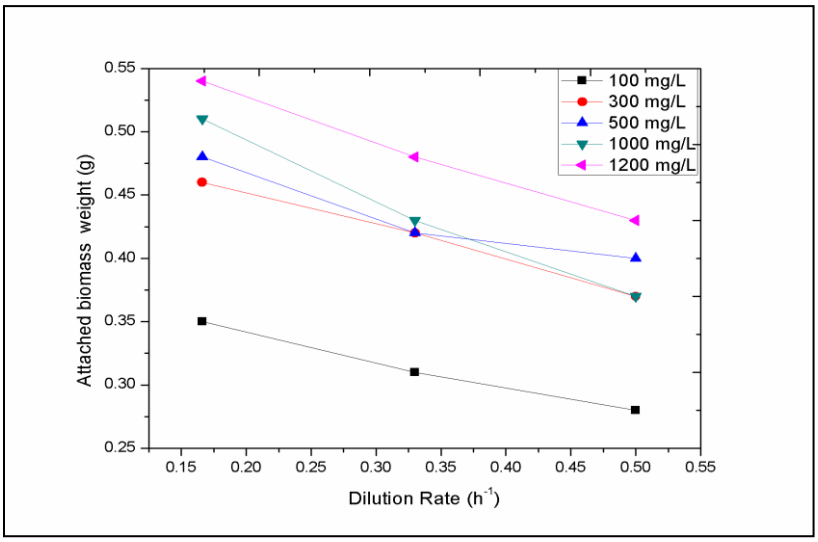



**Figure 4:** Study of attached biomass on dilution rate and influent nitrate concentration.

***Study of biofilm thickness for different dilution rate and influent nitrate concentration.***

It is observed from Figure 5 that the thickness of the biofilm rose as the concentration of influent nitrate increased and dropped as the rate of dilution increased. It is evident that the biofilm thickness was 0.19 µm for an 100 mg/L initial nitrate concentration at a dilution rate of 0.166/h and 5.57 µm for an 1200 mg/L initial nitrate concentration at the same dilution rate.

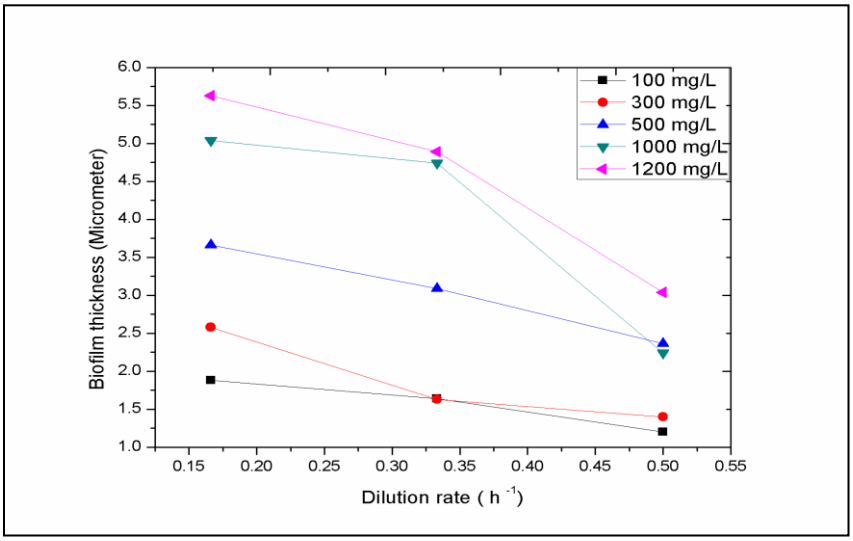



**Figure 5:** Study of biofilm thickness for different dilution rate and influent nitrate concentration.

***Study of suspended biomass for different dilution rate and initial nitrate concentration.***

From Figure 6, it is evident that when the dilution rate and influent nitrate content increases, so does the suspended biomass. As can be seen, the suspended biomass was 4 g at 100 mg/l and rose to



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10.5 g at 1200 mg/L of nitrate at a dilution rate of 0.166/h.

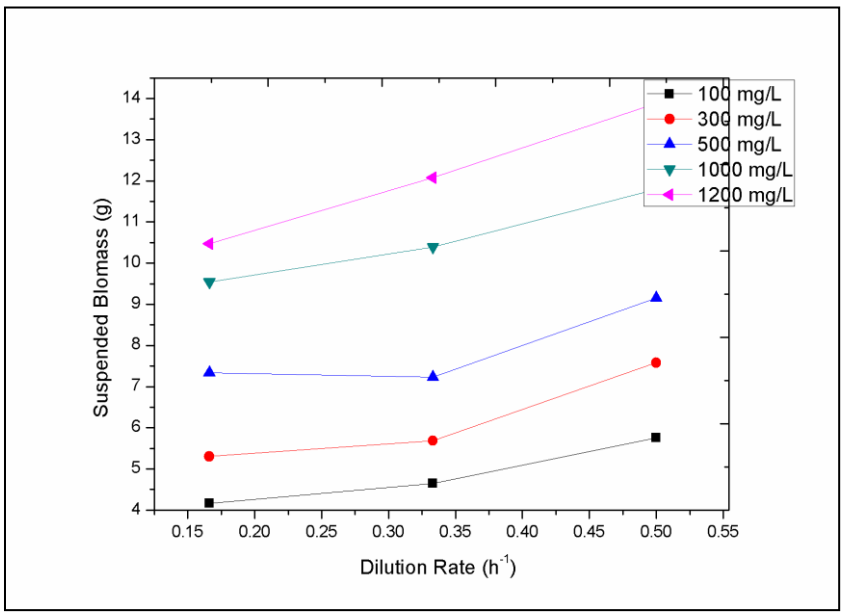



**Figure 6:** Study of suspended biomass for different dilution rate and initial nitrate concentration.

***Morphological characteristics of biofilm and EPS formation***

When examining the impact of biofilm thickness on biomass at varying dilution rates, it was observed that biofilm thickness decreased as the dilution rate rase. According to Figure. 7, the biofilm thickness was 7.02 µm, 6.76 µm, and 5.56 µm for dilution rates of 0.166/h, 0.333/h, and 0.5/h, respectively.

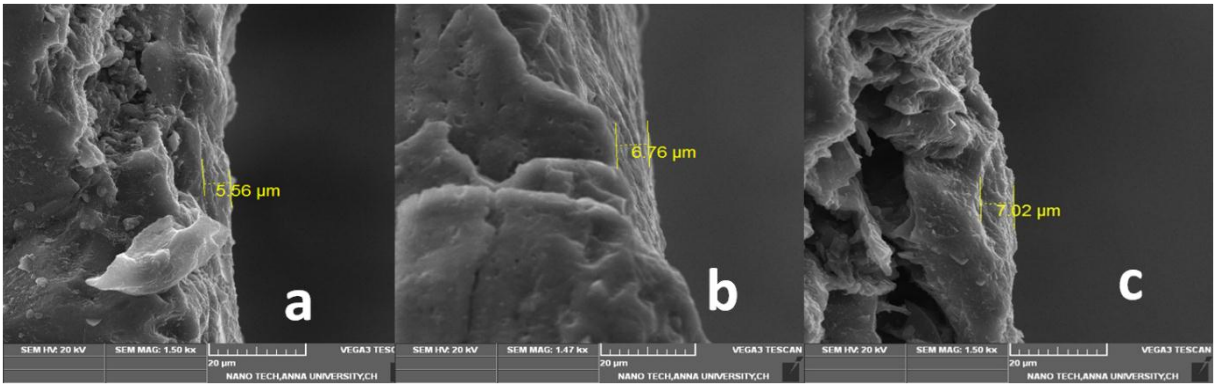



**Figure 7:** Morphological characteristics of biofilm (a) 0.5/h (b) 0.333/h (c) 0.166/h

## Discussions

***The study of dilution rate on nitrate reduction:*** Nitrate adsorption onto the biofilm surface could be the cause of the initial abrupt decline. Cell walls, cell membranes, cytoplasm, and extracellular polymeric materials are potential sorption sites in biofilms. According to Aksu, these sites support the sorption capabilities of biofilms for both organic and inorganic substances[25]. It has been found that the organic compounds and microbial cell walls interact physiochemically to speed up the sorption step[26]. The amount of nitrate removed by biodegradation may be less substantial during this stage. These cells are subjected to an

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increase in shear stress, flowing liquid, and continually flowing air when they are placed in a continuous reactor after being acclimated, grown, and immobilized in a batch setting. The abrupt rise of shear stress on a biofilm stimulates the secretion of EPS-polysaccharides, resulting in sloughing of the biomass and attached biomass decreases in the adsorption phase[27, 28].

***The study of attached biomass on nitrate reduction:***

Since nitrate is one of the nutrients that increases metabolic activity, an increase in the concentration of nitrate in the inlet results in more nitrate available for the microorganisms and, consequently, higher growth. On GAC particles, this increased biomass development creates a denser and thicker biofilm. A lower percentage of nitrate is removed because of the thicker and denser biofilm's increased barrier to mass transfer [29]. The flow velocity past the particle increases with the dilution rate. It is possible that the higher flow velocity caused biomass to be eroded off the film surface [30]. The reduction in attached biomass with increased dilution rate is due to increased cell detachment by erosion.

***The study of attached biomass on dilution rate on nitrate reduction:***

Due to attrition between GAC particles and biofilm shearing, the amount of connected biomass reduced as the dilution rate increased [31, 32]. Additionally, it is evident that a higher influent nitrate content resulted in more attached biomass since there were more nutrients available for biofilm formation. The stream velocity near the particle increases as the dilution rate rises. Higher biomass degradation from the biofilm surface may have been caused by this increased flow velocity [33].

***The study of biofilm thickness on dilution rate:***

The continuous flow of GAC particles in the draft tube part of the spouted bed reactor causes attrition and shear, which in turn results in the sloughing of associated biomass from the biofilm. The conditions under which the biofilm is produced determine its structure and morphology. The cohesion forces are provided by the EPS molecules on the biofilm and adhesion to the substratum, and hence the stress distresses the composition[34, 35, 36]. The detachment of the biomass occurs when the tensile strength of the EPS matrix is affected by tensile forces caused by the external shear [37, 38].

***Effect of dilution rate on suspended biomass:***

More nutrients become available as the initial nitrate concentration rises, which promotes the growth of biomass [39]. A higher dilution rate will result in more particle attrition, which will shear the biomass from the solids more and cause more biomass to wash out of the reactor [40].

***Effect of Morphology and EPS Formation:***

The dry weight of the attached biofilm rose as the bacteria multiplied during the first phase, progressively increasing the thickness over time. The biomass content was directly correlated with the thickness of the biofilm; as biofilming grew, dry density dropped, increasing the biofilm's porosity and resulting in increased roughness [41]. It was found that when the dilution rate decreased, the nitrate reduction rate rose. Biofilm thickness rises with decreasing dilution rate, which enhances EPS generation and, consequently, nitrate reduction [42, 43].

The EPS has a significant impact on the structure, adsorption capacity, dehydration, and other chemical and physical characteristics of the biofilm. Because there was less cell decomposition in the current study, there was less organism degradation, which resulted in the production of humic material. It was observed that the production of EPS rose in tandem with the rate of nitrate reduction over time. Moreover, the nitrate that the microbes used to enable cell proliferation resulted in the formation of EPS in the first place, overlaying the biofilm and producing a significant quantity of EPS. As a result, the quantity of EPS rose over time with each component. After 70–80 hours, it was found that the EPS stayed consistent, signifying that the steady state has been achieved by the bioreactor has reached a steady state. A higher dilution rate would cause the biofilm to shred, which would minimize the generation of EPS. Additionally, a shorter residence time would result in less nitrate-cell interaction. The studies

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have found that EPS produced largely polysaccharides in pure culture [44] and proteins in the mixed culture system [45].

## Conclusion

The bioreactor chosen in the present study is complex in terms of circulation, mixing, holdup (hydrodynamics), DO level variation in the draft tube and annular region, and mass transfer aspects in terms of resistances in the bulk on the GAC surface and intraparticle diffusional resistance. The investigation shows that the rate of bio-denitrification reduces during start-up as the dilution rate increases. The time to attain a steady state is enhanced by an increase in dilution rate and influent nitrate concentration. At 300 mg/L influent nitrate concentrations and a dilution rate of 0.166/h, the bio-denitrification achieved was 98%. The biofilm thickness and attached biomass dry weight reduce with an increase in dilution rates as reduced cell growth and erosion are caused by increased cell detachment. The increase in dilution rate from 300 to 1200 mg/L increases the biofilm thickness and biomass weight due to the rise in growth rate of organisms at higher nitrate concentrations. The EPS formation has a significant impact on the bio-denitrification, resulting in minimizing the EPS at higher dilution rates. Also, the biofilm thickness rises with decreasing dilution rate, which enhances EPS generation and, consequently, nitrate reduction. Hence, a draft tube-spouted bed reactor could be a promising and sustainable approach to nitrate reduction.

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## Authors' contributions

All authors have contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Keshava Joshi. The first draft of the manuscript was written by Dr. Lokeshwari Navalgund and Dr Vinayak Shet, Dr. Keshava Joshi commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Authors ethically approve the submission and declared that it was not submitted elsewhere.

## Competing interests

The authors declared no conflict of interests.

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