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Development of a Denitrifying Biofilter with Short Start-up Period

Ali Oboodi¹, Ehsan Fathi², Hassan Nezammahalleh^{3*}¹ Faculty of Engineering, Science and Research Branch, Islamic Azad University, P. O. Box. 14515/775, Tehran, Iran² Faculty of Mechanical Engineering, Tarbiat Modares University, P. O. Box. 14115-114, Tehran, Iran³ Young Researchers and Elite Club, South Tehran Branch, Islamic Azad University, P. O. Box. 15655/461, Tehran, Iran*Correspondence should be addressed to Hassan Nezammahalleh, Young Researchers and Elite Club, South Tehran Branch, Islamic Azad University, P. O. Box. 15655/461, Tehran, Iran; Tel: +982182883452; Fax: +982182884718; Email: nezammahalleh@ut.ac.ir.

ABSTRACT

In the present work, a novel down-flow biofilter with four identical layers was developed for the de-nitrification of 100 mg L⁻¹ nitrate contaminated wastewater, flowing horizontally from one layer to another in a continuous fashion. This biofilter can be applied for long-term continuous wastewater treatment without any need to whole system shutdown and backwashing. The clogged layer or the deactivated section can be easily spotted and repaired while the system is under continuous operation. To attain a short startup period, the effect of two main constructional parameters, namely, filter media and inoculants source, were then investigated. Two studied filter media were sand and woodchips. The pure culture of *Saccharomyces cerevisiae* and the mixed cultures of activated sludge and wastewater were three common inoculants under investigation. The biofilter packed with woodchips seeded with the wastewater resulted in the least startup period of about 3 days with a steady nitrate removal efficiency of 90 %.

Key words: Biofilter, Denitrification, Nitrate, Startup period, Wastewater.

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1. INTRODUCTION

Nitrate introduction into the environment mainly by aquacultural and agricultural activities limits the direct use of groundwater resources, intensification of agriculture, and further industrialization. Consumption of nitrate contaminated water can cause such health problems as methemoglobinemia in infants. The maximum amount of nitrate and nitrite in the water has been set to 10 mgNO₃-N L⁻¹ and 1 mgNO₂-N L⁻¹ by the American Public Health Association (1) and to 11.3 mgNO₃-N L⁻¹ and 0.03 mgNO₂-N L⁻¹ by the World Health Organization (2) for human consumption. Some general guidelines have also been suggested for water reuse applications in aquaculture industry (3). The treatment of nitrogen contaminated water can be performed in a sequence of three main steps. The first step in the wastewater treatment is physical removal of suspended solids. The pretreated water flows into a nitrification tank in which ammonium oxidation to nitrate occurs. This nitrification step can be performed by both chemical ozone disinfection and biological nitrifying microorganisms.

Various technologies have been developed for this process as submerged filters, trickling filters, reciprocating filters, rotating biological contactors, rotating drums, and fluidized bed reactors (4). The final step in the wastewater treatment for reuse applications is denitrification in which various chemical (5, 6), physical (7), and biological processes can be applied (8). The biological nitrate removal is the preferred process for denitrification since the other alternatives are of such disadvantages as poor nitrate selectivity, concentrated waste disposal issues, expansive, and susceptibility to fouling in the case of reverse osmosis. The biological nitrate removal can be performed by a variety of microorganisms upon three metabolic pathways: 1) Assimilatory nitrate reduction, 2) Dissimilatory nitrate reduction, and 3) Anammox. The assimilatory pathway does not cause a pure nitrogen reduction from the system. It results in the conversion of nitrate to ammonium and organic nitrogen compounds instead. The dissimilatory pathway reduce nitrate to molecular nitrogen and ammonium. The direction of the dissimilatory pathway towards denitrification or the

development of anammox process is the final step in the wastewater treatment for recirculation purposes. Various process techniques of membrane bioreactors (9, 10), fluidized bed bioreactors (11), up-flow sludge blanket bioreactors (12-14), and biofilter were developed in this regard. Development of the biofilter has many advantages compared to the other technologies as simplicity, inexpensive installation, low operating and maintenance cost, and improved biological activity without any excessive area requirements (15). A number of denitrification biofilters were developed in technical literature depending on filter media (16-19), flow scheme arrangements (20), type of biological process (21, 22), and type of inoculants (18, 23). These design parameters have a substantial influence on the performance of the biofilter and on the economics of the plant. Yang *et al.* (20) developed a biofilter and investigated its performance regarding aquaculture water treatment under three different media and four flow scheme arrangements. It was found that biocompatible media with surface and structural characteristics suitable to capture suspended matters and to support biological activity are more critical than the flow scheme arrangements. Zhou *et al.* (23) conducted a study on the feasibility of autotrophic denitrification using sulfur limestone in a lab scale up-flow biofilter and evaluated the effect of process parameters on the system performance. They concluded that the efficient treatment of contaminated water with low nitrate concentration, lower than 50 mg L⁻¹, can be achieved at higher hydraulic retention time (HRT) than 3 h and ambient temperature. Jing *et al.* (18) developed a double layer system including a trickling biofilter for soluble carbon removal and a submerged biofilter for denitrification of oxidized ammonium. The biofilter was packed with coal fly ash ceramic granules with numerous nanometer pores and inoculated with river sediment. A high soluble carbon and total nitrogen removal rate was achieved without any external carbon source supplementation. Chu *et al.* (19) used the biodegradable biopolymer polycaprolactone acting as both biofilm carriers and carbon source supplement for groundwater denitrification. This solid phase denitrifying system removed more than 95 % of total nitrogen at HRT of 3-6 h after one month startup period. The removal efficiency and volumetric rate were decreased about 5 % and 48 %, respectively, with prolonging operation time more than a year due to an increase in the biofilm thickness and a corresponding decrease in biofilm activity and clogging in the system. The bioreactor backwashing was found essential for successful denitrification and clogging prevention which corresponds

to a high operational cost as well. Development of a biofilter without any need to intermittent shutdown and backwashing can be economically appealing to the industry. A novel denitrifying biofilter consisting of multiple layers through which wastewater flows horizontally was developed in the present work. This system provides the opportunity to separate and repair the blocked layer without any need to whole system shutdown and backwashing. The long startup period is one of the problems which limit the rapid operation of such systems. The effects of two different filter media and three types of inoculants were studied to set up a system with short startup period. Two biofilter media, namely, woodchips serving as both a carbon source and biofilm carrier and the other media sand as just biofilm carrier were considered in this investigation. The inoculants were the pure culture of *Saccharomyces cerevisiae* and the mixed cultures taken from activated sludge and indigenous wastewater microorganisms. To the best of our knowledge, this is the first report assessing the effects of filter media and inoculants type on biofilter startup period.

2. MATERIALS AND METHODS

2.1. Materials

Two types of natural media, namely, sand and woodchips were used for biofilter fabrication. The sand medium was nearly spherical and had an average diameter of 5 mm. The woodchips were not uniform and had an average length of 4 mm. The woodchips were obtained from the available stockpiles at a local sawmill (Tehran, Iran) which utilizes a variety of walnut and beech tree species. Table 1 shows the physical properties of the biofilter media measured by the proposed methods in literature (24). The biofilter media were washed several times with distilled water to remove impurities and were dried in an oven at 373 K before packing into a reactor. The cleansed media were then packed into a bench scale reactor for the biofilter preparation. The wastewater influent to the biofilter was collected from the effluent of the secondary clarifier in the wastewater treatment plant at Science and Research Branch, Islamic Azad University (Tehran, Iran). The influent nitrate concentration was fixed at 100 mg L⁻¹ in all the experimental tests by dissolving KNO₃ to each feeding influent. The KNO₃ was purchased from Merck (Darmstadt, Germany) and used as received without any further purification. Three inoculants type were used in this study. The activated sludge and the indigenous anoxic microorganisms of the wastewater were used as the mixed culture source. The pure culture of *S. cerevisiae* was used as another source to seed the bio-filter media.

Table 1. Physical properties of the biofilter media

Medium	Bulk density, kg/m ³	Average media diameter, mm	Total porosity, %
Sand	1210	5	76
Woodchips	305	4	84.5

2.2. Experimental setup and operation

An anaerobic down-flow biofilter with four layers was constructed for denitrification of the nitrate contaminated wastewater. The bench scale biofilter image and the schematic of the system were shown in Figure 1. The biofilter was made of a cubical frame with dimensions of $100\text{cm} \times 37\text{cm} \times 32\text{cm}$ which is divided into four identical layers with a working volume of 42.4 L. The layers were separated by a 15 cm gap for sampling and feeding purposes. The bottom of each layer has two rows of pores (diameter: 2 mm) in one end so as to provide horizontal flow in each layer. The influent is distributed throughout the first biofilter layer by 9 nozzles installed at the top. The biofilter surface was covered with a black sheet prior to operation to eliminate the possible growth of oxygenic phototrophic microorganisms. The biofilter was

prepared by seeding the denitrifying anoxic inoculants into each woodchips or sand filter media, soaking them in 100 mg L^{-1} nitrate solution in a stirred tank bioreactor and then cultivating at 303 K for a day in an incubator. A 10.6 L cultivated media were then installed into the first layer and the other ones were filled with the same uncultivated media. The biofilter was then started by feeding the system at hydraulic loading rate (HLR) of $0.93\text{ m}^3\text{ m}^{-2}\text{ d}^{-1}$ with the $100\text{ mg NO}_3\text{ L}^{-1}$ contaminated water. The biofilter operated at the ambient condition without any temperature control in order to simulate the natural temperature fluctuations. The experiments were replicated three times for each media and inoculants type. The startup period of the biofilter was recognized as the time in which the effluent nitrate concentration from the system reaches an steady value lower than $10\text{ mgNO}_3\text{ L}^{-1}$ (25).

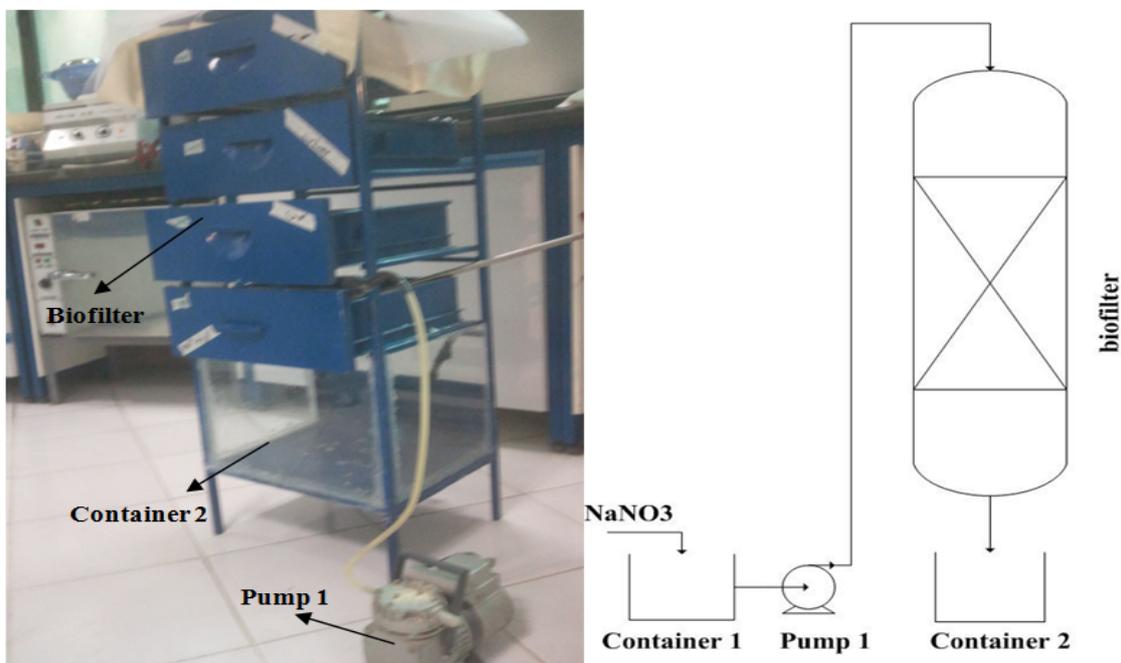


Figure 1. (Right) Schematic of the biofilter system; (Left) Real image of the system prior to operation

2.3. Analytical methods

The influent nitrate concentrations were measured routinely to be sure of a fixed nitrate concentration at the inlet to the biofilter. Samples from the effluents were taken at different time intervals and were centrifuged at 6000 rpm for 15 min. The supernatant were then collected and analyzed for nitrate and nitrite concentrations according to the APHA standard methods (1). A Hack spectrophotometer (Model DR5000, Hack Co., Colorado, USA) was applied for colorimetric determination of the nitrate and nitrite concentrations using analytical kits (NitraVer5 2106169 for nitrate and NitriVer3 1407899 for nitrite). The pH of the effluent samples was determined by a pH meter (Metrohm, Switzerland). The temperature fluctuations were monitored by sampling from the flow input and output to and from each layer. The average of

these temperature measurements at each time was considered in the bio-filter performance evaluation.

3. RESULTS AND DISCUSSION

3.1. Biofilter flow scheme arrangement

The biofilter was constructed in four layers each one packed with the same media in all the following experimental runs:

- First run: Biofilter was packed with the sand and inoculated with *S. cerevisiae*.
- Second run: Biofilter was packed with the sand and seeded with the activated sludge.
- Third run: Biofilter was packed with sand and seeded with the wastewater.
- Forth run: Biofilter was packed with woodchips

and inoculated with the wastewater.

The influent was distributed throughout the first biofilter layer by nine nuzzles installed at the top. The wastewater then flows horizontally in each layer to be exited from the pours produced at one end of a layer's bottom. This flow scheme provides an increase in the HRT without a

reduction in the HLR. Fig. 2 shows this flow scheme in the biofilter. The height of each layer has enough depth to provide anaerobic conditions for facultative anoxic microorganisms. The air flow from each biofilter layer bottom was limited by the black sheets that tightly cover the biofilter.

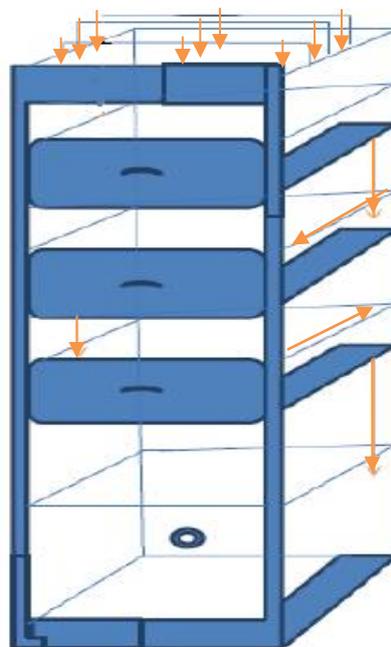
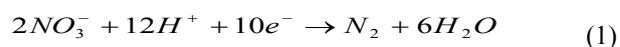


Figure 2. Flow scheme arrangement in the biofilter. The arrows in orange color show the flow direction

3.2. Biofilter startup with different inoculants and filter media

The filter media has a strong influence on a biofilter performance. The filter media should be biocompatible and possess surface and structural characteristics conducive to the development of biofilms and the capture of organic suspended matters. The natural media of coarse sand and woodchips were used for the biofilter preparation. The recent research focus is on the use of coarse particle media due to high permeability and minimized maintenance requirements for long term operation (26). The nitrate contaminated water at a concentration of 100 mgNO₃ L⁻¹ and HLR of 0.93 m d⁻¹ was flowed through the biofilter throughout the experiments. The biofilter was operated at this low velocity to promote the microbial growth through the filter bed. Aslan and Cakici found the same velocity as the optimal one for denitrification of drinking water using a biofilter packed with sand (27). The nitrate concentration changes in the biofilter with sand media with three different inoculation sources were shown in Figure 3 and Figure 4 during the startup period. The HRT of the wastewater was 4 h in each test. Each test was replicated three times and different nitrate concentration was measured at each time point but the startup period had low deviations from the average. The results of one test were drawn in Figure 3 and 4. The biofilter inoculated with the yeast, Figure 3A, has shown no nitrate removal activity.

The biofilter prepared with the sand media inoculated with *S. cerevisiae* was stopped after a day due to complete disruption of the yeast cells. The anoxic bacteria begin to grow slightly in the system and further denitrification activity progressed by the anoxic microbial community. With the dominance of the other microorganisms and the occurrence of different metabolic activities in the system, the pH was nearly constant without any increase in alkalinity. The Figure 4A has shown an increasing trend in nitrate concentration at the initial times due to nitrification of ammonium and the other organic nitrogen sources in the effluent. With the adherence of the denitrifying microorganisms to the media and dominance of their population, the nitrate was reduced into nitrogen gas and released out of the system. The similar trend was observed in Figure 3B with the activated sludge inoculants. The pH of the system was increased in both cases of inoculation with the mixed cultures due to the following denitrification metabolism.



This alkalinity increase can be an indication of the biological activities in the system. The population of microorganisms was also viewed under microscope and rod shaped microorganisms were dominant ones in the system, the results were not shown here.

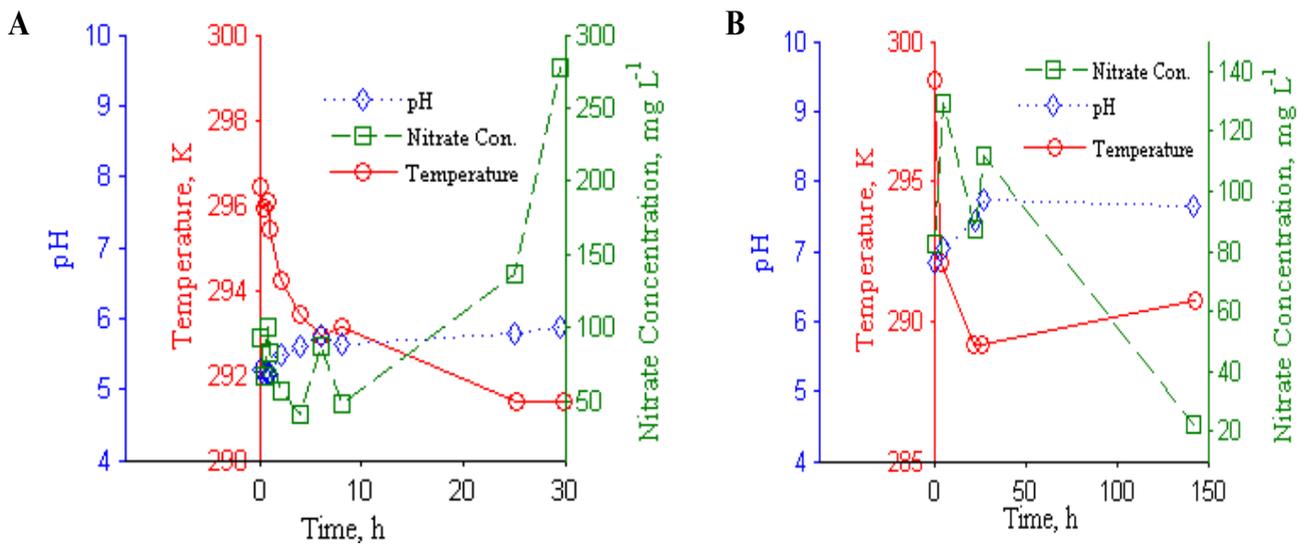


Figure 3. The nitrate concentration and pH changes in the effluent and the temperature fluctuations of the biofilter systems. The biofilters prepared by sand media were inoculated with *S. cerevisiae* (A) and with the activated sludge (B)

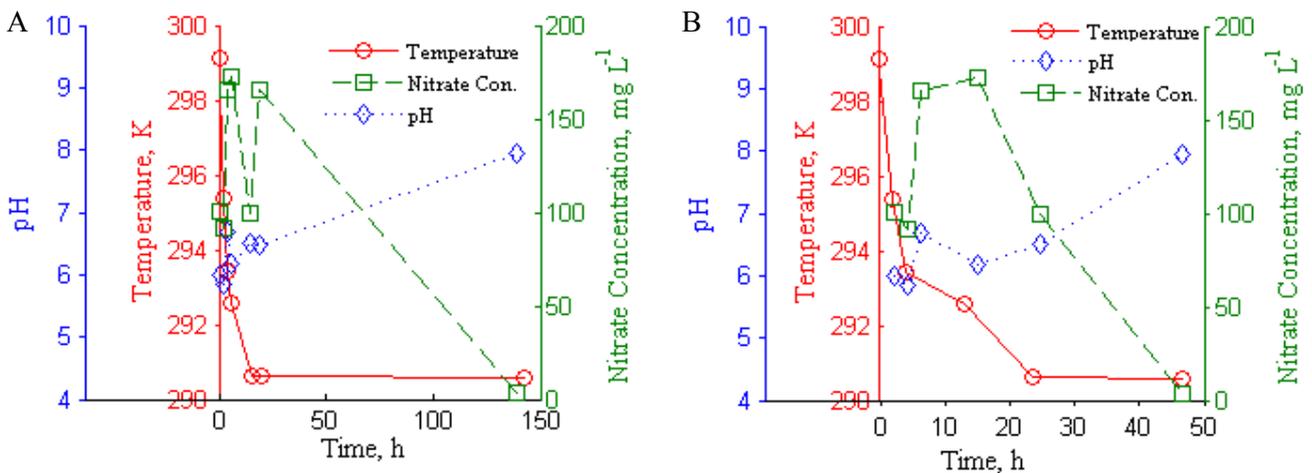


Figure 4. The nitrate concentration and pH changes in the effluent and the temperature fluctuations of the biofilter systems; the biofilters prepared by sand medium (A) and woodchips medium (B) were both inoculated with the wastewater

The nitrate reduction by seeding the filter media with the indigenous wastewater microorganisms resulted the least startup period, Figure 4A. The inoculants were then used to seed the woodchips medium. The denitrification activities were examined and shown in Figure 4B. The nitrate was reached to the permissible limit in three days that is the required time span for system startup. This short startup period is because of the structural characteristics of the packing media, suitable for the biofilm formation. This medium also provides a supplementary carbon source for the microorganisms which support their further growth in the absence of reducing carbon nutrients. The air filled

space between the layers in the system provides an appropriate condition for rapid release of nitrogen gas from the system. The other advantage of this air gap is prevention of strict anaerobic condition that may shift the dissimilatory pathway in the denitrifying bacteria to ammonia production (Table 2). The down-flow arrangement with high operational period usually causes the filter clogging. This biofilter can be easily repaired in the case of clogging without whole system shutdown. The strict anaerobic conditions in the biofilters with high aspect ratio led to reduced denitrifying activity at the depths (27).

Table 2. Biological nitrate removal (21, 22)

Metabolic pathway	physicochemical conditions	Organisms
Assimilatory nitrate reduction $(NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+)$	Low N in media; high C/N ratio	Plants; fungi; algae; bacteria
Dissimilatory nitrate reduction Dissimilatory nitrate reduction to ammonia $(NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+)$	High C/N ratio; high sulfide concentration;	Anaerobic and facultative anaerobic bacteria
Denitrification $(NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2)$	Low C/N ratio; high reduced media;	Aerobic and facultative aerobic bacteria
Anammox $(NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O)$		Facultative bacteria

3.3. Comparison of the bio-filter performance at the operational conditions

Table 3 presents performance comparison of the biofilters developed in this study regarding nitrate removal efficiency and startup period. The biofilter inoculated with *S. cerevisiae* cannot be applied for denitrification purposes due to lack of microbial tolerance to the severe condition of the polluted water. The system operated with the media seeded with the wastewater led to a shorter startup period than the activated sludge one. This observation is a result of aerobic microorganisms' dominance in the activated sludge system. The growth and adherence of anoxic microorganisms to the biofilter media is decreased and a longer startup period is resulted. The biofilter installed with the woodchips media seeded with the wastewater led to the best denitrification activity with a short startup period of 45 h. The startup period of the biofilter

developed in the present study was compared in Table 4 with the other ones reported since 2010 in Scopus database. The startup period of the biofilters inoculated with activated sludge was higher than the ones inoculated with the wastewaters that are in complete agreement with the results of this study. The short startup period in this work compared with the other reports is mainly because of fewer microorganisms washout from the system. Most of the biofilters developed by the other researchers were in the lab scale and the bacterial seeds were mixed totally with the media leading to loose microorganisms' adherence and fast washout from the system. The biofilters in the bench scale have shorter startup periods than the ones in the lab scale. A short column height and/or low working volume require a lower HLR to reach the same performance. The biofilter with short startup period in the other studies requires a high HRT in the order of few days so as to reach the steady performance.

Table 3. The biofilter performance comparison regarding the startup period and nitrate removal efficiency

Biofilter	Inoculants	Medium	Nitrate removal, %	Nitrite, mgNO ₂ -N/L	Startup period, hr
1	<i>S. cerevisiae</i>	Sand	NA*	---	NA
2	Wastewater	Sand	90	0.62 ± 0.05	134 ± 15
3	Activated sludge	Sand	88	---	158 ± 20
4	Wastewater	Woodchips	90	0.035 ± 0.01	45 ± 6

* NA: Not Available

The nitrite concentration at the end of startup period was measured in the effluents of the biofilters prepared by the woodchips and sand media seeded with the wastewater. The biofilter prepared by the sand medium resulted in a high effluent nitrite concentration of 0.62 mgNO₂-N L⁻¹ while the concentration was measured 0.035 mgNO₂-N L⁻¹ in the case of woodchips medium. The reducing power limitation in the biofilter packed with sand led to incomplete reduction of nitrate to molecular nitrogen gas. The reducing power in the other one was supplemented by the woodchips and the nitrite accumulation reached to the regulated limit. The biofilter system developed in this work provided the right condition and time for microbial adherence to the media. This biofilter can also be applied for long term operation without any need to system shutdown and backwashing. The clogged layer or the

deactivated section can be easily spotted and repaired while the system is under continuous operation with a slight reduction in the HLR. The short startup period is a key success to this biofilter development and utilization in the industrial settings. The startup period can be further reduced by the use of anaerobic sludge from a digester due to the fast acclimation of the microorganisms to the new system. This biofilter has the potential to be applied for simultaneous nitrification and denitrification due to presence of both aerobic and anaerobic conditions in each layer. The annual performance of the biofilter, the other pollutants concentration in the effluent, and the effect of HLR on the biofilter performance need to be investigated prior to successful application of this novel system configuration.

Table 4. Startup period comparison of the biofilters prepared by various media and inoculants

Description	HLR	Nitrate, mgNO ₃ L ⁻¹	Temperature, °C	Media	Inoculants	Startup period, d	Nitrate removal, %	Reference
Double layer cylindrical biofilter (diameter: 1.5 m; height: 2.05 m)	4 m ³ m ⁻² d ⁻¹	10.6-30.3	9-26	Fly ash ceramic granules	River sediment sludge	16	90	(18)
Cylindrical solid phase denitrification (SPD) bioreactor (diameter: 6.3 cm; height: 60 cm)	0.9-1.06 Ld ⁻¹	13.7	21-23.5	Woodchips	River water	> 10	100	(26)
Cylindrical SPD bioreactor (working volume: 3.0 L)	2.06 L d ⁻¹	266-354	30	Ellipsoid polycaprolactone granules	Anoxic sludge	30	96	(19)
Cylindrical biofilter (working volume: 3.2 L)	Batch operation	443	30	Sulfur limestone	Digested sludge	6-8	90	(23)
Two cylindrical biofilter (diameter: 4.9 cm; height: 10 cm)	14.4 L d ⁻¹	50	18	Granular biologically activated carbon	Groundwater and the biofilm of a granular activated carbon filter	> 15	100	(28)
Six discrete compartments biofilm reactor (working volume: 70 L)	2.9 L h ⁻¹	3000	26	Biologically activated Raschig Rings	Anaerobic sludge	14	100	(29)
Cylindrical SPD biofilter (diameter: 5 cm; height: 90 cm)	0.5 L d ⁻¹		20	Brewer's spent grain	Anaerobic sludge	30	99	(30)
Cylindrical biofilter (diameter: 9 cm; height: 2 m)	6 L h ⁻¹	30	15-20	Granular ceramsite	Biological anoxic filter effluent	28	84	(31)
Cylindrical SPD bioreactor	2.4 m ³ m ⁻² h ⁻¹	30	20.1-22	Granular polycaprolactone	Activated sludge	35	99	(32)
Cylindrical SPD bioreactor (diameter: 4.5 cm; height: 40 cm)	15 L d ⁻¹	50	24	Biodegradable snack ware	Groundwater	23	100	(33)
Cylindrical denitrification column (diameter: 9 cm; height: 35 cm)	0.08-1.9 m d ⁻¹	160	22	A mixture of 70% agricultural soil and 30% date palm leaf	Wastewater and agricultural soil	25	88	(34)
Cylindrical biofilter (diameter: 10 cm; height: 160 cm)	2 L h ⁻¹	200		Sulfur limestone	Anaerobic sludge	30	100	(17)
Cylindrical biofilter (working volume: 8 L)	1.8 L d ⁻¹	25	30	Granular sulfur particles	Activated sludge	30	79	(35)
Cylindrical biofilter (diameter: 10 cm; height: 3 m)	0.7 L d ⁻¹	17.6	~20	Polycaprolactone	Activated sludge	53	87	(36)
Cubical four layers biofilter (working volume: 42.4 L)	0.93 m d ⁻¹	100	16- 25	Woodchips	Wastewater	3	90	This work

4. CONCLUSION

A down-flow denitrifying biofilter with four layers was developed in the present work. The wastewater was distributed throughout the first layer top and flowed horizontally through the other ones. This bio-filter was packed with two different media and seeded with three inoculation sources and the system with woodchips medium resulted in the best performance regarding startup period and nitrate removal efficiency. The bio-filter packed with the woodchips seeded with the indigenous wastewater microorganisms were resulted in the least startup period. A steady 90 % nitrate removal efficiency was also achieved from an influent 100 mg L⁻¹ nitrate contaminated wastewater during 3 days of continuous operation. The results of this study were compared with the conclusions of

the other publications. The least startup period reported for a denitrifying bio-filter was about 1 week which achieved under a batch system operation. The short startup period achieved in this work made possible the efficient application of this bio-filter for continuous long-term treatment of the nitrate contaminated wastewater without any need to intermittent system shutdown and backwashing.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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