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Nitrification and Denitrification Processes for Biologic Treatment of Industrial Effluents

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Abstract

Nitrification process performance was evaluated using a three-phase fluidized-bed bioreactor. A synthetic effluent was used for this experiment containing 180–230 mg/L of chemical oxygen demand (COD), 25–30 mg/L of $N\text{-}NH_4^+$, 12 to 13 mg/L of total phosphorous, and micronutrients. The bioreactor used for denitrification behaved as completely mixed. The results indicate that the nitrification process was efficient, reaching efficiencies of about 98%. The best results related to the efficiency of the denitrification process were obtained when the processes were supplemented with the carbon source. The results indicated an efficiency of 86–98% COD removal.

Index Entries: Nitrification; denitrification; three-phase fluidized-bed bioreactor; *Nitrobacter*; *Nitrosomonas*.

Introduction

Biologic treatment is by far the most common method used to treat sewage, and two distinct process arrangements are used. Bacteria can be grown either in suspension (e.g., activated sludge) or attached to the surface of large solid medium (e.g., a biologic filter).

Biologic fluidized bed is a combination of these two processes because bacteria are grown on the surface of small solid particles that are held in fluidized suspension. The liquid to be treated is passed upward through a bed of solid medium at a velocity sufficiently high to fluidize the particles. A high concentration of bacteria leads to much greater rates of reaction per unit volume compared to either biologic filters or activated sludge. Consequently, a smaller reactor can be used, thereby reducing capital cost *(1)*.

Domestic sewage and many kinds of wastewater from pharmaceutical, agricultural, and food industries contain great amounts of carbonaceous

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and nitrogenous substances as pollutants. Recently, much attention has been paid to the removal of ammonia from wastewater because ammonia promotes eutrophication in both terrestrial and littoral waters *(2)*. Ammonia is toxic to fish at very low concentrations. In general, it is recommended that no more than 0.02 mg/L of free ammonia be permitted in receiving waters *(3)*.

Biologic ammonium removal is a nitrification process, i.e., the conversion of ammonium into nitrate. Nitrification is initiated by two different functional groups of bacteria: the ammonium oxidizers, which convert ammonia into nitrite using an O_2-dependent ammonium monooxygenase; and the nitrite oxidizers, which oxidize nitrite into nitrate using a molybdenum-containing nitrite oxidoreductase.

Both physiologic groups contain Gram-negative bacteria with obligate chemolithoautotrophs that make use of the energy released from the two oxidation reactions. The nitrifying bacteria assimilate carbon dioxide into the cell material via the ribulosebisphosphate cycle. Both oxidations have relatively high redox potentials and these, coupled with the requirement for reverse electron flow for synthesis of reducing power, lead to low yields. These organisms also have low maximum specific growth rates with doubling times typically in the range of 10–24 h. These factors have limited studies on their physiology and growth kinetics, but a number of continuous-flow studies have been conducted *(4)*. The oxidation of ammonia into nitrite and its subsequent oxidation to nitrate are carried out by *Nitrosomonas* and *Nitrobacter*, respectively. The growth rate of nitrifying bacteria is controlled by substrate concentration, temperature, pH, and oxygen tension *(5)*.

The increase in nitrate concentration in public water supplies is becoming a serious problem in some parts of the world. Nitrate concentration in groundwater reached threateningly high levels 20 yr ago, and it has continued to increase ever since. Nitrate is a cause and an inorganic nutrient for the growth of algae, and it can represent a danger to public health if present in excessively high concentrations in drinking water. It was found that nitrate could cause methemoglobinemia in infants (blue baby syndrome) *(6)*.

Biologic denitrification of nitrates and nitrites present in wastewater is important and necessary. It is a process of nitrate and nitrite reduction in which nitrite serves as the terminal exogenous hydrogen acceptor when the oxygen tension in wastewater is sufficiently low. The normal end product of this nitrate and nitrite respiration is elementary nitrogen or nitrous oxide gas, which, being inert, can be allowed to escape into the atmosphere *(7)*.

The literature contains numerous data concerning the influence of different denitrification conditions on the rate of the process *(8)*. Environmental conditions that must be optimized for denitrification are temperature, pH, and type of carbon substrate. In the present work, the system of biologic treatment was based on the oxidation of organic and nitrogen matter of synthetic wastewater. Micronutrients were added for good per-

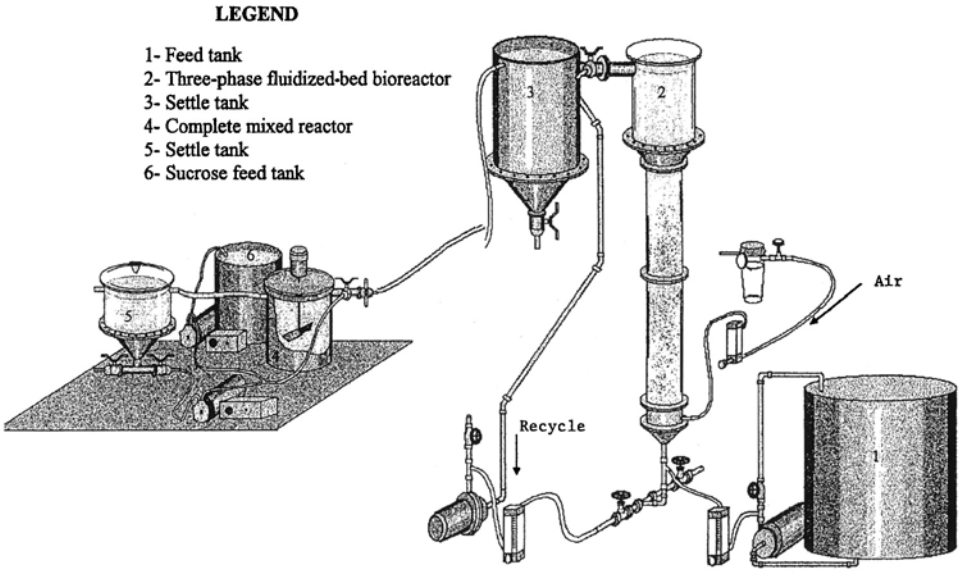

Fig. 1. Experimental unit.

formance of microorganisms. Dissolved oxygen, pH, and temperature were monitored for the nitrification process.

Materials and Methods

In the present work, the system of biologic treatment was based on the oxidation of organic and nitrogen matter ($N\text{-}NH_4^+$) of synthetic wastewater, and the pilot unit was operated for 50 d. For the removal of nitrogen matter, nitrification and denitrification processes were used, in series.

Experimental Setup

The experimental setup, as shown in Fig. 1, consisted of an acrylic column measuring 2 m high and 0.11 m in internal diameter (three-phase fluidized-bed nitrification reactor). In its upper part was an area of velocity reduction (0.5 m high and 0.24 m in internal diameter) that permitted separation of the three phases. There was a sedimentation column, made of polyvinyl chloride (PVC), that measured 0.8 m long and 0.24 m in internal diameter, coupled to the reduction area.

Concentrated substrate, stored in a reservoir, was diluted with tap water and continuously fed to the three-phase fluidized-bed reactor. The diluted synthetic effluent was distributed to the reactor by means of a gas-liquid distributor. This distributor consisted of a nylon cone through which the ascending liquid came in. On this cone there was a nylon tube whose diameter was the same as that of the column containing a gas distributor. This distributor was made of a 4-mm-diameter copper tube shaped like a spiral. Perforations of 0.5 mm in this spiraled tube allowed the gas to be dispersed in the surrounding liquid. Above the distributor was a

Table 1
Composition of Artificial Wastewater

Component	Concentration (mg/L)
Glucose	180–220 of COD
NH_4Cl	24–56 of $N\text{-}NH_4^+$
KH_2PO_4	50
$FeSO_4 \cdot 7H_2O$	13.7
$NaHCO_3$	750
Na_2CO_3	500
$CaCl_2 \cdot 2H_2O$	10

plate regularly perforated with 235 holes 3 mm in diameter, resulting in 16.10% free area. This plate had two main purposes: to improve fluid distribution in the fluidized-bed and to support the particles in the bed. The plate was flanged between the gas-liquid distributor and the fluidized bed.

Part of the flow that passed through the sedimentation column, coupled to the exit of the three-phase fluidized-bed, was recirculated to the base of the three-phase fluidized-bed reactor by means of a centrifugal pump, to promote the fluidization of the particles in suspension. Another part of the supernatant fed the denitrification reactor. Pumping was not necessary owing to the free fall of 2.5 m between the exit of the nitrification system and the denitrification process entrance.

The denitrification system consisted of a completely mixed reactor, which was made of PVC, with a volume of 35 L. A decanter was coupled to this reactor. The decanter was made of an acrylic column having at the base a stainless steel cone connected by a flange, with a total volume of 20 L. This system also had a storage reservoir for glucose, used as a supplementary source of carbon. A solution of glucose was then continuously fed to the denitrification reactor by a centrifugal pump. The sludge of the decanter from the denitrification system was recirculated to the denitrification reactor, to ensure a high retention time for the microbial cells.

Support

The support used in the three-phase fluidized-bed bioreactor for the development of the biofilm was cylindrical PVC particles, with a specific mass of 1.37 g/cm^3 and equivalent diameter of 2.94 mm.

Bacteria

The fluidized-bed support was inoculated in batch for 24 h with aerobic sludge from an effluent reservoir from Londrina, PR, Brazil, and nitrifying bacteria from activated sludge unit from the Refinary Getúlio Vargas (Araucária, PR, Brazil). Aerobic sludge and nitrifying bacteria were previously acclimatized to the synthetic substrate (Table 1) for 7 d.

The completely mixed bioreactor for denitrification was also inoculated in batch for 24 h. The inoculum used for the denitrification process

was obtained from anaerobic sludge from an effluent treatment plant in Londrina, PR, Brazil. Anaerobic sludge was previously acclimatized for 7 d to a solution of KNO_3 (1.119 g/L), glucose (0.21 g/L), and KH_2PO_4 (0.02 g/L).

Wastewater

The composition of the synthetic wastewater used in this work is given in Table 1. In the experiments, sodium bicarbonate was used as a buffer for the medium during the nitrification process.

The formation of ion H^+, during ammonia oxidation, reduces the alkalinity of the medium. To sustain the optimal pH range, a well-buffered medium is necessary *(5)*. Bicarbonate was used as reported by others *(9–11)*. All the compounds that formed the synthetic effluent were dissolved daily with tap water in the feeding tank (500 L).

Operation

The experimental run was operated for 50 d by continuous flow.

Nitrification Process

The three-phase fluidized-bed reactor worked at a hydraulic retention time of 5 h and a gas flow of 6.72 L/h. The concentration of the dissolved oxygen in the liquid was from 3.5 to 4.5 mg/L, and the recycling flow was 313 L/h. The liquid temperature was controlled at 27–32°C by means of an electric heater. The pH value of the liquid was controlled at 7.0 to 8.0 by adding NaOH and $CaCO_3$ to the artificial substrate (feed tank) when needed. The dissolved oxygen of the wastewater was adjusted to 6 mg/L, which is optimal for the nitrifying bacteria *(5)*.

Denitrification Process

The complete mixed reactor was operated at a hydraulic retention time of 4 h and 30 min and a flow feed of 8 L/h. The liquid entering the denitrification process, which was the effluent of the nitrification process, was kept in an anoxic condition, and there was no need to manipulate oxygen flow inside the complete mixed reactor. The quantity of extra carbon source (glucose) was monitored, so as to give concentrations of 41–292 mg/L of COD for the process.

Analyses

COD was determined according to Micro-Methods from Tavares *(12)*. Values of ammonia were followed by Koroleff *(13)*. Nitrite and nitrate concentrations in the liquid were determined by the Test Kit (HACH) model NI-15 (cat. no. 21820-00) and model NI-11 (cat. no. 1468-03), respectively.

Attached biomass in the support was determined by protein and polysaccharide concentration according to the Lowry and Dubois methods in Tavares *(12)*, respectively. Each method made use of 60 particles taken from the interior of the three-phase fluidized-bed reactor, and the extrac-

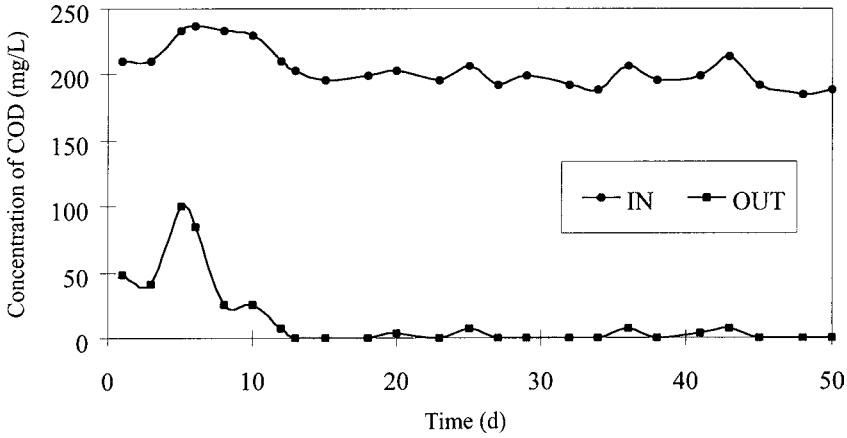

Fig. 2. COD reduction concentration vs time: nitrification process.

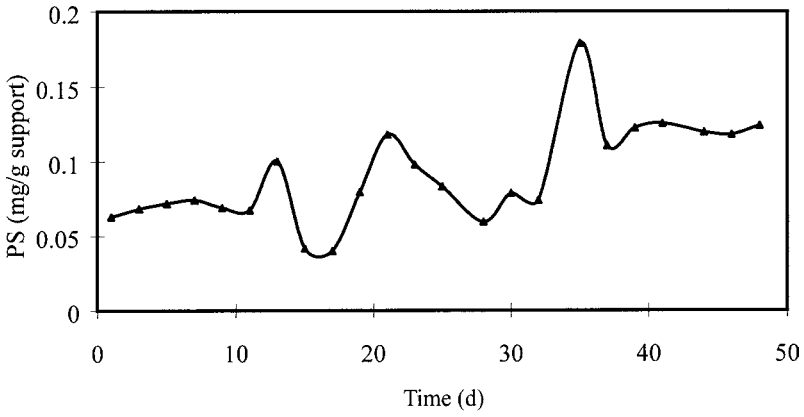

Fig. 3. Variation in polysaccharide (PS) content in the attached biofilm with reactor operation time.

tion was carried out in water bath at 80°C with specific reactants for each analysis (*see* ref. *12*).

Results

Figure 2 shows COD concentration in the influent and effluent wastewater vs time (days) during the nitrification process. Mean values of COD concentration fell from 204 to 15 mg/L. Biofilm was estimated by the values of the polysaccharides and protein attached to the support vs time (Figs. 3 and 4, respectively). Removal efficiency was calculated as (CODin – CODout)/CODout.

Figure 5 shows the evolution of the nitrification process. These data indicate that the ammonia was converted into nitrate and nitrite. The results of COD reduction concentration vs time for the denitrification process are presented in Fig. 6; mean values fell from 173 to 95 mg/L. Figure 7 shows

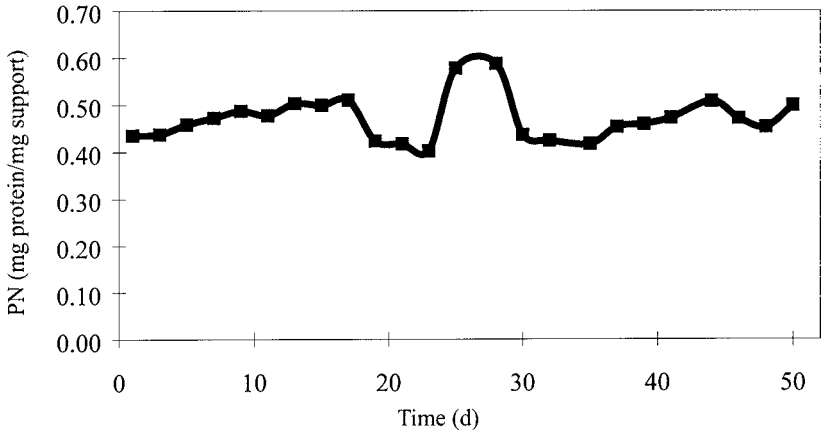

Fig. 4. Variation in protein (PN) content in the attached biofilm with reactor operation time.

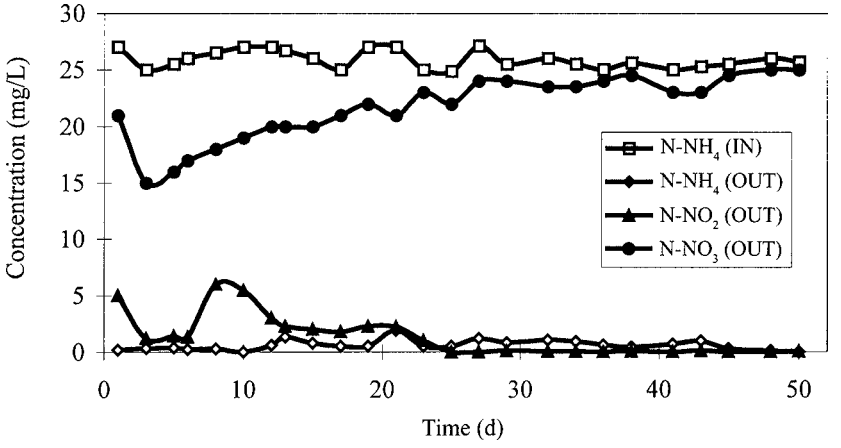

Fig. 5. Evolution of nitrification process.

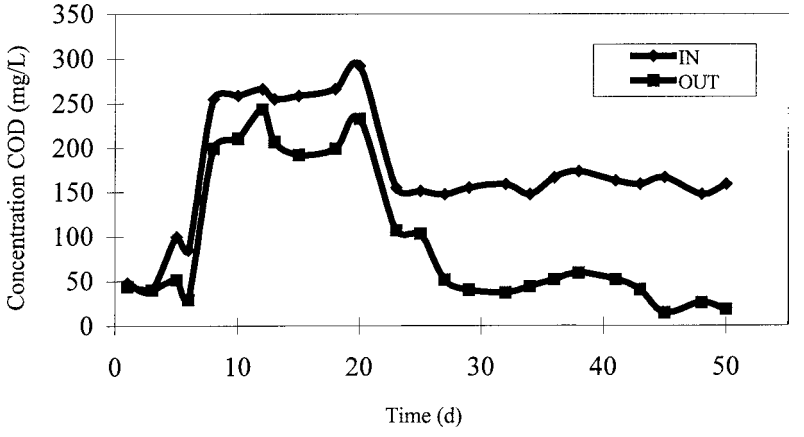

Fig. 6. COD reduction concentration vs time: denitrification process.

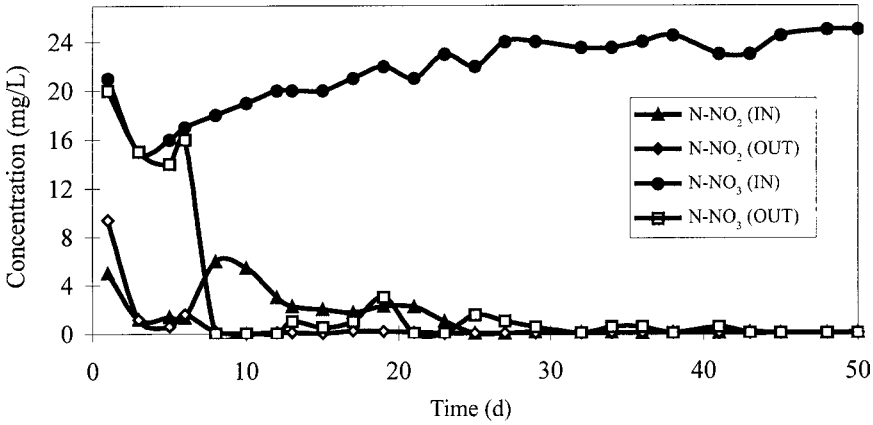

Fig. 7. Evolution of denitrification process.

the evolution of the denitrification process, in which inhibition of the process was verified between 1d and 6d. After 6d, additional carbon source (glucose) was added, and mean values fell from 148 to 292 mg/L. With a sufficiently high concentration of carbon, the nitrate and nitrite were converted to nitrogen gas.

Discussion

Throughout nitrification no organic substance was needed; however, some organic compounds may be assimilated to a limited extent. Perhaps the microbiologic oxidation of organic carbon, contained in the substrate that fed the nitrification process, was also produced by nitrifying organisms (autotrophic), even though most of it was produced by heterotrophic organisms from the fluidized-bed reactor. This hypothesis, though, would need further experiments to be confirmed.

It is a known fact that nitrification takes place at a considerably slower reaction rate than microbial oxidation of organic carbon *(3)*. To induce and increase nitrification, it was necessary to operate the fluidized bed with a high hydraulic retention time (4 h).

The dissociation balance for ammonium-ammonia is dependent on the temperature and pH. Thus, it is necessary to control the pH and temperature in the nitrification process. According to Abeling and Seyfried *(14)*, the nonionized forms of ammonium and nitrate have—as ammonia (NH_3) and as nitrous acid (HNO_2)—an inhibition effect on the *Nitrosomonas* and on *Nitrobacter*.

In the present study, we observed that all the ammonia consumed was converted into nitrate or nitrite and a small amount used for assimilation into nitrifiers. In terms of the performance of a denitrification system, optimal pH and anoxic conditions are important environmental conditions. A nonoptimal pH condition will prolong the lag phase of nitrite reductase in the denitrification process. Limiting of the carbon source would

Table 2
t-Test for Dependent Sample of Fluidized-Bed Inflow and Outflow

	COD (mg/L)	N-ammonium (mg/L)
Average values of the reactor inflow	204.88	25.91
Average values of the reactor outflow	15.06	0.59
p^a	0	0

[a]Marked differences are significant at $p < 0.05$.

Table 3
t-Test for Dependent Sample of Denitrification Bioreactor Inflow and Outflow

	COD (mg/L)	N-nitrite (mg/L)	N-nitrate (mg/L)
Average values of the reactor inflow	173.81	1.40	21.56
Average values of the reactor outflow	95.66	0.54	3.00
p^a	0.00	0.03	0.00

[a]Marked differences are significant at $p < 0.05$.

cause the accumulation of nitrate, which is dangerous to health. Therefore, we must be able to supply enough carbon source to reach a high efficiency of nitrite and nitrate removal of about 98%. A small amount of nitrite and nitrate incorporated into the sludge was considered, following the assumption that no stripping out occurs in the denitrification bioreactor.

The biologic fluidized bed has been shown to be technically viable for an effluent that needs upgrading to complete nitrification.

A *t*-test was carried out to evaluate the efficiency of the process of COD, nitrification, and denitrification removal. The *t*-tests for matched data were carried out to verify whether the inlet media values (μ_{in}) were equal to the outlet media values (μ_{out}):

$$H_0: \mu_{in} = \mu_{out}$$
$$H_1: \mu_{in} \neq \mu_{out} \ (\mu_{in} > \mu_{out})$$

in which H_0: is the null hypothesis and H_1: is the no null hypothesis. Tables 2 and 3 show the inlet and outlet media values of the fluidized-bed reactor and of the complete mixed bioreactor. They also show the significance levels (p) of the values.

All p values are <0.05. This indicates that for all the variables, the treatments were efficient, once the inlet media values of the process were statistically greater than the outlet ones.

Conclusion

The results indicate that the nitrification and denitrification processes in series were efficient for reducing ammonia in the treatment of effluents.

The nitrification process reached efficiencies of about 98%. The best results related to the efficiency of the denitrification process were obtained when a carbon source (glucose) was supplemented to the process. COD removal was satisfactory, showing the efficiency of the three-phase fluidized-bed bioreactor in the biologic treatment of effluents. The results indicated an efficiency of 86–98% COD removal.

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