JKAU: Eng. Sci., **vol. 6**, pp. 137-151 (1414 A.H./1994 A.D.)

Nitrate Removal from Drinking Water Using Anoxic Packed Reactor

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ABSTRACT. An anoxic upflow packed-bed reactor was operated to denitrify water having a nitrate-nitrogen concentration of 120 mg/l. A methanol to nitrate ratio of 0.55, *i.e.*, less than the stoichiometric requirements was utilized throughout the present investigation. The effects of hydraulic retention time, oxygen tension, and nutrient concentrations on the performance of the process were investigated. The minimum hydraulic retention time needed to meet the nitrate standard in drinking water was 6 hours. A higher hydraulic retention time of 12 hours was needed to meet the nitrate standard of 1 mg/l as nitrogen. No detectable methanol concentration was found in the effluent of the reactor when the system was operated at a hydraulic retention greater than or equal to 9 hours. The results of the study indicated that once denitrification was established, the dissolved oxygen concentrations in the feed water did not affect the performance of the process and effective denitrification occurred at a dissolved oxygen concentration as high as 8 mg/l. Thus, oxygen tension was not found critical for the process. The phosphorus concentration in the feed did not significantly affect the efficiency of the system. High denitrification efficiencies were achieved at phosphorus concentrations in the feed as low as 0.05 mg/l. Based on the findings of this research and other evidence, a biochemical model for the denitrification process is proposed.

KEY WORDS. Biochemical model, Denitrification, Drinking water, Nitrate, Nitrite, Oxygen tension, Phosphorus.

1. Introduction

Nitrate concentration of groundwater resources in excess of the drinking water standard of 10 mg/l as nitrogen, set by the Saudi Arabia Standard Organization (SASO),

is becoming a major problem in some parts of Saudi Arabia. Nitrate contamination of drinking water is also reported in many areas of the U.S. and several parts of Europe[1-2]. Nitrate has been shown to cause methamoglobinemia (blue baby syndrome) as it reacts, after reduction to nitrite, with hemoglobin in blood and produces methamoglobin which cannot transfer the oxygen to the cell[3]. Nitrate is also suspected to produce carcinogenic nitrosamines[4].

Several treatment alternatives are available for nitrate removal including ion exchange, reverse osmosis, chemical reduction, electrodialysis, distillation and biological processes[1,5-6]. Denitrification is considered as one of the most economical methods of nitrate removal from drinking water[7]. In denitrification, nitrate serves as a terminal electron acceptor in the absence or presence of limited oxygen concentrations (anoxic condition). A wide variety of microorganisms can reduce nitrate to nitrite (nitrate respiration) in metabolic reactions catalyzed by the enzyme nitrate reductase. A lesser number of bacteria can reduce nitrate all the way to elemental nitrogen (denitrification). Both the process of nitrate respiration and denitrification are collectively referred to as dissimilatory nitrate reduction. Type and characteristics of bacteria that are capable of denitrifying have been summarized by others[1,8]. Since groundwaters are usually low in organic carbon, an external carbon source (substrate) is needed for the denitrification processes. A variety of substrates have been used as the electron donor such as methanol, acetic acid, ethanol, carbon monoxide, methane, thiosulfate and hydrogen. The following stoichiometric relationships for the utilization of methanol in wastewater denitrification processes have been formulated[9] :

Energy reaction

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad (1)$$

Synthesis reaction

$$14CH_3OH + 3NO_3^- + CO_2 + 3H^+ \rightarrow 3C_5H_7O_2H + 19H_2O \quad (2)$$

Overall, empirical, reaction,

$$1.08CH_3OH + NO_3^- + H^+ \rightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O \quad (3)$$

when both nitrite and dissolved oxygen are present, the overall methanol requirement for wastewater denitrification is described by the following empirical equation[9].

$$C_m = 2.47N_o + 1.53N_1 + 0.87D_o \quad (4)$$

where

C_m = Required methanol concentration, mg/l.
N_o = Initial nitrate-nitrogen concentration, mg/l.
N_1 = Initial nitrite-nitrogen concentration, mg/l.
D_o = *Initial* dissolved-oxygen concentration, mg/l.

The stoichiometric relationships for various substrates in the denitrification process are summarized elsewhere[1,9].

Although a number of research studies have been conducted on the subject, several aspects of the process such as optimization of reaction conditions with respect to substrate and nutrient concentrations, oxygen tension and pH need to be investigated[1]. In this study, the effects of both oxygen tension and nutrient concentration (phosphorus) on the denitrification of high nitrate drinking water, using static bed upflow reactor were investigated. The effects of the hydraulic retention time (HRT) on the effluent nitrate and nitrite concentrations were studied. Consequently, the optimum hydraulic retention time for the process was determined. Based on the results of this study, and other evidence, a possible biochemical model for denitrification of water is proposed.

Material and Methods

Experimental System

A laboratory-scale anoxic upflow reactor was constructed using a 150 mm diameter and 1500 mm long PVC pipe (Fig. 1). The column was packed with 35 mm diameter plastic balls. Four sampling ports were located along the reactor at 300 mm intervals. Two positive displacement peristaltic pumps were utilized to operate the system, one used to pump the water with high nitrate concentration and the second to pump the substrate.

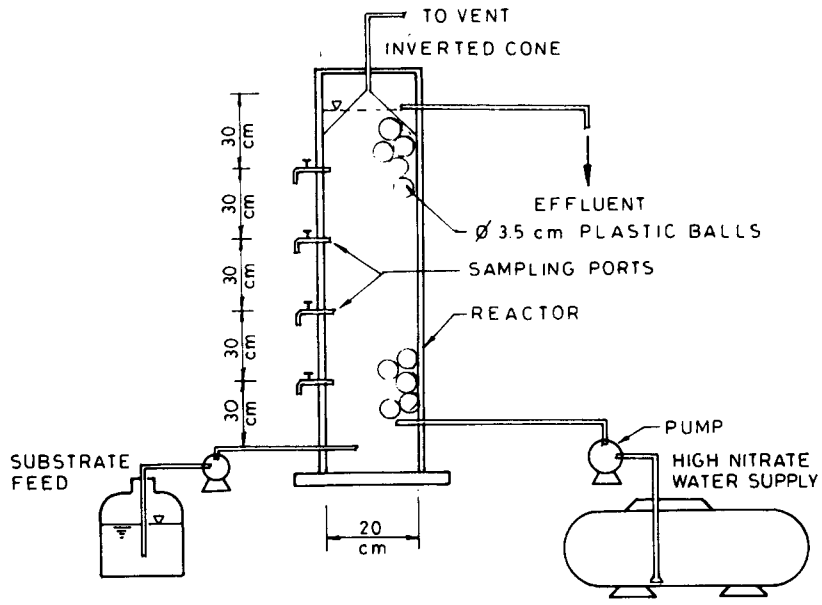

FIG. 1. Schematic diagram of the experimental system.

Influent Water

Tap water with a nitrate concentration of 520 mg/l was used throughout the study to simulate the majority of the groundwaters analyzed in several areas of Saudi Arabia. Potassium nitrate was used as the nitrate source. The desired phosphorus concentration in the feed water was maintained using a potassium diphosphate solution. The D.O. of the feed was reduced to less than 1 mg/l, when needed, by bubbling nitrogen gas for about 20 minutes, through the feed water using four stone diffusers. A sufficient amount of methanol solution was added to maintain a methanol to nitrate ratio of 0.55 throughout the study. This ratio was selected below the stoichiometric level of 0.57 to avoid an excess concentration of methanol in the effluent.

Start-up and Operation

The reactor was seeded using an activated sludged sample obtained from anoxic reactor of a laboratory scale anaerobic-anoxic-oxic (A^2/O) activated sludge system. After seeding, the system was operated at a flow rate of 35 ml/min for 3 months before significant nitrate removal, more than 90%, was accomplished. For each experiment, the steady-state conditions were reestablished. Steady-state conditions were considered when the effluent nitrate concentrations were consistent over a period of one week. The main variables during this study were the hydraulic retention time and the dissolved oxygen plus phosphorus concentrations in the feed.

During the first phase of the study the system was operated at various flow rates to determine the optimum hydraulic retention time for the system. The D.O. in the feed was reduced to less than 1 mg/l and the phosphorus concentration was maintained at 1 mg/l. In phase 2 of the study, the system was operated under aerobic conditions, that is, the D.O. of the feed was not reduced. In phase 3 the system was also operated under aerobic conditions, but at various phosphorus concentrations in the feed (Table 1). The range of phosphorus concentrations studied (0.05 to 1.0 mg/l) was chosen based on the average volatile suspended solids measured during phases 1 and 2 of the study and utilizing the approximate formula for cell tissue, $C_5H_7NO_2P_{0.083}$ to estimate phosphorus requirements for bacterial biosynthesis. The temperature ranged from 20-22°C throughout the study.

Analytical Methods

During the course of the study, influent and effluent samples were analyzed for nitrate, nitrite, volatile suspended solids, alkalinity, pH, turbidity and total coliforms. Nitrate was determined using the ultraviolet spectrophotometric screening method (section 418(19), APHA[10], 1980). Nitrate was measured by the colorimetric technique, using diazotized sulfanic acid (section 419, APHA[10], 1985). A YSI model 54A oxygen meter was used for monitoring the dissolved oxygen concentrations. The pH was measured using a Model 610A Fisher pH meter. The total coliforms was determined by the membrane filter technique. Other tests were also performed according to the procedures described in the APHA[10], 1985. Methanol con-

TABLE 1. Phases of the study.

Phase	Run	HRT, hours	NO_3^- – N mg/l	M : N*	PO_4^{---} – P mg/l	D.O. Duration	
						mg/l	Days
Start - up		22	120	0.55	1.0	0 - 1	90
I	1	22	120	0.55	1.0	0 - 1	20
	2	17	120	0.55	1.0	0 - 1	22
	3	12	120	0.55	1.0	0 - 1	26
	4	9	120	0.55	1.0	0 - 1	20
	5	6	120	0.55	1.0	0 - 1	18
	6	3	120	0.55	1.0	0 - 1	12
II	1	22	120	0.55	1.0	8 - 9	21
	2	17	120	0.55	1.0	8 - 9	18
	3	12	120	0.55	1.0	8 - 9	18
	4	6	120	0.55	1.0	8 - 9	10
III	1	22	120	0.55	0.50	8 - 9	14
	2	22	120	0.55	0.10	8 - 9	12
	3	22	120	0.55	0.05	8 - 9	12

*Methanol : Nitrate.

centration in the effluent of the reactor was determined using a Model 5840-A Hewlett Packard Gas Chromatograph equipped with flame ionization detector and 1% SP-1000-80/100 Casbopack-C column.

Results and Discussion

To facilitate presentation of the results, a separate discussion of the results obtained from effect of hydraulic retention time, oxygen tension and phosphorus concentration on the denitrification process will be presented. The overall system performance with respect to effluent pH, alkalinity, suspended solids and total coliforms during the course of investigation will also be presented.

Hydraulic Retention Time

High nitrate removal efficiencies (98 to 99%) were achieved when the system was operated at hydraulic retention greater than or equal to 9 hours. The effect of the hydraulic retention time on the steady state effluent nitrate-nitrogen concentrations is presented in Fig. 2. About 92% of the influent nitrate concentration of 120 mg/l was removed in a hydraulic retention time as low as 6 hours, and with a methanol to nitrate ratio of 0.55. When the hydraulic retention time was reduced to 3 hours, only 60% of the influent nitrate was removed. Methanol concentration in the effluent of the reactor was essentially zero when the system was operated at a hydraulic retention greater than or equal to 9 hours (Fig. 2). Because of its toxicity, methanol concentration in the treated water should be seriously considered when methanol is used as the carbon source for denitrification process of drinking waters. The curve which

describes the relationship between the hydraulic retention time and the effluent nitrate concentrations (Fig. 2) is very similar to the profiles of nitrate concentrations along an anoxic reactor reported by others[2]. The same researchers reported that system operation at a hydraulic retention time of 9 hours resulted in a complete removal of nitrate from water having nitrate-nitrogen concentration of 100 mg/l. However, no information was given on the effluent nitrite concentration. Because of its high toxicity, a nitrite accumulation problem is a concern in denitrification operations[1].

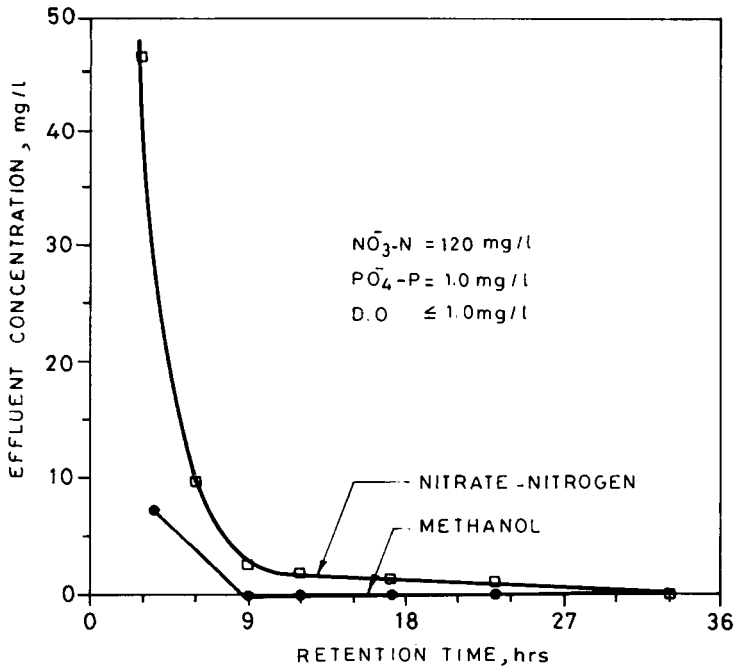

FIG. 2. Effluent nitrate-nitrogen and methanol concentrations at various hydraulic retention times.

Figure 3 illustrates the variations of the effluent nitrite concentrations as a function of the hydraulic retention time of the system. The nitrite removal pattern is very similar to that obtained for nitrate concentration profile. When the hydraulic retention time was less than 12 hours, the effluent nitrite-nitrogen concentration was more than 1.0 mg/l, which is the nitrite standard in drinking water. At a hydraulic retention time of 22 hours or more, the effluent nitrate was essentially zero. The present investigation showed that about 92% of the influent concentration of 120 mg/l nitrate-nitrogen can be removed when the system is operated at a hydraulic retention time as low as 6 hours (Fig. 2). However, at that retention time the effluent nitrite-nitrogen concentration of 2.7 mg/l was above the 1 mgN/l (MCL) set by the U.S. Environmental Protection Agency (Fig. 3). Hence a hydraulic retention time of 12 hours is suggested by this research (Fig. 2 and 3) to reduce nitrate-nitrogen concentration as

high as 120 mg/l to the allowable nitrate and nitrite concentrations. In summary, excellent denitrification efficiency of high nitrate drinking water could be obtained without supplying enough methanol provided that the appropriate hydraulic retention time is allowed. Both nitrite accumulation problems and organic contamination of the denitrified water could be avoided by utilizing a methanol to nitrate ratio below the stoichiometric requirements while operating the system at the appropriate hydraulic retention time. Variation of nitrate and nitrite concentrations along the

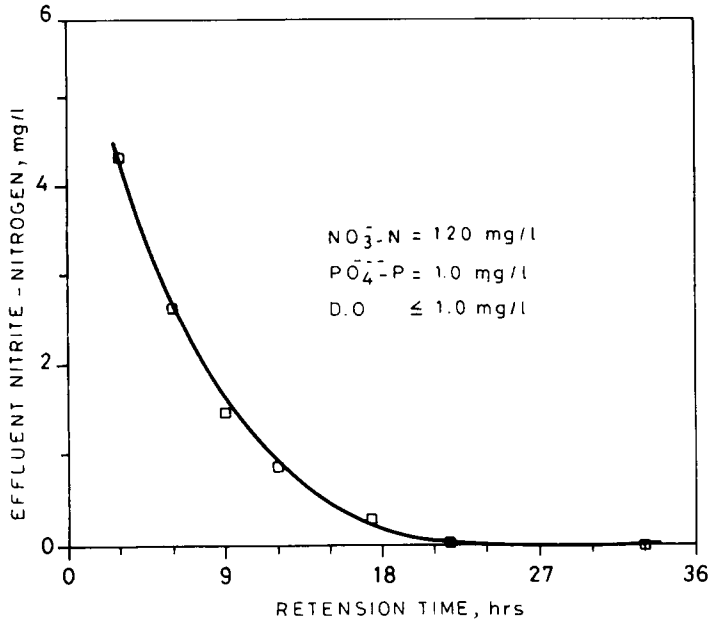

FIG. 3. Effluent nitrite concentrations at various hydraulic retention times.

column at a hydraulic retention time of 6 hours is shown in Fig. 4. The pattern of the nitrate profile along the column is very similar to that reported by others[2]. Most of the nitrate was removed in the first third of the column. Beyond that little nitrate reduction occurred, which suggested that the upper layers of the bed were not as effective as the bottom layers. The results also suggested that it is advantageous to operate anoxic filters in series for better utilization of the process. The nitrite concentration profile showed a maximum concentration at a column depth of 50 cm from the inlet followed by a decrease in the nitrite concentration towards the effluent of the filter.

Oxygen Tension

The effect of the dissolved oxygen concentrations on the effluent nitrate and nitrite concentrations at various hydraulic retention times is summarized in Fig. 5. As shown in the figure, the dissolved oxygen concentration in the influent water caused

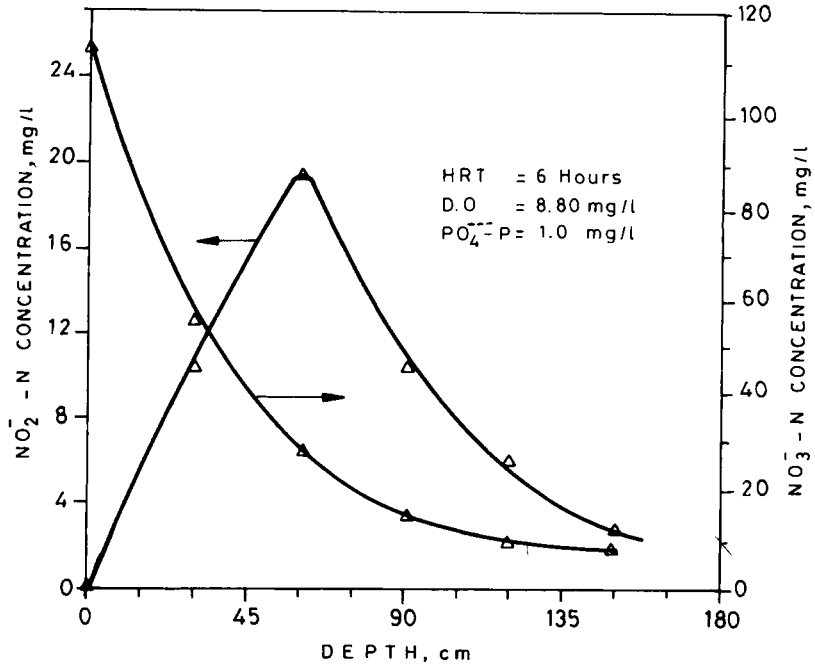

FIG. 4. Profiles of nitrate and nitrite concentrations along the reactor.

no effect on the performance of the denitrification process. The results of this study (Fig. 5) suggested that once a system establishes nitrate removal the dissolved oxygen concentration in the water does not have any effect on the effluent nitrate and nitrite concentrations. Up to date, no information is reported on the effect of dissolved oxygen concentration, or oxygen tension, on the performance of drinking water denitrification processes. Most of the studies on the effect of oxygen on the denitrification process were conducted using pure culture under specific environmental conditions.

While many investigators considered oxygen as an inhibitor of denitrification, several investigators have reported that in microbial cells with preformed nitrate reductase enzymes, the presence of dissolved oxygen may prevent further-synthesis of the enzyme, but does not cause it to be inactivated[11-13]. Some species have been reported to denitrify in systems with oxygen tensions as high as 153 mm of mercury (0.2 bar)[11]. Also, there is evidence that both nitrification and denitrification in soil can occur simultaneously[12-13]. It was also reported that nitrite could be reduced by some species in a concentration of dissolved oxygen as high as 8 mg/l[11.14]. Thus, several reports supported the findings of this research, although they were carried out using pure cultures which may not represent a mixed culture in an experimental system.

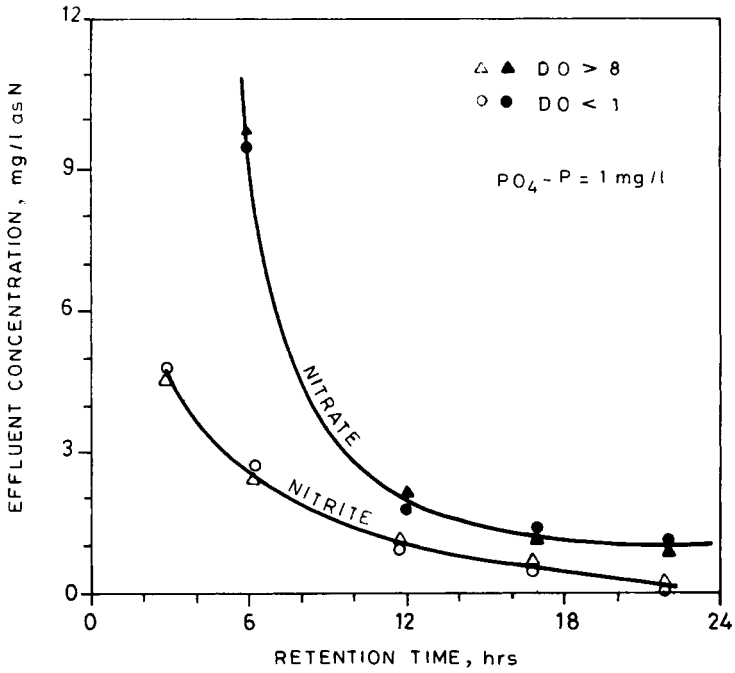

FIG. 5. Effect of D.O. concentrations on the effluent nitrate and nitrite concentrations.

However, the species diversity in a water denitrification system is expected to be low as compared with that of wastewater denitrification.

Phosphorus

Figure 6 shows the effect of phosphorus concentrations in the feed water on the performance of the denitrification process, *i.e.*, the effluent nitrate and nitrite concentrations at a hydraulic retention time of 22 hours. The difference between the mean values of effluent nitrate concentrations obtained during the course of operation for both the low and high phosphorus concentrations in the feed water was tested at 5 percent significance level with the student t-test. From results obtained, it was concluded that phosphorus concentration in the feed does not have a significant effect on the effluent nitrate concentrations. The same test was also applied to compare the collected data for effluent nitrite concentration. It was also found that the effect of phosphorus on the effluent nitrite is insignificant at 5 percent significant level.

System Performance

Table 2 summarizes the performance of the system during the study period with respect to total coliforms, suspended solids, turbidity, alkalinity and pH in the effluent of the reactor. The data indicated that the process resulted in a relatively high col-

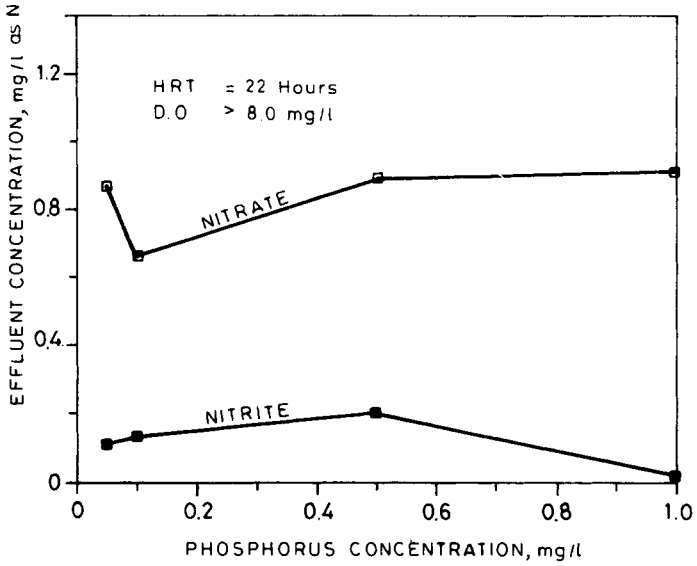

FIG. 6. Effect of phosphorus concentration on the effluent nitrate and nitrite concentration.

iforms concentrations (0-11 coli/100 ml). Although no coliforms were present in effluent water for several experiments, the water can not be considered safe for drinking and disinfection should be considered as a post-treatment. The total suspended solid concentrations and the turbidity in the effluent were also high suggesting that both solids and colloidal removal processes should also be included in the

TABLE 2. Summary of the experimental data.

Phase	Run	Total coliforms / 100 ml		V.S.S. mg/l		Turbidity N.T.U.		Alk. mg/l as $CaCo_3$		pH	
		Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.
1.	1	0.0	11	0.0	14	0	4	37	434	7.6	9.1
	2	0.0	9	0.0	9	0	3	37	443	7.1	9.3
	3	0.0	0.0	0.0	18	0	5	32	392	7.8	9.3
	4	0.0	0.0	0.0	–	0	–	38	440	7.9	9.2
	5	0.0	0.0	0.0	33	0	10	32	440	7.9	9.2
	6	0.0	0.0	0.0	26	0	8	37	310	7.6	9.0
2.	1	0.0	10	0.0	10	0	4	37	410	7.1	9.5
	2	0.0	8	0.0	8	0	2	37	430	7.9	9.4
	3	0.0	0.0	0.0	14	0	5	34	420	7.7	9.0
	4	0.0	0.0	0.0	44	0	7	37	440	7.2	9.3
3.	1	0.0	5	0.0	10	0	2	38	399	7.5	9.2
	2	0.0	0.0	0.0	18	0	5	37	416	7.9	9.4
	3	0.0	0.0	0.0	40	0	5	34	410	7.1	9.3

post-treatment. Alkalinity of the effluent water was in the range of 310 to 440 mg/l as $CaCO_3$ which should be considered in the design and selection of the required post-treatment.

Although no methanol concentrations in the effluent water were found when the system was operated at a hydraulic retention greater than or equal to 9 hours, still the posttreatment should include a process of organic removal such as GAC (granular activated carbon) to insure a continuous organic-free effluent water.

Biochemical Model

It is believed[15-17] that nitrate is reduced to nitrite by the enzyme nitrate reductase, utilizing electrons from cytochrome B. The nirtrite is further reduced to nitrogen gas, by the enzyme nitrite reductase, utilizing electrons from cytochrome C. Based on these reports and the results of this study, a possible biochemical model for water denitrification is proposed and illustrated by Fig. 7. The proposed model is intended to provide a possible biochemical pathways to explain an observation reported by this research, *i.e.*, how both oxygen and nitrate can be utilized as electron donors in water denitrification processes.

The proposed model suggests, mainly, that both oxygen and nitrate can be used simultaneously as electron acceptors in water denitrification processes. The proposed model also suggests that nitrate can only accept electrons from cytochrome B and nitrite accepts electrons from cytochrome C. In the proposed model, nitrate is reduced to nitrite by the enzyme nitrate reductase utilizing electrons from cytochrome B. The produced nitrite is further reduced to nitrogen gas by the enzyme nitrite reductase utilizing electrons from cytochrome C. At the same time electrons could pass to oxygen from cytochrome A. Thus, a total of three moles of ATP are formed per one mole of NADH oxidized. The proposed model may explain the effective denitrification observed in the presence of high dissolved oxygen.

Summary and Conclusion

The anoxic upflow packed-bed reactor was found very effective in removing nitrate from drinking water. About 92% of the influent nitrate-nitrogen concentration of 120 mg/l was removed in a 6 hours retention time and using a methanol to nitrate ratio of 0.55, *i.e.*, less than the stoichiometric requirements. However, a higher retention time of 12 hours was needed to reduce the effluent nitrite concentrations to the allowable limit of 1 mgN/l. Methanol free effluent can be obtained when the system is operated at a hydraulic retention greater than or equal to 9 hours and a methanol to nitrate ratio of 0.55.

After establishing denitrification, the system performance with respect to the effluent nitrate and nitrite concentrations was not affected by the dissolved oxygen concentrations in the feed water. Effective denitrification was observed at a dissolved oxygen concentration as high as 8.8 mg/l. Thus, oxygen tension was not found critical for the denitrification process. Phosphorus concentrations in the feed water

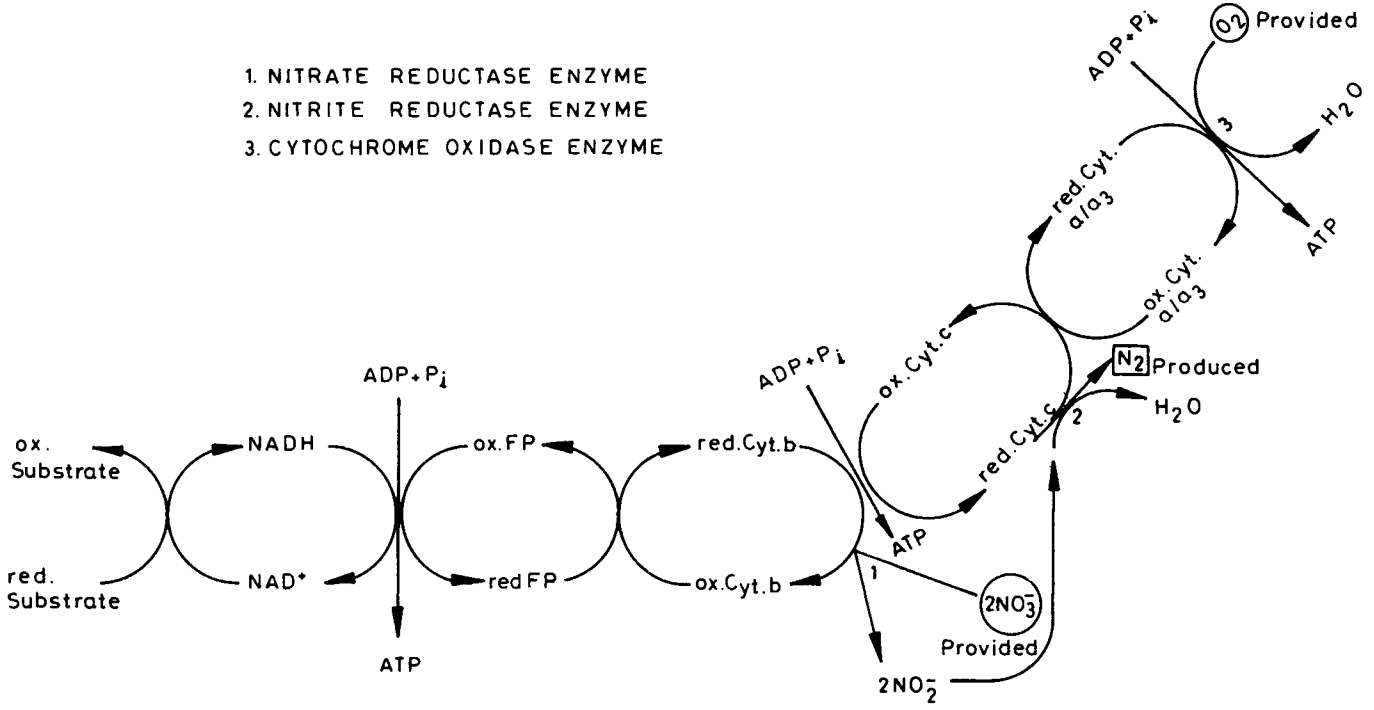

FIG. 7. Proposed biochemical model for bio-dentrification. (Cyt.: Cytochrome, FP: Flavoprotein, ox: Oxidized, red: Reduced).

showed no significant effect at a 5% confidence level on the denitrification process, *i.e.*, effluent nitrate and nitrite concentrations. A high nitrate removal efficiency of 99% was observed at phosphorus concentration as low as 0.05 mg/l.

The results of the study also suggested that the posttreatment for the denitrification process of drinking water should include processes for suspended solids and colloidal particles removals, organic removal and disinfection.

Nomenclature

A^2/O	Anaerobic – anoxic – oxic.
ADP	Adenosine diphosphate.
ATP	Adenosine triphosphate.
Cyt	Cytochrome.
D.O.	Dissolved oxygen.
F.P.	Flavoprotein
GAC	Granular activated carbon.
HRT	Hydraulic retention time.
MCL	Maximum contaminant level.
NAD	Nicotinamide adenine dinucleotide.
NTU	Nephelometric turbidity units.
Pi	Phosphate.

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إزالـــة النيـــترات من ميـــاه الشرب باستخـــدام مفاعـــل بيـــولوجي

زياد حمزة أبو غرارة

قسم الهندسة المدنية ، كلية الهندسة ، جامعة الملك عبد العزيز

جـــــدة - المملكة العربية السعودية

المستخلص . تم تشغيل مفـاعل بيولوجي لإزالة النيترات من مياه الشرب ذات تركيز ٥٢٠ مجم/لـتر . وتمت دراسـة تأثير كلٍ من مدة الاحتباس ، وشد الأوكسجين وتركيز الفسفور على أداء هذا النظام .

دلت نتائج هذه الدراسة على أنه يجب تشغيل النظام بمدة احتباس لاتقل عن ٦ ساعات لخفض تركيز النترات إلى الحد المسموح به ، وذلك عند استخدام نسبة ميثانول إلى نيترات ٠,٥٥ . إلا أن المدة اللازمة لخفض تركيز النتريت إلى أقل من ١ مجم/نتروجين/لتر يتطلب مدة احتباس أطول وقدرها ١٢ ساعة .

كما دلت الدراسة على أنه بعد تحقيق المعالجة البيولوجية للنترات فإن تركيز الأوكسجين في المياه المعالجة لا يؤثر على كفاءة النظام ، حيث يمكن الحصول على إزالة فعّالة للنترات عند تركيز للأوكسجين في المياه المعالجة يصل إلى ٨ مجم/لتر . كما دلت الدراسة على أن تركيز الفسفور في المياه المعالجة ليس له أي تأثير على كفاءة النظام .

وبنـاءً على نتـائج هذه الدراسة ونتائج بعض الدراسات الأخرى ، تم اقتراح نموذج بيوكيميائي لوصف ما يحدث في عملية إزالة النترات بيولوجيًا من المياه .