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ИСПОЛЬЗВАНИЕ *OPUNTIA IMBRICATA* В КАЧЕСТВЕ ПОДЛОЖКИ АНАЭРОБНОЙ ПЛЕНКИ В UASB-РЕАКТОРЕ ДЛЯ ДЕНИТРИФИКАЦИИ ПРИ ВЫСОКОЙ КОНЦЕНТРАЦИИ НИТРАТА

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Исследовано влияние времени гидравлического задержания (ВГЗ) и концентрации нитрата на процесс денитрификации в реакторе с восходящим потоком через слой анаэробного ила (UASB-реактор) с биопленками, выращенными на *O. imbricata.* Процесс биологической денитрификации изучали на модельных сточных водах при ВГЗ 12, 18, 25, 26, 31,5 и 62 ч. Концентрация нитрата и ХПК в поступающей жидкости составляли соответственно 1,5 и 3,5 г/л. Реактор UASB-типа, заполненный *Opuntia imbricata,* с затравкой из гранулированного ила показал лучшие результаты по сравнению с UASB-реактором, содержащим только гранулированный ил. У последнего реактора наблюдалась низкая скорость денитрификации при всех исследуемых ВГЗ. Время гидравлического задержания оказалось одним из наиболее важных операционных параметров в анаэробных реакторах при удалении нитрата, оптимальное значение ВГЗ для денитрификации составило 25 ч. Наибольшая скорость денитрификации 255 мг NO_3/л/ч наблюдалась для UASB-реактора, заполненного *Opuntia imbricata,* с затравкой из гранулированного ила. Показано, что использование *Opuntia imbricata* в качестве носителя для биопленочных реакторов значительно (в десять раз) увеличило эффективность денитрификации и удаления ХПК.

INTRODUCTION

Increased nitrate contamination of available and future drinking water sources constitutes an important and rapid growing environmental problem in many countries. Concern for possible health consequences has led to the recommendation of an upper limit of 10 mg/l of NO_3–N/l in water for human consumption [9], and the development of a number of techniques for lowering nitrate concentration to acceptable levels. Among these techniques, microbial remotion (denitrification) stands out as being the most economical and environmentally sound, as well as being feasible on a large scale [17]. Denitrification is the reduction of nitrate to a gaseous product, usually nitrogen gas, through a sequence of enzymatic reactions. Bacteria capable of denitrifying are ubiquitous in nature [10] and have the capacity to use nitrate in place of oxygen as terminal electron acceptor in their respiratory processes under anoxigenic conditions. Microbial nitrate remotion treatments take advantage of this anaerobic respiration process and aim to maximize the rates of nitrate consumption by assuring a steady supply of carbon and energy sources which are usually simple organic compounds [17]. The nature of the carbon source determines the route of nitrate reduction. Indeed, the competition between denitrification and dissimilatory nitrate reduction to ammonium in media with low dissolved oxygen concentrations seems to be largely controlled by the nature of the electron donor [5]. Improving biological systems to eliminate nitrogen compounds is one of the main challenges in the modern waste water treatment. The development of stable microbial populations with great activity is the goal to obtain better results in the residual water treatment [4] and the use of biofilms it is a good alternative. Recently, immobilized-cell processes have been receiving increasing attention in the field of wastewater biodenitrification [7]. In this investigation it Coyonoxtle (*Opuntia imbricata*) was used as support for biofilm. Coyonoxtle is an abundantly available shrub in the northeast region of Mexico. The objective of this work was to investigate the effects of HRT and nitrate concentration on denitrification process in an USAB reactor packed with support mentioned above.

MATERIALS AND METHODS

Inoculum

An anaerobic granular sludge coming from a mesophilic full-scale UASB reactor treating wastewater from a beer industry was used like inoculum. The sludge which was added to the reactor with coyonoxtle was liquefied to help biofilm formation. In the reactor without coyonoxtle the inoculum was the same granular sludge without liquefying; the inoculum volume was of 200 ml in both cases

Wastewater composition

In order to evaluate the nitrate removal and the consumption of the organic matter was necessary to prepare a synthetic wastewater with the next composition 1.5 g of nitrate/l (except for studying the effect of the nitrate concentration), 3.5 g of COD/l, pH 7.0±2 and added of the one microelements trace solution (potassium, iron, magnesium, manganese and molybdenum).

Carrier preparation

For the study of the nitrate removal in reactor UASB with biofilm, was selected a natural carrier (the dry and ligneous part of the *Opuntia imbricate* cactacea) it was washed using potable water and distilled water, before to add the coyonoxtle at the reactor it was dried at 50°C by twelve hours and weighed

Chacaracteristic of the reactors

The synthetic wastewater treatment system consisted in the use of three thermostated reactors UASB; with. 3.4 l capacity and equipped with twelve sampling ports along the reactor height. One of the reactors (R1) was packed with natural carrier (*Opuntia imbricata)* (14 cm length × 1.5–3 cm diameter) and containing biofilm from granular sludge; the second (R2) contained only granular sludge and the third control reactor (R3) it only containing *Opuntia imbricata* without granular sludge.

Reactors operation and sampling

All reactors were fed with the same synthetical water by means of the use a peristaltic pump at different feeding flows depending on HRT studied (i.e. for a HRT of 24 hours the feeding flow was of 2.37 ml min^{-}) The temperature (37°C) in the reactor was thermostatically controlled by pumping water from the thermostat through the jacket surrounding the reactor

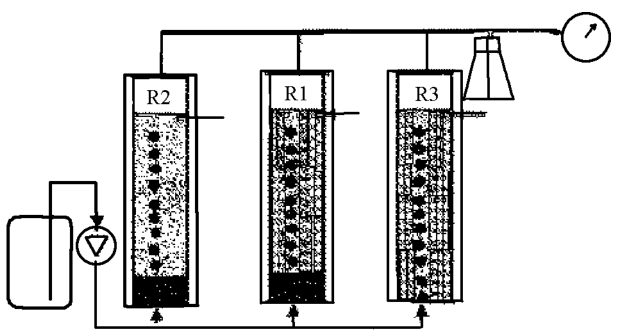

Fig. 1. Reactors structures and operating conditions

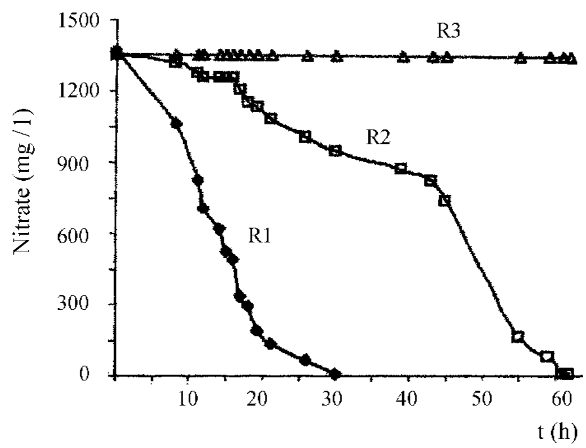

Fig. 2. Comparative kinetic of denitrifying reaction in three column reactors: (R1) packed with *Opuntia imbricate* and consortium; (R2) Consortium without support; (R3) Reactor packed with *Opuntia imbricata* only

[16]. The sampling of the reactors was made by means of the use of keys in the selected ports (four ports) as too in the influent and effluent (the reactor structures and operating conditions are shown in Fig. 1)

Analytical procedures

The denitrifying activity was determined by measurement of NO_3^-–N and NO_2^-–N concentration present in the synthetic wastewater by the spectrophotometric method of chromotropic acid and Griess's technique respectively, for measurement of organic matter consumption was used the method of chemical oxygen demand (COD) as described in *Standard Methods* [3]. Methane and molecular nitrogen were determined by gas chromatography (GC) on a Varian GC model 3400, equipped with a thermal conductivity detector and using helium as the carrier gas. Measurement of pH was performed electrometrically using a potentiometer equipped with an electrode selective (Orion model 710A)

RESULTS AND DISCUSSION

As shown in Fig. 2, we observed that R1 exhibited a rapid nitrate consumption compared to reactor R2. The removal efficiency was 100% for R1 and R2 at 30 h and 62 h HRT respectively. Fig. 3, *a* exhibits the nitrate removal efficiency and the nitrate load rate curves as function of HRT for R1. The nitrate removal efficiency was above 95% at 12 h HRT even at a high nitrate concentration. The denitrifying velocity at different ports of the reactor at 31.5 h HRT is shown in Fig. 3, *b*. It can be seen that velocity increase was rapid up to port 2 and very slow from port 2 to effluent. Fig. 4 shows the denitrifying velocity as function of hydraulic time retention. It is clear that the best velocity was reached at 25 h, although 97% nitrate removal was obtained efficiency at 12 h. Fig. 5

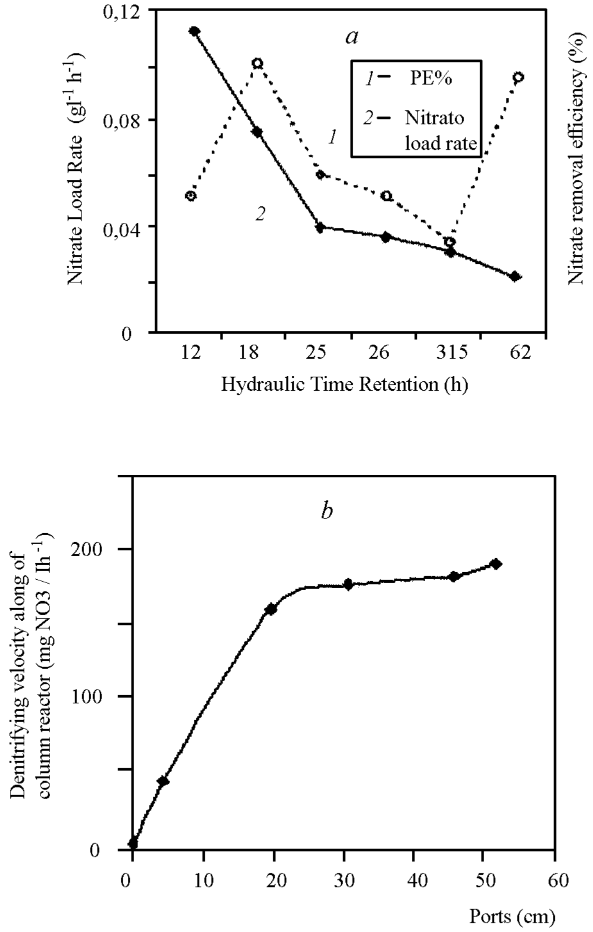

Fig. 3. *a* - Nitrate load rate and removal efficiency of nitrate as function of HRT; *b* - Denitrifying velocity as function of column height at 31.5 h of hydraulic time retention for R1

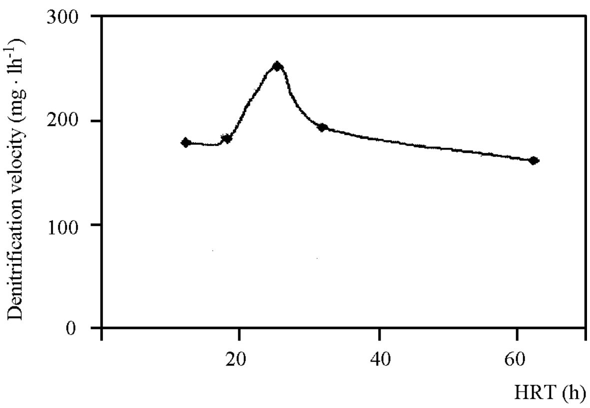

Fig. 4. Effect of hydraulic time residence on denitrifying velocity in the reactor 1

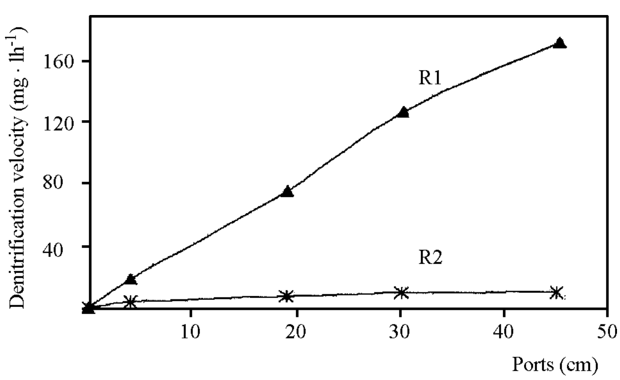

Fig. 5. Denitrifying velocity (12 h HRT) at different ports of R1and R2

Table 1

Comparative values of initial nitrate concentration

Reference	Initial nitrate concentration (mg/l)
This work (2005)	1500
Aboutboul et al. (1995) (1)	5–20
Akuna et al. (1993) (2)	200
Bilanovic et al. (1999) (6)	500
Hendriksen and Ahring (1996) (11)	336
Her and Huang (1995) (12)	50
Mosquerra et al. (2003) (14)	700
Qian et al.(2001) (15)	20
Volokita et al. (1996) (17)	200
Zhang and Verstraete (2001 (18)	300

shows that denitrifying velocity in R1 was ten times that of R2. In the reactors with biofilms, the biomass is fixed on the support offering the advantage of low maintenance, low start up time, easy operation and reduced production of biomass [8]. All these factors seemed to improve the process of nitrate removal when biofilm was present. Fig. 6 shows the denitrifying velocity as function of nitrate concentration in the reactor 1, the reaction order for denitrification was 0.5 and the K_V was 2.68 h^{-1}. It should be observed that the initial nitrate concentration was of 1.5 g/l, which is considerably high when compared to existing studies (Table 1). The denitrifying velocity and activity were better in the R1 at 62 h of HRT (Table 2).

With respect to COD, the effect is not marked although the use of the support improved the removal efficiency, the maximum being 75% for R1. This can be due to the fact that

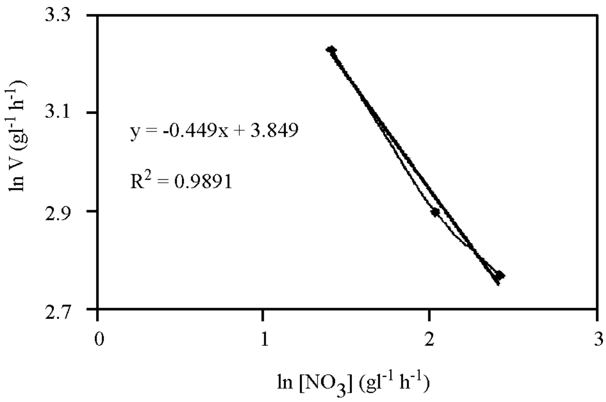

Fig. 6. Denitrifying velocity as function of different initial concentration of ion nitrate

not all of the COD can be used during the denitrification process [15]. Eventually, the efficiency of denitrification will be strongly affected by the loading rate of the external carbon source [13]. In this investigation there was no accumulation of nitrite at none of the HRT. Formation of nitrogen and methane was dependent on HRT. The maximum velocity of formation of N_2 and CH_4 reached were respectively 0.0162 g/l/h and 0.1491g/l/h. Zhang and Verstraete [18] mentioned that the degrees of both methanogenesis and denitrification depended on substrates and types of reactors used. Since the pH of the effluent was 8.0±0.5, it is possible that there was formation of ammonium but this ion was not determined.

Conclusions

The influence of HRT and the use of biofilm on the denitrification process were studied in this investigation. The results show that HRT is one of the most important operating parameters for nitrate removal in anaerobic reactors. It was demonstrated that the use of *O. imbricate* (coyonoxtle) as carrier for biofilm remarkably improved the nitrate removal efficiency by ten times besides substantial improvement in COD removal. Further studies are needed to evaluate the influence of denitrification on methanogenesis with denitrification to optimize the process.

Table 2

Denitrifying velocity and activity at 62 h of HTR in the three reactors

Reactor	HTR (h)	Denitrifying velocity (mg NO_3/ l h)	Denitrifying activity (mg NO_3/ g VSS /d)	g VSS/l
R1	62	166.76	27.8	6
R2	62	13.66	2.28	6
R3	62	0	0	0

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OPUNTIA IMBRICATA AS SUPPORT FOR ANAEROBIC BIOFILM IN AN UASB REACTOR FOR DENITRIFICATION UNDER HIGH NITRATE CONCENTRATION

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The objective of this work was to investigate the effects of hydraulic residence time (HRT) and nitrate concentration on denitrification process in an upflow anaerobic sludge bed (UASB) reactor with biofilms growed on *O. imbricata*. Biological denitrification in a model water was investigated at 12, 18, 25, 26, 31.5 and 62 HRT hours. The nitrate and the chemical oxygen demand (COD) concentration in the influent were 1.5 and 3.5 g/l respectively. The UASB type reactor packed with *Opuntia imbricate* and inoculated with granular sludge showed the best results since the UASB reactor contained only granular sludge had low denitrification rate in all of tested HRT. HRT was one of the most important operating parameters for nitrate removal in anaerobic reactors and 25h was found to be optimum for denitrification. The highest denitrification rate, 255 mg NO_3/l/h, occurred in UASB type reactor packed with *Opuntia imbricata* inoculated with granular sludge. It was demonstrated that the use of coyonoxtle (*O. imbricata*) as carrier for biofilm generation remarkably improved the efficiency of denitrification by ten times and improvement of COD removal.