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Full Length Research Paper

Nitrification in a submerged attached growth bioreactor using *Luffa cylindrica* as solid substrate

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A laboratory-scale submerged attached growth bioreactor using *Luffa cylindrica* as support material for the immobilization of nitrifying bacteria was applied for polishing the effluent of an UASB reactor treating domestic wastewater under the tropical conditions of northeast Brazil, in the City of Campina Grande (7° 13' 11" South, 35° 52' 31" West, 550 m above m.s.l.), Paraiba state. *L. cylindrica* showed a very good performance as a solid substrate for the development of the biofilm aggregating microorganisms capable of metabolizing both organic and inorganic compounds adsorbed on it, particularly those responsible for nitrification. The efficiency of nitrification varied within the range of 82 - 95% with a mean of 88%. The final effluent had an ammonia nitrogen mean concentration of 5 mg.L^{-1}, which is less than that recommended by the National Council for the Environment for discharges into receiving bodies in Brazil.

Key words: Nitrification, Batch reactor, Attached growth, Post-treatment, *Luffa cylindrica*.

INTRODUCTION

Many technological methods such as polishing ponds, submerged aerated biofilters, flotation, Bardenpho activated sludge system and sequential batch reactors (Cybis and Pickbrenner, 2000), have been proposed and in a certain extent, successfully applied for treating the effluent of UASB reactor. Batch systems of activated sludge have been applied since 1914, but only from the beginning of the decade of 1980 that such a technology has become more widespread (EPA, 1993; von Sperling, 1997). In Brazil, only few studies (Dornellas and Figueiredo, 1993; Sousa, 1996) from the 1990s on sequential batch reactors have been reported, and these were also the first studies on their application for the treatment of UASB effluents (Sousa and Foresti, 1996; Coura, 2002; Guimarães, 2003).

In a submerged attached growth bioreactor, a biofilm develops on a solid substrate aggregating a great diversity of microorganisms capable of metabolizing both organic and inorganic compounds adsorbed on the biofilm. As epiphytic microbes, nitrifying bacteria may play an important role in this type of reactor. Nitrification consists of the biological oxidation of ammonia nitrogen to nitrate through the action of aerobic chemoautotrophic nitrifying bacteria (Abreu, 1994), which have low cell yield coefficient because they grow slowly demanding a high cell residence time (Barnes and Bliss, 1983).

The combination of an anaerobic reactor, which has ordinarily a very good performance in removing influent organic matter and produces a less amount of sludge, with an aerobic biological treatment technology, which has a better performance in removing nutrients, for polishing its effluent, may lead to both a less overall consumption of electrical energy and a lower cost associated with an effluent quality complying with standards for discharge into receiving bodies.

However, experience has indicated that the anaerobic effluent appears to have an inhibitory effect on nitrifying

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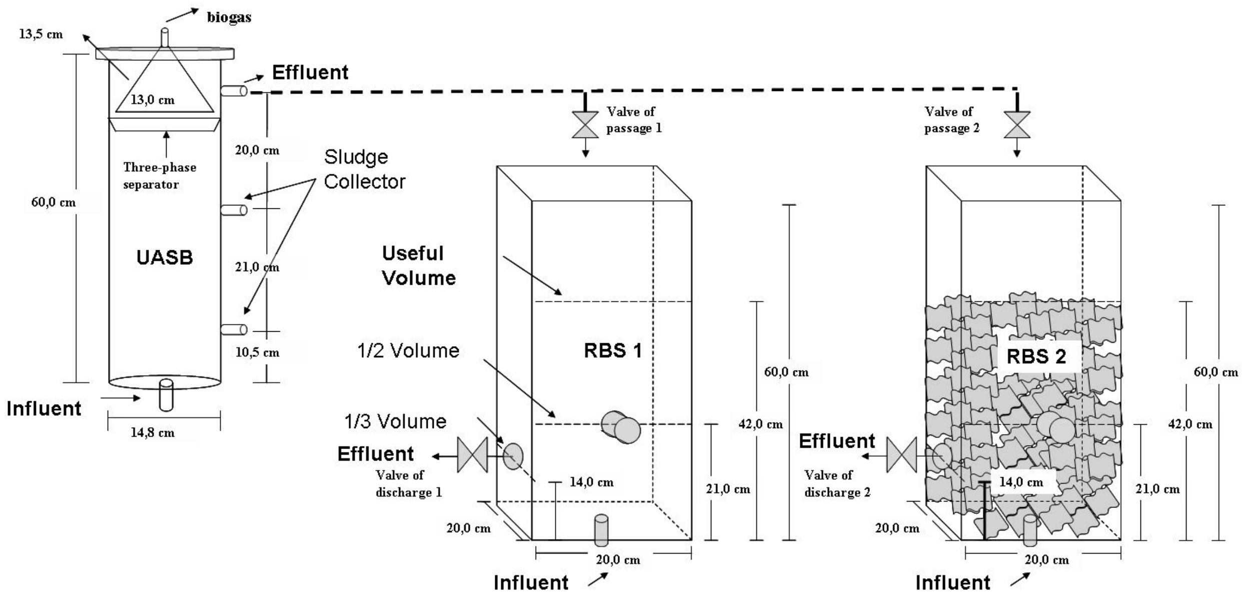

Figure 1. Schematic representation of laboratory-scale experimental system.

bacteria. Guimarães (2003), operating a sequential batch aerobic system following an UASB reactor treating sewage, reported a *Nitrosomonas* yield coefficient of 0.23 d^{-1} which is much lower than that (0.76 d^{-1}) commonly reported in studies on nitrification in conventional activated sludge reactors.

Operating an UASB reactor with a hydraulic retention time as short as 2 h is a means of producing an effluent with a reduced concentration of total sulphide (in fact, a higher relationship between carbonaceous matter and total sulphur), which presumably inhibits nitrification. The sequential batch aerobic reactor adequately designed may reach higher removals of both carbonaceous matter and suspended solids, and also provide a better nitrification. In some cases, according the literature (EPA, 1993; Costa et al., 2005), denitrification and phosphorus removal can also be obtained.

In Brazil, *Luffa cylindrica* fruit is an abundant and low cost local material which becomes quite large and fibrous at maturity and after removing the outer skin may be cut into desired sizes and used as sponges for washing objects and nearly all kinds of surfaces. In scientific research, throughout the world, *L. cylindrica* has been investigated in many types of experiments: Ogbonna et al. (1994) have been successful in using it as a carrier for yeast cells immobilization reaching a high cell concentration applied for ethanol production from both sucrose and molasses media. Akhtar et al. (2003) developed a new biosorbent for the removal of nickel from aqueous solution by immobilizing *Chlorella sorokinian.* According to Pekdemir et al. (2003), *L. cylindrica* showed suitable characteristics for use as a support matrix for formation of a *Thiobacillus ferrooxidans* biofilm for biooxidation of ferrous iron (Fe^{2+}) from strongly acidic industrial wastewater with a high Fe^{2+} content, demonstrating promising potential as an ecological and sustainable alternative to existing synthetic support materials.

This paper is based on a research work whose aim was the evaluation of the performance of a treatment system made up of an UASB reactor treating domestic wastewater followed by a submerged attached growth activated sludge reactor using *L. cylindrica* as support material with a large specific area (850 $m^2.m^{-3}$).

MATERIALS AND METHODS

The experiment was conducted, from October 2007 to January 2008, by a research group funded by the Brazilian Sanitation Programme (PROSAB), working at the area belonging to Paraiba Water and Wastewater Company (CAGEPA), where about thirty years ago the Experimental Station for the Biological Treatment of Sewage of the Federal University of Campina Grande (EXTRABES-UFCG) was established in the City of Campina Grande (7º 13' 11" South, 35º 52' 31" West, 550m above m.s.l.), Paraiba state, northeast Brazil.

The laboratory-scale experimental system, constructed with plexyglass, illustrated in Figure 1, was made up of a cylindrical UASB reactor having an useful volume of 7.8 L and two 16.8 L-prismatic biological aerobic reactors with an useful capacity of 12.8 L each. Both aerobic reactors were sequential batch reactors, being the first a dispersed bed reactor and the second a submerged attached growth reactor. Only results concerning UASB reactor and the second sequential batch reactor will be described herein.

The UASB reactor, with a hydraulic retention time of 2 h, was treated continuously with wastewater (predominantly domestic) from the sewerage of Campina Grande. Raw sewage was collected from a well adjacent to an interceptor pipe crossing the site and pumped to a 200 L-storage tank from where it flowed by gravity into an equalizing tank and later fed into the anaerobic reactor.

The effluent of the anaerobic reactor was treated in a batch aerated submerged attached growth bioreactor, filled with trunk of

Table 1. Standard techniques used for the determination of monitoring parameters.

Parameter	Technique
BOD_5 (mg/L)	Direct dilution in standard BOD bottles
COD (mg/L)	Closed potassium dichromate reflux
TSS (mg/L)	Total suspended solids dried at 103 - 105ºC
VSS (mg/L)	SS fraction volatilized at 500ºC
TALK ($mgCaCO_3$/L)	Volumetric method by titration with 0.01 M H_2SO_4
TKN (mgN/L)	Kjeldahl semi-micro digestion method
$N\text{-}NH_4^+$ (mgN/L)	Volumetric method by titration after distillation of ammonia
$N\text{-}NO_3^-$ (mgN/L)	Instrumental method using a spectrophotometer
$N\text{-}NO_2^-$ (mgN/L)	Instrumental method using a spectrophotometer
TOTALP (mgP/L)	Instrumental method using a spectrophotometer
SORTHOP (mgP/L)	Instrumental method using a spectrophotometer
DO (mg/L)	Instrumental method using a YSI DO meter
OCR (mg/L.h)	Instrumental method using
pH	Instrumental method using a bench pH-meter
T (ºC)	Direct thermometer reading

cone–shaped pieces of *L. cylindrica* sponge, in four cycles of 6 h for each batch. Sponge pieces were previously washed with abundant tap water, three times, for 10 min each time. Each cycle comprised the following steps: (**1**) Filling – 1 h; (**2**) Aerated reaction – 3½ h; (**3**) Sedimentation – 1 h; (**4**) Discharge – 15 min; (**5**) Interval between cycles – 15 min. Cell retention time was about 12 days and during step **2**, aeration provided a dissolved oxygen concentration within the range of 3 - 5 $mg.L^{-1}$, measured among the pieces of the vegetable sponge.

Table 1 summarizes the analytical techniques, whose procedures are described in standard methods for the examination of water and wastewater (1998), used for determining the parameters; biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), Total suspended solids (TSS), Volatile suspended solids (VSS), total alkalinity (TALK), total kjeldahl nitrogen (TKN), ammonia nitrogen ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), total phosphorus (TOTALP), soluble orthophosphate (SORTHOP), dissolved oxygen (DO), oxygen consumption rate (OCR), pH, and temperature (T) monitored during the experimental period.

RESULTS AND DISCUSSION

A very important parameter controlling any attached growth reactor is the quantity of solid substrate inside the reactor in order to support the growth of biofilm. Already, there is a practical knowledge on the ratio between the volume of some support materials and the volume of the reactor. While stone occupies 50% of the volume and sand occupies 63%, the vegetable sponge of *L. cylindrica* occupies only 8% and has a specific surface area of about 850 $m^2.m^{-3}$. However, this type of reactor still needs to be better characterized in terms of its efficiency with relation to the operational parameters hydraulic retention time, the ratio volume of support material/ volume of reactor, as well as the concentration of oxygen used in the process.

Oxygen rate transfer is an important factor which can limit the rate of biomass conversion into end products. During the experimental period, the oxygen consumption rate varied between 28 and 38 $mgO_2.L^{-1}.h^{-1}$, but the availability of oxygen for microorganisms depends on its solubility in water, mass transfer and the velocity it is consumed by the biomass. An oxygen average concentration of more than 2 $mg.L^{-1}$ (Metcalf and Eddy, 2003) is ordinarily recommended in order to achieve an adequate operation.

Biofilm growth

Extracellular polymeric substances (proteins, polysaccharides, lipids and nucleic acids) play an important role in biological treatment systems; in conventional activated sludge they are responsible for the mechanical stability of flocs, while in reactors based on biofilms they make the adhesion of biological material on support material easier. In fact, the complexity of biofilms lays on a variety of factors including the nature of both substrate and support material and the diversity of microorganisms involved in the treatment process (Flemming and Winglinder, 2001).

L. cylindrica sponge specific surface area and empty volume of 850 $m^2.m^{-3}$ and 92%, respectively, were determined before the aerated reactor started operating. During four months of operation the experimental system treated raw sewage having average concentrations of 61.7 $mgN.L^{-1}$ of total kjeldahl nitrogen, 59 $mgN.L^{-1}$ of ammonia nitrogen, 580 $mgCOD.L^{-1}$ and 290 mg $BOD_5.L^{-1}$, producing an effluent with the following mean concentrations: 42 $mgN\text{-}NO_3^-.L^{-1}$, 1.8 $mgN\text{-}NO_2^-.L^{-1}$, 70 $mgCOD.L^{-1}$ and 30 $mgBOD_5.L^{-1}$.

Table 2 shows mean values and standard deviation for the operational parameters sludge retention time (SRT) and hydraulic retention time (HRT) of both UASB and

Table 2. Mean values and standard deviation of operational parameters and physico-chemical variables monitored in the experimental system from October 2007 to January 2008.

Parameter	Raw sewage	UASB influent	SBR effluent
SRT (day)	-	30 ± 0.50	12 ± 0.50
HRT (hour)	-	2 ± 0.001	6 ± 0.001
COD ($mg.L^{-1}$)	620 ± 80	238 ± 46	79 ± 26
TKN ($mgN.L^{-1}$)	68 ± 15	61 ± 14	5 ± 1.8
$N\text{-}NH_4^+$ ($mgN.L^{-1}$)	59 ± 11	53 ± 10	4 ± 1.5
NO_3^--N ($mgN.L^{-1}$)	-	-	42 ± 1
NO_2^--N ($mgN.L^{-1}$)	-	-	1.8 ± 1
TSS ($mg.L^{-1}$)	180 ± 90	106 ± 80	12 ± 10
VSS ($mg.L^{-1}$)	120 ± 70	82 ± 30	10 ± 5
TALK ($mgCaCO_3.L^{-1}$)	390 ± 40	392 ± 50	103 ± 70
pH	7.5 ± 0.22	8.0 ± 0.23	7.3 ± 0.34
TOTALP ($mgP.L^{-1}$)	7.0 ± 3.0	6.3 ± 3.0	6.0 ± 3.0
SORTHOP ($mgP.L^{-1}$)	6.0 ± 2.5	5.8 ± 3.0	5.5 ± 3.0

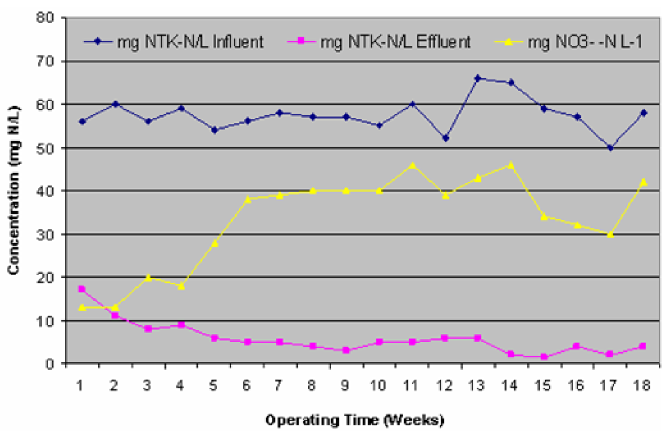

Figure 2. Variation of TKN in both influent and effluent compared to effluent nitrate.

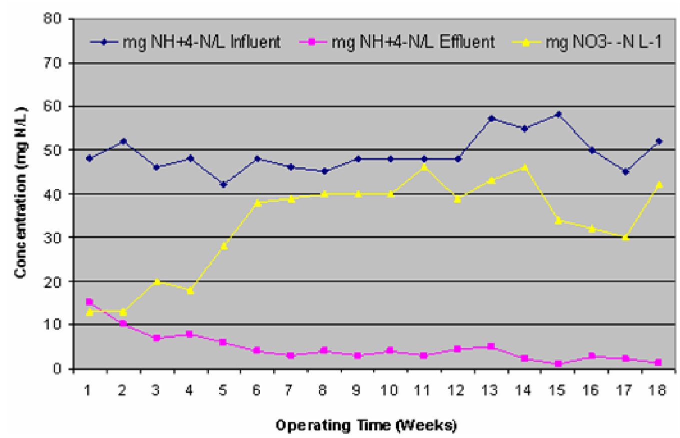

Figure 3. Variation of ammonia in both influent and effluent compared to effluent nitrate.

SBR and variables monitored in raw sewage, UASB effluent and in the effluent of the SBR. Figures 2, 3, 4 and 5 illustrate the variation of TKN, ammonia, total alkalinity and pH, respectively, along the experimental period of 18 weeks from October 2007 to January 2008.

From Figures 2 and 3, it can be seen that from the second week of operation nitrification occurred vigorously in experimental system, with both TKN and ammonia nitrogen in the final effluent reducing gradually and, thereafter, maintained below 5 mgN/L. Figure 4 shows that, in SBR, alkalinity reduced, as a consequence of nitrification, dropping from more than 250 $mgCaCO_3.L^{-1}$, at the beginning, down to near 100 $mgCaCO_3.L^{-1}$ in the fourth week of operation, being kept around 50 $mgCaCO_3.L^{-1}$ in the following weeks. In some occasions, total alkalinity was very close to 35 mg $CaCO_3.L^{-1}$ which is, according to van Haandel and Marais (1999), the minimum limit for guaranteeing buffering capacity and avoiding drop of pH. In Figure 5, it can be seen that pH

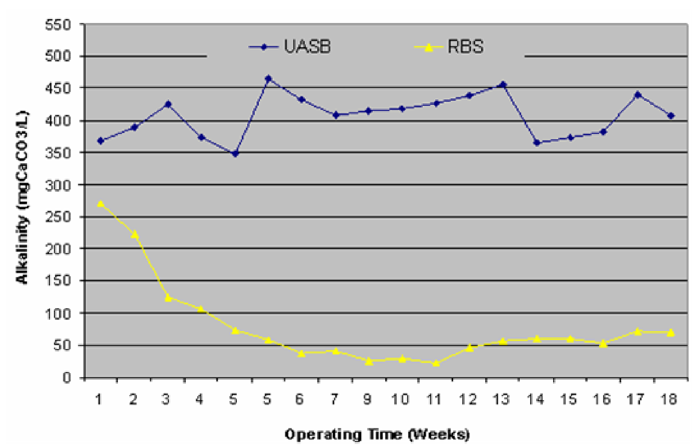

Figure 4. Variation of effluent total alkalinity in both UASB and SBR.

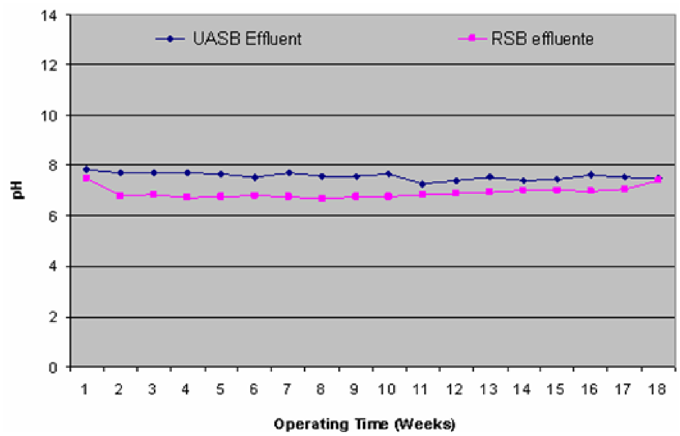

Figure 5. Variation of effluent pH in both UASB and SBR.

was maintained without significant variation throughout the operation of both UASB and SBR with mean values above neutrality.

Conclusion

L. cylindrica showed a very good performance as a support material for the development of the biofilm aggregating microorganisms capable of metabolizing both organic and inorganic compounds adsorbed on it, particularly those responsible for nitrification. The efficiency of nitrification varied within the range of 82 - 95% with a mean of 88%. The final effluent had an ammonia nitrogen mean concentration of 5 mg.L^{-1}, which is less than that recommended by the National Council for the Environment for discharges into receiving bodies in Brazil (Brasil, 2005).

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