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Performance of an Internal-Loop Airlift Bioreactor for Treatment of Hexane-Contaminated Air

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Abstract

Hexane is a toxic volatile organic compound that is quite abundant in gas emissions from chemical industries and printing press and painting centers, and it is necessary to treat these airstreams before they discharge into the atmosphere. This article presents a treatment for hexane-contaminated air in steady-state conditions using an internal-loop airlift bioreactor inoculated with a *Pseudomonas aeruginosa* strain. Bioprocesses were conducted at 20-mL/min, a load of 1.26 g/m^3 of C_6H_{14}, and a temperature of 28°C. The results of hexane removal efficiencies were presented as a function of the inoculum size (approx 0.07 and 0.2 g/L) and cell reuse. Bioprocess monitoring comprises quantification of the biomass, the surface tension of the medium, and the hexane concentration in the fermentation medium as well as in the inlet and outlet airstreams. The steady-state results suggest that the variation in inoculum size from 0.07 to 0.2 g/L promotes hexane abatement from the influent from 65 to 85%, respectively. Total hydrocarbon removal from the waste gas was achieved during experiments conducted using reused cells at an initial microbial concentration of 0.2 g/L.

Index Entries: Air treatment; hydrocarbons; hexane biodegradation; airlift bioreactor; *Pseudomonas aeruginosa.*

Introduction

The inappropriate release of gaseous effluents into the atmosphere promotes air contamination. The understanding of this phenomenon is complicated by the high reactivity and hydrodynamic complexity of the atmosphere, which makes it difficult to ascertain the short- and medium-term effects of the discharges on human health. On other hand, it is estimated that in the United States alone 1,000,000 kg of chemical substances is released annually into the atmosphere, and US$1,200,000 is spent annually on the treatment of these effluents *(1)*.

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Among the great diversity of atmospheric contaminants, chemical compounds and some living organisms can be found. The first case comprises the biatomic gases such as CO, NO_x, and SO_x, and several inorganic and organic substances. In the second case, microorganisms such as fungi and bacteria can contribute to air pollution, associated or not with chemical substances *(2)*.

Regarding air pollution associated with the release of chemical, the volatile organic compounds (VOC) stand out owing to their toxicity, high number of producing facilities, and high-quantity discharge. The toxicity of VOC can vary, with some only slightly toxic and others mutagenic, carcinogenic, and teratogenic *(3)*. Some chemical process industries, such as the petroleum and food industries, generate vast amounts of gaseous effluents that contain VOC, mainly light hydrocarbons, with high toxicity *(4,5)*.

To decrease the productivity of and minimize the environmental contamination caused by the release of these substances into the atmosphere, industries use a series of physical and chemical operations to recover VOC. However, such processes are limited by the concentration of the compound in the gas stream. After the recovery and reutilization stages, these effluents must be treated in physiochemical treatment units, also limited because of efficiency matters and the generation of toxic byproducts. These facts impel and justify the VOC biologic treatment alternatives, which require little initial capital, have low operation cost, promote the absence of secondary pollutants, and present good treatment efficiencies in a wide range of organic load *(6)*.

Among the biologic air pollution control alternatives, there are the air-phase bioreactors with fixed bed, the biofilters; and the bioreactors with fluidized bed, the bioscrubbers *(6–8)*. The biofilters present efficiency-limiting factors related to the control and monitoring of processes, the establishment of preferential flows, and clogging owing to excess biomass growth. By contrast, bioscrubbers do not present these limitations and are therefore recognized as alternatives for VOC-contaminated air treatment.

Besides being only slightly biodegradable in biofilters, hexane is toxic and presents risks of serious damage to the human neurologic system *(2,3)*. There are, in fact, some reports of moderated contamination events related to the inappropriate discharge of this substance *(9)*. These facts impel and support the development of effective bioprocesses for hexane-contaminated airstream treatment.

This article reports the performance of an internal-loop airlift bioreactor inoculated with a *Pseudomonas aeruginosa* strain for the treatment of gas stream containing 1.26 ± 0.1 g of hexane/m^3 of air. The experiments were carried out at 28°C using two different inoculum size. After this stage, air treatment experiments were carried out utilizing reused bacterial cells from the immediately preceding treatment tests at 0.2 g/L of initial biomass.

Materials and Methods

Microorganism

A *P. aeruginosa* strain obtained from petroleum-contaminated soil was maintained on nutrient agar slants at 4°C and regularly subcultured and monitored for purity. After a period of 30 d, the cells were grown in 500-mL Erlenmeyer flasks containing 100 mL of mineral medium comprising 0.5 g/L of KH_2PO_4; 4.5 g/L of Na_2HPO_4; 2.0 g/L of NH_4Cl; 0.01 g/L of $MgSO_4 \cdot 7H_2O$; and 1.0 mL of hexane and pH 7.0 ± 0.2. The flasks were agitated at 150 rpm for 4 d at 28°C. The object of this procedure is to maintain hydrocarbon-degrading activity. (Note that *P. aeruginosa* is a known opportunistic pathogen and, therefore requires extra precautions for its industrial use.)

Treatment of Hexane-Contaminated Air

The internal-loop airlift bioreactor developed by Oliveira and de França *(10)* was used and is illustrated schematically in Fig. 1. The bioreactor was made of clear acrylic plastic and consisted of a 9.4-cm-id outer tube 100 cm high. An 80-cm-high concentric tube was located within the outer tube and was fastened at a 9-cm distance from the air sparger. The reactor had a total capacity of about 7.5 L and was operated with 6.5 L. A perforated plate with 12 holes of 0.5 mm diameter was used as the air sparger. To enable the control and monitoring of the process, the equipment received ports for electrodes and for liquid and gas sampling.

Hexane-contaminated air treatment experiments were conducted using fresh cells or reused cells from preceding air treatment experiments. The initial tests began with the cleaning of the bioreactor with bleach solution at 2% (v/v), while allowing air to flow for 1 h. Next, the solution was drained from the bioreactor, and the equipment was rinsed five times with sterile distilled water at 121°C for 20 min. The mineral medium was sterilized (at 121°C for 20 min) and transferred to the bioreactor under aseptic conditions. Before introduction into the bioreactor, the inoculum was prepared using *P. aeruginosa* cells transferred from the nutrient agar slants to test tubes containing 10 mL of nutrient broth (0.001; Difco, Detroit, MI). After incubation at 30 ± 1°C for 24 h, the inoculum was propagated to 500-mL Erlenmeyer flasks containing 100 mL of the same mineral medium supplemented with 1.0 mL of hexane. The flasks were incubated at 150 rpm to exponential growth phase at 30 ± 1°C. Microbial cells were harvested from the broth by centrifuging at 8000*g* for 20 min, followed by two washes with saline solution (9 g/L of NaCl). The cells were resuspended in the mineral medium and, according to the dry weight curve, the inoculum volume was calculated to obtain 0.07 or 0.2 g/L of initial biomass.

The tests carried out reusing bacterial cells began with collection of part of the fermented medium from the immediately preceding air treatment experiments and subsequent centrifugation at the conditions just described.

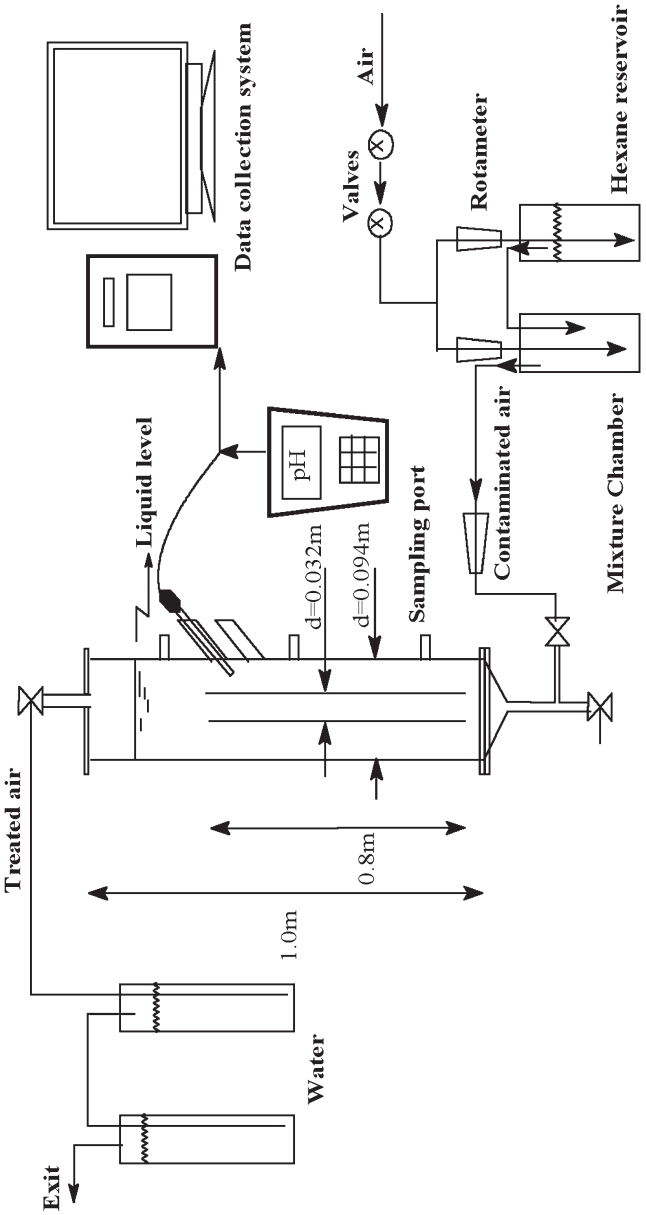

Fig. 1. Scheme of hexane-contaminated air plant.

The following stages were cleaning the equipment, adding the sterile mineral medium, and inoculation the bioreactor. The objective of this procedure was to verify the effect of cell reuse on treatment efficiency. Experiments were carried out with an initial biomass of 0.215 and 0.212 g/L.

Gaseous influents were prepared by flowing synthetic air through an air filter and using needle valves and flowmeters to adjust and control the flow. The airflow was used to drag the hexane from a reservoir. In a mixture chamber, the contaminated airflow was mixed with a dilution airflow that had gone through a humidifying chamber, and then it was directed to the base of the bioreactor, which contained the mineral medium inoculated with the microorganism. All the experiments were carried out with air contaminated at 1.26 ± 0.1 g/m^3, using a total constant flow of 20 ± 1 mL/min at 28 ± 1°C.

At specific time intervals, samples were collected from both the influent and effluent airstreams with a 5.0-mL gas-sample syringe (Hamilton). Hexane in the gas flow was measured by injecting 1.0-mL samples in an HP gas chromatograph, model HP6890 *plus*, equipped with a flame ionization detector, and a capillary column HP-plotter Al_2O_3 (30 m × 0.25 mm × 0.15 μm), maintained at 150°C. Nitrogen was used as the carrier gas at a flow of 30 mL/min, and the temperature for both the injector and the detector was kept at 300°C. To measure the hexane concentration in the fermentation medium, the samples were filtered using a 0.22-μm membrane, and 1 μL was injected into the gas chromatograph under the same conditions as just described. Hexane concentration was measured by comparison with a previously established calibration curve.

Biomass quantification was done through the relation between the dry weight of the cells and the absorbancy at 440 nm (HACH spectrometer; Odyssey). The surface tension of the free-cell medium was determined using a tensiometer Sigma70 System Unit equipped with a platinum-iridium ring.

Results and Discussion

The initial hexane-contaminated air treatment tests were carried out with fresh cells. Two inoculum sizes, 0.07 and approx 0.2 g/L, were used to investigate the effect of the initial cell concentration on the bioprocess. These tests were followed by air treatment experiments, carried out to verify the behavior of the process when the inoculum cells were reused. The reuse of cells removes the stages of microbial propagation and reduces the amount of biologic material to be discarded, thus minimizing the cost of the bioprocess.

Fig. 2 shows the profile of the biomass in the experiments of hexane-contaminated air treatment. In the tests carried out with fresh cells, it can be seen that, in steady state, the biomass reached 0.25 and 0.82 g/L, respectively, when inoculum sizes of 0.069 and 0.243 g/L were used. In the experiments carried out using reused cells, the initial biomass was 0.2 g/L and

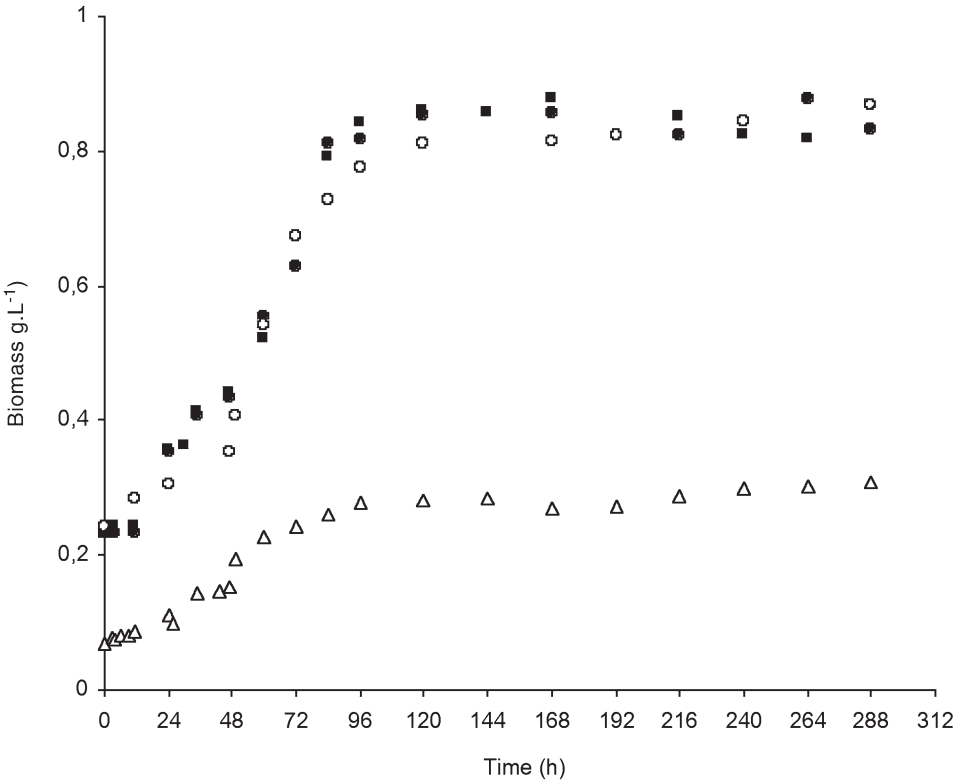

Fig. 2. Evolution of *P. aeruginosa* concentration during hexane-contaminated air treatment (1.26 ± 0.1 g/m^3) under tests employing fresh cells in different initial concentration ([Δ] 0.069 and [o] 0.243 g/L) and reused biomass of immediately preceding experiments at inoculum sizes of (●) 0.215 (■) 0.212 g/L.

reached 0.83 g/L. It can also be noted that the steady-state condition of the process was reached for all the tested parameters at 96 h of treatment. These results clearly show that the initial cell concentration and reuse of biomass do not affect the process lag-phase extension and the time to reach the steady-state condition. It is important to emphasize, however, that the microbial concentration obtained in steady state is related to the inoculum size, irrespective of cell reuse. In addition, cell growth in a mineral medium containing hexane as a sole carbon source does not corroborate the assertions of microbial inhibition presented by Balba et al. *(11)*, who related the limitations of the biodegradation of short carbon chain ($<C_{10}$) linear hydrocarbons to the liposolubilizing properties.

During steady-state conditions, a slight increase in biomass was observed, which can be related to the predominant substrate consumption for byproduct production of the hydrocarbon metabolism and/or even for cell maintenance. This corroborates the results of hydrocarbon degradation not associated with microbial growth that were presented by Cunha and

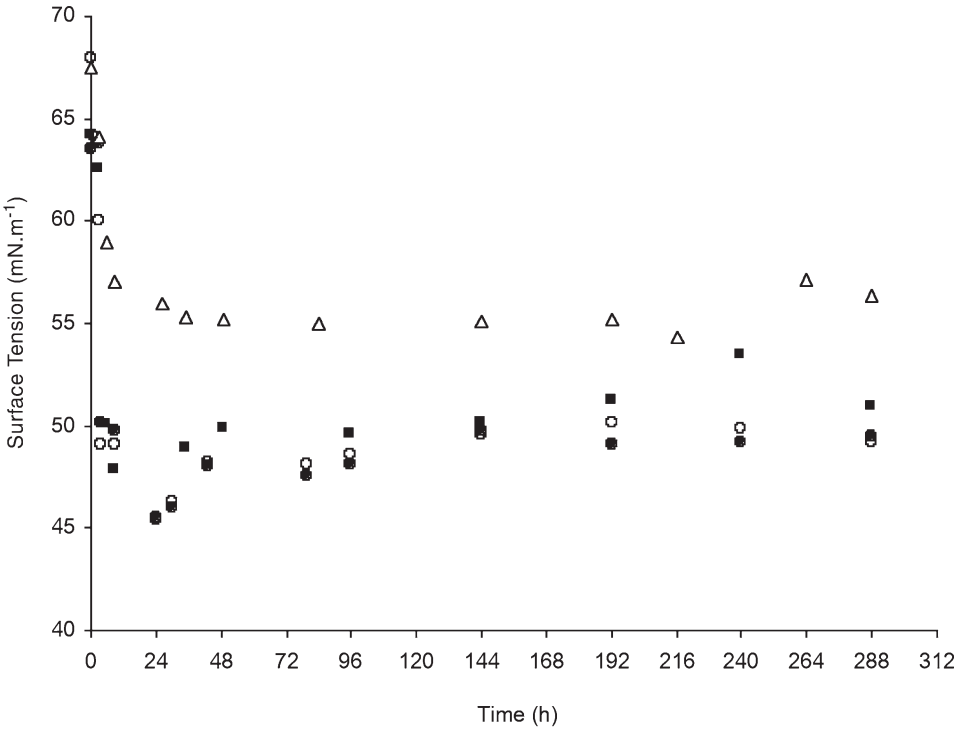

Fig. 3. Influence of initial cell concentration ([Δ] 0.069 and [o] 0.243 g/L) and reuse of biomass of immediately preceding experiments at inoculum sizes of (●) 0.215 and (■) 0.212 g/L on evolution of substrate concentration in fermentation medium during hexane-contaminated air treatment (1.26 ± 0.1 g/m^3).

Leite *(12)* during gasoline-contaminated soil bioremediation experiments using *Pseudomonas* cultures.

Fig. 3 presents surface tension profiles of the fermentation medium. During the 24-h period of the process, surface tension showed a marked reduction from 66 to 55 and from 66 to 47 mN/m, respectively, during the experiments carried out with an initial bacterial concentration of 0.069 and 0.243 g/L. In the tests carried out with reused cells, a similar profile of surface tension can be seen, and a reduction from 66 to 46 mN/m was reached. Therefore, these results demonstrated that the reuse of cells does not influence the surface tension of the medium. A reduction in surface tension suggests the production of surfactant substances, which can increase the bioavailability of hydrocarbon. Mulligan et al. *(13)* and Alexander *(14)* reported that several strains of the genus *Pseudomonas* are capable of producing biosurfactants from hydrocarbons, which facilitates the biodegradation process.

The profiles of hexane concentrations in the fermentation medium in Fig. 4 show that, irrespective of the employed inoculum size and of their reuse, the hexane concentration increased with a decrease in the surface

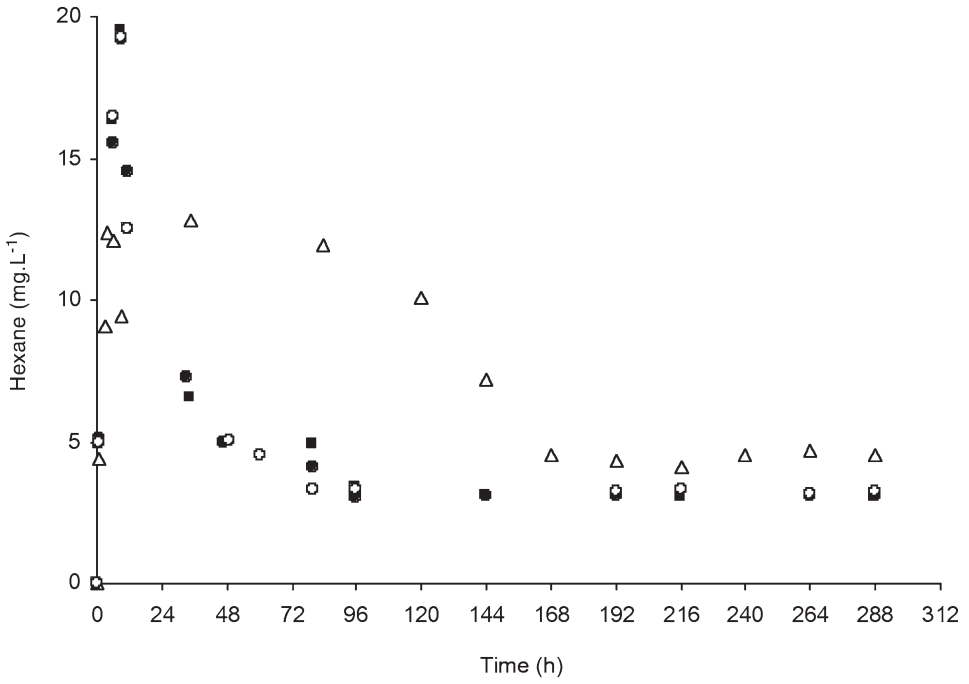

Fig. 4. Influence of initial cell concentration ([Δ] 0.069 and [o] 0.243 g/L) and reuse of biomass of immediately preceding experiments at inoculum sizes of (●) 0.215 and (■) 0.212 g/L on evolution of surface tension of fermentation medium during hexane-contaminated air treatment (1.26 ± 0.1 g/m^3).

tension of the medium. However, we point out that the hydrocarbon degradation rate was more pronounced for the experiments carried out with the greatest inoculum size, and that the steady-state condition was observed at 60 h of the process, irrespective of the reuse of biomass. For the minor initial cell concentration, the steady-state condition was observed only after 168 h of the process. These data suggest that the process is limited by the hexane mass transfer from the gas to the liquid phase. Because of its low polarity, the hexane presented low solubility in water and in polar solvents. The reduction in surface tension during the air treatment could have favored the mass transfer of the pollutant to the liquid phase and, consequently, could have reduced the resistance to the biodegradation process.

Fig. 5 shows the efficiency results for the removal of hexane from the influent in the experiments conducted with different inoculum sizes, and with cell reuse. It can be seen that the increase in the initial biomass promoted an increase in treatment efficiency, which increased from values of about 60 to about 85%. These results reveal that a smaller initial bacterial concentration is not capable of treating the effluent satisfactorily. The first reuse of *P. aeruginosa* cells allowed the total removal of the pollutant from

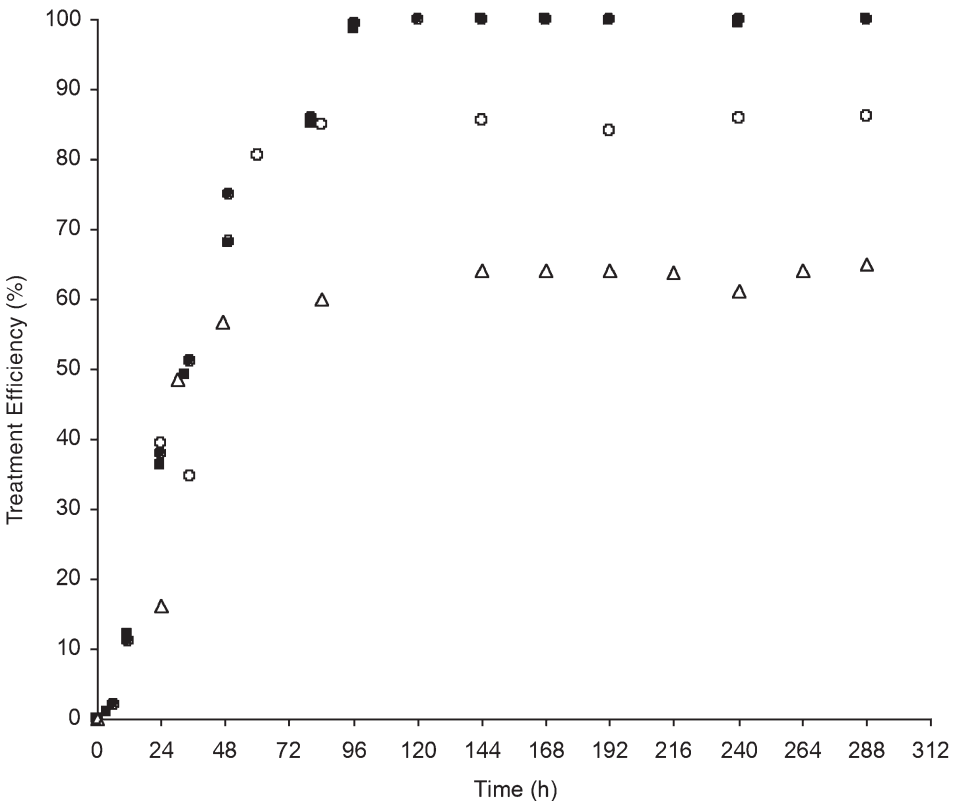

Fig. 5. Efficiency of hexane-contaminated air (1.26 ± 0.1 g/m^3) treatment as function of use of fresh *P. aeruginosa* inoculum at (Δ) 0.069 and (o) 0.243 g/L and by use of reused biomass of immediately preceding experiments at inoculum sizes of (●) 0.215 and (■) 0.212 g/L.

the effluent, indicating that culture adaptation and or acclimation to the bioprocess took place; in other words, the effects of the transposition of the cultures from the agitated flasks to the bioreactor were overcome, as defended by Devinny et al. *(2)*. When the bacterial cells were reused a second time, the efficiency of the removal of the pollutant showed a similar profile, which indicates the stability of the biomass and its acclimation.

The literature provides little research on the treatment of hexane-contaminated air *(15–17)*. Spigno et al. *(15)* presented treatment efficiencies from about 70 to 80% for a system operating with two serial biofilters, that were inoculated with the *Aspergillus niger* filamentous fungi to treat effluent with a load of 2–7 g/m^3. On the other hand, Kastner et al. *(16)* and Kibazohi et al. *(17)* reported total removal of hydrocarbon from hexane air mixtures employing biofilters. However, these high performances were not maintained throughout the monitored period. The bioprocess presented here is quite promising because it efficiently treated hexane-contaminated air just by applying a bioreactor, and because in the literature consulted no

research was found on the use of such a bioreactor configuration for the treatment of this kind of effluent.

During all the experiments, the pH of the fermentation medium remained in the neutral range, from 6.9 to 7.2, which is recognized as favorable for the degradation of hydrocarbons *(14)*. This pH stability can be related to the capacity of the mineral medium buffer or even to the consumption of intermediate acids formed in the hydrocarbon metabolism, generating neutral pH products.

Conclusion

An airlift bioreactor inoculated with a *P. aeruginosa* strain was demonstrated as an effective biologic alternative for hexane (1.26 ± 0.1 g/m^3) treatment. It was concluded that the bioprocess could reach steady hexane removal efficiencies up to 80% by the use of a 0.2 g/L inoculum size. The results also showed that the bioprocess efficiency diminished to 60% when the bioreactor was inoculated with 0.07 g/L. The utilization of 0.2 g/L of reused biomass from the immediately preceding treatment experiments promoted the total removal of the hydrocarbon from the influent, which can be related to the adaptation of the bioreactor culture. Thus, the results presented here allow one to conclude that the inoculum size and bacterial reuse influenced the removal of the hexane from the airstream. However, the effects of the reuse of cells on the reduction of the surface tension of the medium, stability of the pH, and cell mass in steady state were not verified.

The results regarding hexane concentration in the liquid medium and the surface tension allow one to conclude that the mass transfer of the substrate to the liquid phase is the limiting factor in the treatment process. The reduction of the fermentation medium's surface tension indicates biosurfactant production by *P. aeruginosa* during the bioprocesses, which contributed to the decrease in resistance of hexane mass transfer from the gas to liquid phase, resulting in an increase in the efficiency of the treatment process.

Acknowledgments

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