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Microbial removal of xylene using free and immobilized *Streptomyces* sp. AB1: bioreactors application

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ABSTRACT

Xylene is a hydrophobic and aromatic organic compound widely used as a solvent in various industries and medical technologies. While its use is of a practical and economic significance, it is also considered as a highly toxic compound and a severe source of pollution, especially when it is discharged into nature. The aim of our study was to contribute toward xylene biodegradation using microorganisms that belong to the *Streptomyces* group. These microorganisms have been subject of an increasing interest in basic research and in biotechnology applications. Our current research is primarily focused on the biodegradation and removal of xylene using free and immobilized *Streptomyces* sp. AB1 strain, isolated from the Mitidja plain soils (north of Algeria). The follow up of xylene removal kinetics showed higher elimination rates in medium containing xylene as the sole source of carbon (200 and 300 mg l^{-1}). Biodegradation percentage varies between 80 and 99%. GC spectra showed changes during incubation time which is an indication of a microbial attack. Production of biodegradation metabolites was also detected in the medium. In a similar way, the *Streptomyces* sp. AB1 strain was encapsulated in sodium alginate beads, the result of these investigation shows high xylene removal rate (90%) from contaminated water and that *Streptomyces* sp. AB1 can regenerate sodium alginate beads without loss of sorption capacity.

Keywords: Xylene; Biodegradation; Streptomyces; Alginate-immobilization

1. Introduction

Environmental contamination by aromatic organic compounds from petrochemical and energy-producing industries is continually increasing. Among aromatic compounds such as benzene, toluene, and xylenes are the most severe contaminants because of their considerable use, their low water solubility, acute toxicity to the liver, kidney, and the central nervous system [1], and genotoxicity, these compounds have been

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classified as priority pollutants by the European Environmental Agency [2]. Bioremediation techniques are becoming the most recommended because of their relatively low cost in comparison with other techniques that are not only expensive but also deliver low reduction yield [3,4]. Biodegradation technology uses microorganisms to treat contaminants by degrading organic compounds to less toxic material, such as CO_2, methane, water, and inorganic salts, and it can be performed in situ or ex-situ under aerobic or anaerobic conditions.

Numerous studies have been carried out using degradation of xylenes by different microorganisms (*Alcaligenes xylosoxidans*, *Pseudomonas putida* [5,6], and *Rhodococcus* sp) [7]. However, only few works have been reported on xylenes degradation using *Actinomycetes* [8,9], in spite of their ability to degrade different compounds [10,11] while synthesizing different bioactive metabolites, such as biosurfactants [12,13].

The use of free strains for the degradation of various toxic compounds has a number of disadvantages but in bioremediation immobilized strains have a great potential for application [14]. Immobilized cells have several advantages over freely suspended cells, including higher activity, higher cell density, and longer stability than free cells. Hence, the immobilized cells have the potential to degrade toxic chemicals at the higher concentration as compared to the freely suspended cells [15].

This article describes the biodegradation kinetics ability of *Streptomyces* sp. strain AB1 to grow and transform xylene as a soluble substrate in liquid medium by free and immobilized cells. The effects of substrate concentration on biodegradation and cell growth were also studied.

2. Materials and methods

2.1. Chemicals

Sodium alginates (SA) with 99% purity and Glucose with 99.5% purity were purchased from Merck, Sigma-Aldrich. Hexane (Alkane mixture, essay GC) from Panreac Quimica and Xylene (isomers mixture, essay GC) from Anlar Normapur were purchased. All other chemicals used in this study were of analytical grade.

2.2. Cultures and media

The strain (Fig. 1) was isolated from Boufarik (humid surface soils in Mitidja plain, north of Algeria) as previously described by Badis et al. [10].

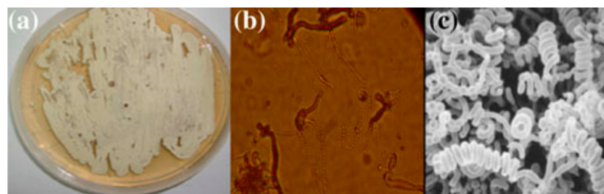

Fig. 1. Streptomyces shown in: (a) strain thallus, (b) microscopic observation to 100 times, and (c) the actinomycetes atlas 1997.

The inocula were obtained after 3 d of incubation, cultured in ISP9 medium (Fig. 2), which contained ($g\,L^{-1}$): 10 g Glucose, 2.64 g $(NH_4)_2$ SO4, 2.38 g KH_2PO_4, 5.65 g $K_2HPO_4{\cdot}3H_2O$, and 1 ml of mineral salts medium.

The original culture was first adapted to grow in the ISP9 with xylene as the sole carbon source in 100 ml of culture medium. After several transfers with gradually increasing xylene concentration, the culture showed good growth. The adapted culture was then studied for their abilities to degrade xylene as a sole carbon source in free and immobilized conditions.

2.3. Biodegradation kinetic studies in free culture conditions

The experiments were carried out in batch cultures using 250-ml glass vials containing 100 ml of culture medium with 2% of adapted inocula, covered by cotton and aluminum caps. Various amounts of xylene compounds, as sole carbon sources for cell growth, were added to ISP9, using a micro-syringe, directly from the stock solution to give desired final concentrations, respectively: 100, 200 and 300 mg L^{-1}(no other organics, such as glucose or yeast extract, were added

Fig. 2. Inocula of AB1 strain in ISP9 medium after three days of incubation.

to the medium). The pH of the medium was adjusted to 6.2 and 7.2, respectively, by adding either HCl or NaOH. Vials were incubated in an incubator shaker (30˚C at 150 rpm). The organic compounds were added after autoclaving to minimize losses from volatilization. The samples from the culture broth were withdrawn under sterile conditions at different incubation periods for the analysis of residual xylene by GC and pH medium changes were monitored during experiments with pH-meter (HANNA pH211).

2.4. Biodegradation kinetic studies in immobilized culture conditions

Streptomyces sp. strain AB1 was grown on ISP9 medium; the cells were harvested during the mid-logarithmic growth phase by centrifugation at 1,000 rpm for 10 min. Cells were immobilized by adding the centrifuged inocula in autoclaved sodium alginate solution before preparing beads. Different types of beads at 2 mm diameter were prepared, respectively; beads with active biomass (Positive beads) by introducing 2% of live inocula in polymer solution (sodium alginate), beads with inactive biomass (Negative beads) were produced by introducing 2% of autoclaved inocula, and alginate beads without biomass (Alg beads).

The preparation of beads was to transfer the solution using a peristaltic pump (with a flow rate of 2.27 ml min^{-1}) into a sterile calcium chloride solution $CaCl_2$ (0.1 M). Maturation beads periods of 10 h were chosen [16]. The whole solution was soaked in sterile distilled water several times to remove excess Ca^{2+} and no encapsulated cells. After beads jellification, 4 mg of different forms of beads were introduced into 100 ml of culture medium with 200 mg L^{-1} of xylene. The whole thing was then covered with cotton and aluminum caps. Vials were shaken (30˚C at 150 rpm) and pH medium was set to 6.2.

2.5. Analytical methods

The content of organic compounds was analyzed by gas chromatography (GC) using SHIMADZU type GC17A, equipped with a flame ionization detector and a capillary column SE30 (FSCW 0.25 mm × 25 m). Nitrogen was used as the carrier gas at a flow rate of 40 ml min^{-1}. GC temperature programming was set as follows: 60˚C for 2 min and then 10˚C min^{-1}–300˚C and kept at 300˚C for 10 min, injector temperature was 250˚C. The content of residual organic compounds was also analyzed using a JENWAY UV–visible spectrophotometer in 1-cm quartz cells at 208 nm. The percentage reduction (%R) was determined by the following formula:

$\%R = (Abs^{\circ} - Abs)/Abs \times 100$. Where Abs°: absorbance at t_0 and Abs: absorbance at $t_{(x)}$.

Each measurement was done in duplicate. Aqueous samples were extracted with hexane 50% (v/v) and shaken for 2–5 min.

3. Results and discussion

3.1. Optimization of optimal conditions of degradation

It is noted that no aeration or any other oxygen source was provided to the medium. It contains: 9–13 mg L^{-1} of dissolved oxygen, which is sufficient for degradation of only 4–6 mg L^{-1} of xylene. However, biodegradation is via anaerobic processes. Additional oxygen can be provided from the air present in the headspace in the bottle.

Fig. 3 shows the biodegradation kinetics for free cells grown on different concentrations of xylene as the sole carbon source, and different initial pHi of medium fixed at 6.2 and 7.2.

The reduction of different xylene concentrations begins the first few hours up to its total exhaustion after 1–5 d. The results show that *Streptomyces* sp. AB1 strain is able to metabolize xylene as a sole carbon and energy source, to grow into different concentrations. Similar results were found in different studies using *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* [17] and *Pseudomonas* spp. [18]. But the biological processes are very sensitive to fluctuations in the concentration of carbon source and by-products of degradation, since limit levels, the microbial activity is inhibited [19]. Fig. 3 shows the difference of reduction rate for the used concentrations, at relatively higher xylene concentrations (200 and 300 mg L^{-1}); the degradation rates were 90% after 1 d of incubation for each pH but at 100 mg L^{-1} of xylene concentration, degradation rate is comparatively low (75%) which decreases to 36%. The fluctuation in the biodegradation of xylene at 100 mg L^{-1} of xylene is probably due to the bioavailability of substrate, it can be clearly seen that the degradation rate increased with increasing substrate concentration and then decreased after reaching a maximum. At initial pH (7.2), in the presence of hydrochloric acid (pH adjustment by HCl), xylenes may undergo chlorination which provides derivatives as hexachlorocyclohexane used as insecticide; as explained by Arnaud [20], the presence of by-products probably have effects on the bacterial growth and on the rate of biodegradation [19].

The microbial process involves substrate consumption, a heat release, and formation of biomass and

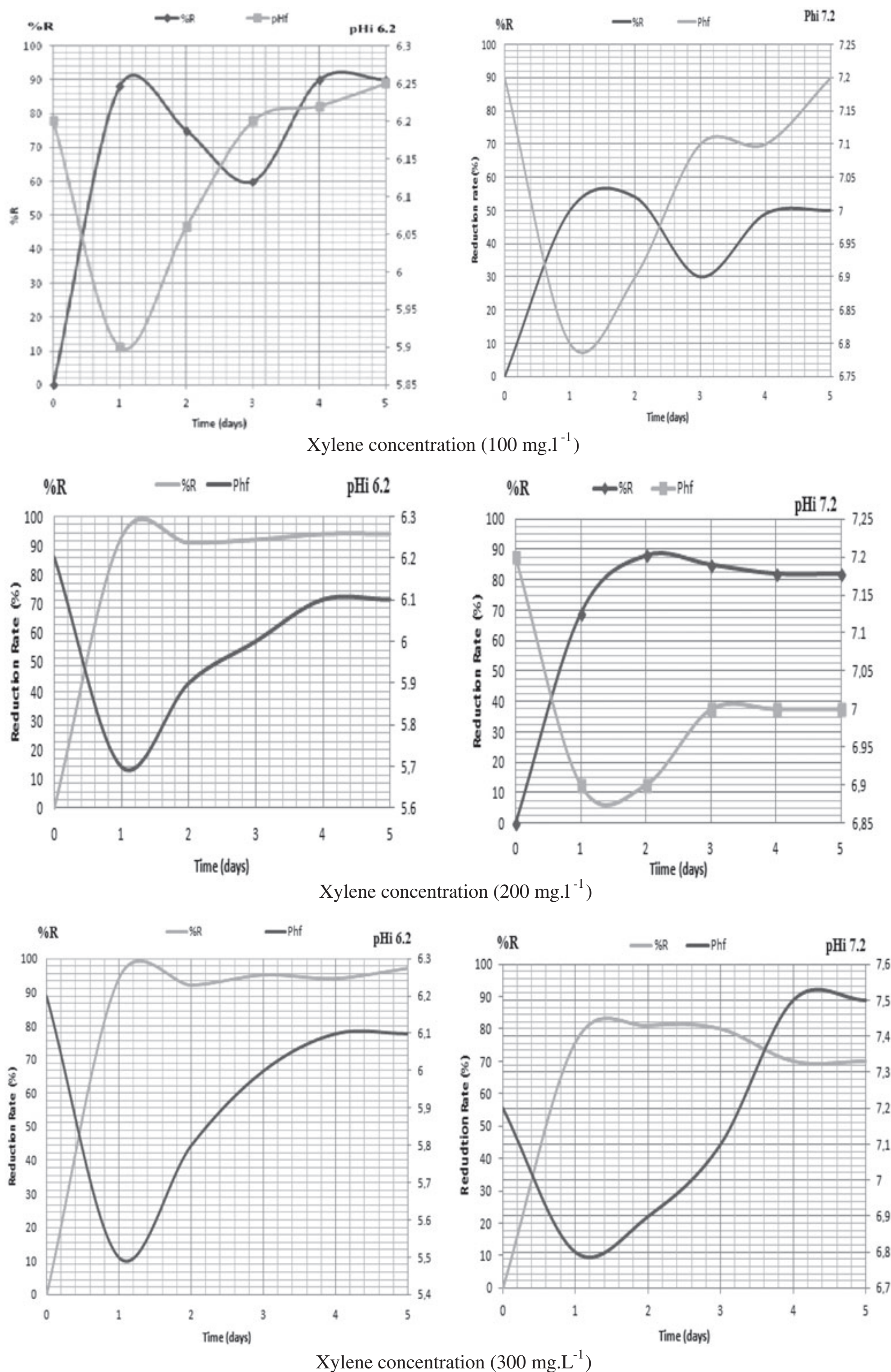

Fig. 3. Effects of substrate concentration and initial PH on degradation rate and medium's PH of free cells grown on xylene.

degradation metabolites [21], and these xenobiotic compounds may cause slowdowns of microorganisms' growth or complete inhibition even at low concentrations [22]. It has been found that after one day of incubation the value of the maximum wavelength (λ_{max}) becomes 318 nm with an absorbance *Abs* = 0.109, this is probably the absorbance value of by-products that were generated during the degradation of xylene.

Fig. 3 shows a decrease in the medium pH during the degradation of the substrate which is explained by the presence of acidic metabolites (carboxyl group) and explains the fermentative way of degradation [22]. It is to be noted that in this study only minimal mineral solution was used and no organic nitrogen source or other nutriments were provided in the medium. Furthermore, no aeration or additional oxygen source was provided to these bacteria in the bioreactor.

The previous histogram (Fig. 4) allows us to conclude that the AB1 strain can metabolize xylene (sole source of carbon and energy) to grow and develop at different concentrations. These results are similar to those found by Labrecque [17], which confirms that all concentrations (100, 1,000 and 2,000 mg L^{-1}) of xylene did not inhibit the growth of *K. marxianus and S. cerevisiae*; the better degradation rate is at relatively higher xylene concentrations (200 and 300 mg L^{-1}), pH equal to 6.2 is the optimum pH of xylene degradation [23]. The results obtained at different pH values show that the strain degrades rapidly xylene present in the medium at this pH as previously reported by Zermane [9] and In-Gyung and Chang-Ho [24]. For all following experiments, the level of contaminant was kept constant at 200, 300 mg L^{-1}, and pH at 6.2.

3.2. Biodegradation kinetic studies in free culture conditions

The strain showed a great removal capacity until total exhaustion and a high resistance to the toxicity of the substrate used (mixed xylene isomers). This part aims to confirm this elimination by estimating the presence of primary metabolites of degradation.

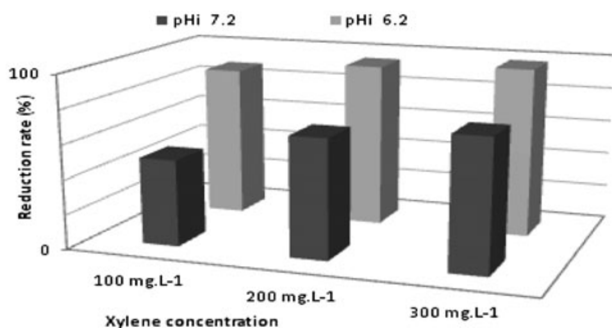

Fig. 4. Xylene concentrations effect and pH on bacterial reduction rate.

The detection of products generated from the degradation of xylene was detected by GC. The medium used is the one containing 200 and 300 mg L^{-1} of xylene. Note that the biodegradation tests were provided in optimal conditions: 30˚C and pH 6.2. The spectra obtained are shown in Fig. 5.

GC spectra showed changes during incubation. A gradual reduction of xylene can be noticed with time, which confirms that xylene is used as source of carbon and this up to its total exhaustion after 5 h. Several degradation metabolites corresponding to new products were found in the culture medium after 2 h of incubation, as revealed by GC where new peaks appeared in the chromatogram.

These degradation metabolites can be attributed to biosurfactants formation according to Ferhat et al. [25]. Table 1 summarizes the different new peaks that appeared overtime.

3.3. Biodegradation kinetic studies in immobilized culture conditions

The introduction of a polymer solution (sodium alginate) into the peristaltic pump with a flow rate of 2.27 ml min^{-1} has enabled us to obtain beads of 2 mm diameter. The choice of this diameter is based on the study of Grattepanche [26], which stated that bead size influences the activity of the immobilized biomass, small diameter beads tend to improve mass transfer and a higher productivity.

According to Daugulis and Janikowski [27], the octanol/water distribution coefficient is the parameter that characterizes the hydrophobicity of a molecule, the more important it is, the more molecules tend to adsorb onto a solid surface. This is actually highlighted in our results where a high adsorption capacity (70%) is noticed for alginate beads without biomasse, 80% of reduction noticed for inactive biomass beads, whereas it reaches 90% for positive beads (Fig. 6). These reduction percentages are relatively high, in comparison with activated carbon that can treat up to 95% of an effluent containing up to 80 mg L^{-1} of benzene [28]. But in all the processes that involve the addition of an adsorbent, the adsorption capacities tend to decrease overtime, due to a decrease of free active sites. Once saturated, the adsorbent must be regenerated or replaced. The chemical or thermal regeneration is a process that can be expensive and limited by the compounds irreversibly adsorbed [29].

The different types of beads show fluctuations in reduction capacity, which can probably be interpreted

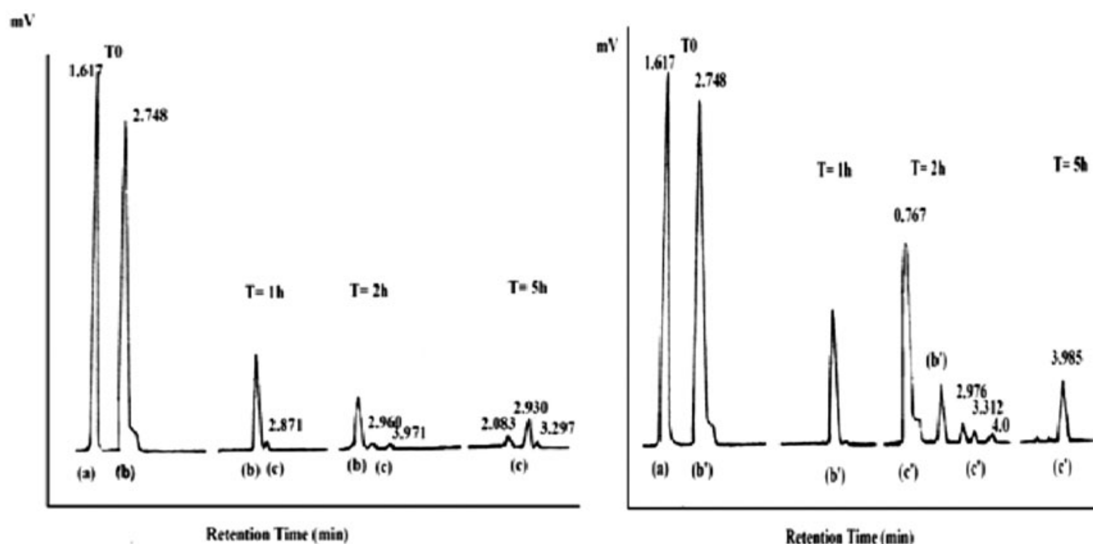

Fig. 5. GC chromatograms of metabolites stem from biodegradation of 200 and 300 mg L^{-1} of xylene, respectively. The retention time correspond to: (a) Solvent; (b), (b′) xylene concentrations; and (c), (c′) metabolites obtained after (T = h) of incubation with *Streptomyces* sp. AB1.

Table 1
New peaks appeared during incubation analyzed by GC

Incubation time xylene concentration	2 h	4 h	5 h	21 h
200 mg L^{-1}	2.960 min 3.571 min	2.998 min	2.083 min 2.930 min	2.947 min
300 mg L^{-1}	0.767 min 2.976 min 3.312 min	1.36 min 2.935 min	2.930 min 3.985 min	–

as an adsorption followed by a desorption of the substrate with a release of active sites after the degradation of adsorbed substrate (xylene), by a favorable concentration gradient [30]. This release allows the recovery of adsorption that is observed after 3 h of incubation, the maximum elimination (90%) by positive beads was observed after 4 h (Fig. 6), this phenomenon is called bioregeneration of adsorbent.

However, dissolution of beads was recorded after 6 h of incubation. For longer time, no follow up can be achieved because of cell release which occurred spontaneously under the influence of bacterial growth on the periphery of the beads and the shear forces due to the mechanical stirring by reducing the thickness of the peripheral layer of the gel containing the active biomass, and collisions between the beads [31]. According to some researchers, the presence of the biological environment can regenerate the adsorbent, but the development of biomass may limit the adsorption phenomena [32].

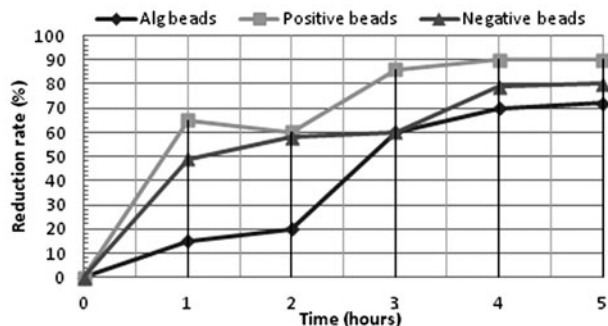

Fig. 6. Reduction percentage of different alginate beads in 200 mg L^{-1} of xylene.

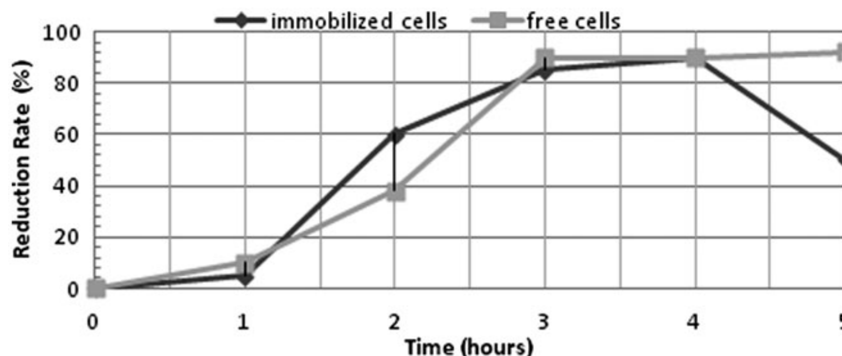

Fig. 7. Comparing xylene percentage of reduction by free and immobilized strains.

The percentage reduction of the different types of beads does not show a large difference (80–90%), but it is clearly notable that the degradation rate is not the same in the first hours of incubation. Active cells beads showed a removal of 60% of xylene, while beads without biomass does not exceed 20%, then it can be concluded that the biomass introduced into alginate beads accelerates reducing xylene. Recent studies indicate high removal efficiencies of BTEX from the liquid phase in biological systems with immobilized biomass, up to 99% with removal rates close to 20 mg L^{-1} h^{-1} [33,34].

Fig. 7 shows the percentage of reduction by free and immobilized strains in 200 mg L^{-1} of xylene.

Percentage of reducing xylene in either free or immobilized culture condition showed significant growth during the first four hours with a reduction rate (90–99%). The difference between free and immobilized cells was especially in the speed of degradation. The higher xylene degradation rates for the immobilized culture were attributed to the higher cell density. In the second hour of reduction, immobilized strains showed faster reduction (60%) than free strains (30%) (double elimination rate), and it probably returns to the protection of strains into the beads against some inhibitory effects [35].

After three hours of incubation, we saw similar removal rates for each type of strains. We can conclude that the immobilization of *Streptomyces* sp. AB1 in alginate sodium beads seems to be effective, as it shows high removal rates (85–90%), but beyond this encapsulation will have no effect; explosion beads were noticed after (6 h), caused by bacterial growth [36]. While free cell degradation continues to reach 99% of degradation, it is clear that the higher degradation rate in the bioreactor can be attributed to the higher cell density in the porous beads bioreactor.

4. Conclusion

This study allowed us to make an initial assessment of xylene biodegradation by *Streptomycetes* sp. AB1 as a potentially biodegradative species of mono-aromatic hydrocarbon. Results have shown a high rate of degradation equivalent to 99% in less than 5 h. The encapsulation of *Streptomyces* has been successfully completed with a significant reduction (90%) of the strains immobilized in sodium alginate beads. The kinetic studies of xylene biodegradation by *Streptomyces* sp. AB1 strains showed that xylene can be effectively degraded in the porous beads bioreactor under hypoxic conditions and with high substrate concentrations. Xylene as a sole carbon source for cell growth showed good kinetics; however, immobilized cells adapted in the bioreactor were less sensitive and more tolerable to the toxic substrate than the free cells under similar conditions also it showed the faster rates of degradation. The immobilized culture in the porous beads could be used to treat industrial waste streams containing high concentrations of xylene. In addition, these results show a production potential of biosurfactants; this will probably allow the possibility for the bioremediation of contaminated sites based on free or immobilized cells.

References

[1] R.R. Eldon, Removal of xylene in gas-phase using compost-ceramic ball biofilter, Int. J. Phys. Sci. 4(11) (2009) 638–644.
[2] P. Negrea, F. Sidea, A. Negrea, L. Lupa, M. Ciopec, C. Muntean, Studies regarding the benzene, toluene and o-xylene removal from waste water, Chem. Bull. Politech. Univ. 53(67) (2008) 1–2.
[3] S. Silva, G. Matthew, R. Lucia Durrant, Degradation of polycyclic aromatic hydrocarbons (2–7 rings) under micro aerobic and very-low-oxygen conditions by soil fungi, Int. Biodeterior. Biodegrad. 63 (2009) 224–229.

[4] C. Lin, L. Gan, Z.L. Chen, Biodegradation of naphthalene by strain *Bacillus fusiformis* (BFN), J. Hazard. Mater. 182 (2010) 771–777.

[5] L. Jecu, A. Gheorghe, G. Epure, F. Popea, A. Rosu, A. Roseanu, A biological treatment for the purification of waste waters containing volatile organic compounds, in: Proceedings of Romanian International Conference on Chemistry and Chemical Engineering RICCCE 15th, Sinaia, Romania, September 20–22, 2007.

[6] S.H. Yeom, A.J. Daugulis, Benzene degradation in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans* Y234, Proc. Biochem. 36 (2001) 765–772.

[7] K.F. Reardon, D.C. Mosteller, J.D. Bull Rogers, Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1, Biotechnol. Bioeng. 69 (2000) 385–400.

[8] M. Rehfuss, J. Urban, *Rhodococcus phenolicus* sp. nov., a novel bioprocessor isolated actinomycete with the ability to degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources, Syst. Appl. Microbiol. 28 (2005) 695–701.

[9] F. Zermane, Study of the Cultural Characteristics of the Actinomycetes Involved in the Biodegradation of Cellulose, Pectic Substances and Synthetic Organic Compounds, Magister Thesis in Applied Microbiology, University Mentouri Constantine Algeria, 2008.

[10] A. Badis, F.Z. Ferradji, A. Boucherit, D. Fodil, H. Boutoumi, Removal of natural humic acids by decolorizing *actinomycetes* isolated from different soils (Algeria) for application in water purification, Desalination 259 (2010) 216–222.

[11] B. Jaouadi, A. Badis, N. Zaraî, S. Bejar, The Bioengineering and Industrial Applications of Bacterial Alkaline Proteases: The Case of SAPB and KERAB. Progress in Molecular and Environmental Bioengineering—From Analysis and Modeling to Technology Applications. Angelo Capri (Ed.), ISBN: 978-953-307-268-5, InTech, Available from: <http://www.intechopen.com/articles/show/title/>, The Bioengineering and Industrial Applications of Bacterial Alkaline Proteases: the Case of SAPB and KERAB. 20 (2011) 445–466.

[12] K. Eddouaouda, S. Mnif, A. Badis, S. Younes, S. Cherif, S. Ferhat, N. Mhiri, M. Chamkha, S. Sayadi, Characterization of a novel biosurfactant produced by *Staphylococcus* sp. strain 1E with potential application on hydrocarbon bioremediation, J. Basic Microbiol. 52 (2012) 408–418.

[13] B. Jaouadi, A. Badis, D. Fodil, F.Z. Ferradji, H. Rekik, N. Zaraî, S. Bejar, Purification and characterization of a novel thermostable keratinolytic serine alkaline proteinase from Streptomyces rochei strain AB1 with high stability in organic solvents, Bioresour. Technol. 101 (2010) 8361–8369.

[14] M. Kulkarni, A. Chaudhari, Microbial remediation of nitro-aromatic compounds: An overview, J. Environ. Manage. 85 (2007) 496–512.

[15] J. Ha, C.R. Engler, J.R. Wild, Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads, Bioresour. Technol. 100 (2009) 1138–1142.

[16] M. Lezhari, Preparation and Characterization of Innovative Adsorbents Based on Pillared Clays and Alginate: Application to the Adsorption of Organic Compounds and Heavy Metals, Industrial Chemistry PhD, University S. Dahlab Blida, Algeria, 2011.

[17] M.H. Labrecque, Study of the Capacity of Two Yeast Strains to Degrade Xylene, Masters in Microbiology Agricultural, Department of Soil and Agricultural Engineering, University Laval, Quebec, 2003.

[18] J. Jiin-Shuh, L. Ming-Kuo, W. Shih-Ming, C. Pabitra, P.M. Jyoti, Effects of inorganic nutrient levels on the biodegradation of benzene, toluene, and xylene (BTX) by *Pseudomonas* spp. in a laboratory porous media sand aquifer model, Bioresour. Technol. 99 (2008) 7807–7815.

[19] J. Sikkema, J.A.M. Bont, B. Poolman, Mechanisms of membrane toxicity of hydrocarbons, Microbiol. Rev. 59 (1995) 12–22.

[20] P. Arnaud, Complementary Chapter of Organic Chemistry Courses, Dunod, eighteenth edition, Paris, 2009.

[21] J.P. Guiraud, Microbiologie Alimentaire, Dunod, Paris, 1998.

[22] M. Farhadian, D. Duchez, C. Vachelard, C. Larroche, Monoaromatics removal from polluted water through bioreactors—A review, Water Res. 42 (2008) 1325–1341.

[23] H. Gardin, J.M. Lebeault, A. Pauss, Biodegradation of xylene and butyl acetate using an aqueous-silicon oil two-phase system, Biodegradation 10 (1999) 193–200.

[24] J. In-Gyung, P. Chang-Ho, Characteristics of *Rhodococcus pyridinovorans* PYJ-1 for the biodegradation of benzene, toluene, m-xylene (BTX), and their mixtures, J. Biosci. Bioeng. 97 (2004) 429–431.

[25] S. Ferhat, S. Mnif, A. Badis, K. Eddouaouda, R. Alouaoui, A. Boucherit, N. Mhiri, N. Moulai-Mostefa, S. Sayadi, Screening and preliminary characterization of biosurfactants produced by *Ochrobactrum* sp. 1C and *Brevibacterium* sp. 7G isolated from hydrocarbon-contaminated soils, Int. Biodeterior. Biodegrad. 65 (2011) 1182–1188.

[26] F. Grattepanche, Study of a Pre-continuous Fermentation System of Milk with a Mixed Culture Immobilized Functional, PhD, Science and Food Technology, University Laval, Quebec, 2005.

[27] A.J. Daugulis, T.B. Janikowski, Scale-up performance of a partitioning bioreactor for the degradation of polyaromatic hydrocarbons by *Sphingomonas aromaticivorans*, Biotechnol. Lett. 24 (2002) 591–594.

[28] M. Farhadian, C. Vachelard, D. Duchez, C. Larroche, In situ bioremediation of monoaromatic pollutants in groundwater: A review, Bioresour. Technol. 99 (2008) 5296.

[29] C.O. Ania, B. Cabal, C. Pevida, A. Arenillas, J.B. Parra, F. Rubiera, J.J. Pis, Effects of activated carbon properties on the adsorption of naphthalene from aqueous solutions, Appl. Surf. Sci. 253 (2007) 5741–5746.

[30] L. Geoffroy, Study of the Elimination of Dangerous Aromatics Substances Coupling Adsorption and Biodegradation Process, PhD Thesis, Engineering Process and Environment, the National Institute of Applied Sciences Toulouse, France, 2009.

[31] Y. Doleyres, Continuous Production of Probiotic Lactic Ferments by Immobilized Cell Technology, PhD thesis, Agricultural Sciences and Food, Laval University, Quebec, 2003.
[32] Ö. Aktaş, F. Çeçen, Adsorption reversibility and bioregeneration of activated carbon in the treatment of phenol, Water Sci. Technol. 55 (2007) 237–244.
[33] K. Ohlen, Y.K. Chang, W. Hegemann, C.R. Yin, S.T. Lee, Enhanced degradation of chlorinated ethylenes in groundwater from a paint contaminated site by two-stage fluidized-bed reactor, Chemosphere 58 (2005) 373–377.
[34] T.F. Guerin, A pilot study for the selection of a bioreactor for remediation of groundwater from a coal tar contaminated site, J. Hazard. Mater. 89 (2002) 241–252.
[35] A. Groboillot, D.K. Boadi, D. Poncelet, R.J. Neufeld, Immobilization of cells for application in the food industry, Crit. Rev. Biotechnol. 14 (1994) 75–107.
[36] A.J. Daugulis, T.B. Janikowski, Scale-up performance of a partitioning bioreactor for the degradation of polyaromatic hydrocarbons by *Sphingomonas aromaticivorans*, Biotechnol. Lett. 24 (2002) 591–594.