Evaluation of m-Nitrophenol and p-Nitrophenol degradation with biological activated carbon by immobilization of *Pseudomona putida* ATCC 700447

Escobar. E. [1], Moreno-Piraján J.C.[2], Pérez. M. [1], Sánchez O.[1*]
[1] Department of Chemical Engineering – Product and Process Design Group
[2] Department of Chemistry – Porous Solids and Calorimetric Group
Universidad de Los Andes, Carrera 1E No. 19 A 40, Bogotá, Colombia
el-escob@uniandes.edu.co, jumoreno@uniandes.edu.co , mar-pere@uniandes.edu.co
*osanchez@uniandes.edu.co

The degradation of m-nitrophenol (MNP) and p-nitrophenol (PNP) with biologically activated carbon (BAC) by the immobilization of *Pseudomonas putida* ATCC 700447 was studied. pH and nitrophenol concentration effect was evaluated on activated carbon adsorption over activated carbon (AC) and biodegradation by *Pseudomonas putida* ATCC 700447, separately. At the best conditions the Langmuir constant for each one was 100 mg g^{-1} for MNP (at pH 9.34 and 150 rpm) and 101 mg g^{-1} for PNP (at pH 8.00 and 150 rpm). The obtained biodegradation rates were 5.192 and 18.284 ppm h^{-1} for 50 and 100 ppm of MNP (at pH 9.34) with a degradation of 79 and 36% within 71 and 6 h, respectively; while, PNP was 100% degraded within 24 and 48h with degradation rates of 5.251 and 2.549 ppm h^{-1} for 50 and 100 ppm (at pH 6.00), respectively. The fixed BACs allowed to get a completely disappearance of both nitrophenolic compounds in the bulk fluid within 16 h, a higher time than the alone adsorption but lower than biodegradation process. In conclusion, BACs by *P. putida* ATCC 700447 offer advantages like greater microorganism resistance to MNP toxicity and capability of their own regeneration due to the microorganisms break out the nitrophenol molecules continuously as they are adsorpted enlarging the carbon adsorption ability.

1. Introduction

On a worldwide basis, textile and pesticide industry generate large quantities of contaminated effluents with nitro-phenols like m-nitrophenol (MNP) and p-nitrophenol (PNP). Due to the toxic character of these substances, the quantity that can be poured is found regulated. According to the Environmental Protection Agency (EPA), the water quality criterion for the aquatic life conservation and protection establishes that the limit of MNP is 200 mg L^{-1} and the level of PNP should not exceed 100 mg L^{-1}, since their easy adsorption by respiratory tract, digestive and cutaneous way (EPA, 1998; Gemini *et al.*, 2006; Kalme *et al.*, 2007).

Biological growth on activated carbon in water and wastewater treatment applications is an expected consequence of the favorable environment provided by this material. Activated carbon surfaces are excellent for colonization by microorganisms and its adsorptive properties serve to enrich substrate and oxygen concentrations, the craggy surface provides recesses that are sheltered from fluid shear forces, and the variety of

functional groups on the surface can enhance attachment of microorganisms. The principle advantage of BAC systems is increased effluent throughput until breakthrough resulting in less frequent regeneration time (Weber *et al.,* 1978).

Working with *Pseudomonas putida* in BAC facilitates the degradation of nitrophenols in contaminated effluents and at the same time offers advantages as the regeneration of these, due to the biological activity of the microorganisms that degrade continuously the compounds involved, enlarging the adsorption of the carbon toward these compounds, given that upon degrading, free spaces of adsorption on the material are liberated (Zeyer and Kearney, 1984).

The development of an efficient method in which adsorption and degradation of nitrophenol compounds are involved is of great interest for the Colombian industry. By this reason, in this work was evaluated MNP and PNP degradation through a BAC by the use of *Pseudomonas putida* ATCC 700447.

2. Materials And Methods

2.1. Chemicals

MNP and PNP were 99.9% of purity obtained from Merck. All the other reagents used were analytical grade. The activated carbon was a bituminous coal from Cesar (Colombia), which surface area and pore volume were determinated by adsorption-desorption of N_2 at 77 K (Quantachrome Sorptometer ACE-3) using BET (Bruner Emmett Teller), acid and basic groups were determinated by Boehm titrations (Titrometer TA 20 Bonus) (Barkauskas and Cannon, 2003), point of zero charge (p.z.c) and immersion enthalpy were determinated according to Bansal and Goyal (2005).

2.2 Microorganism

Pseudomonas putida ATCC 700447 which degrades PNP was obtained from culture collection (ATCC, USA). The organism was activated in a standard nutrient broth (Tryptic Soy Broth (TSB) composition per liter: Soya peptone, 3 g; dextrose, 2.5; casein peptone, 17 g; K_2HPO_4, 2.5 g; NaCl 5 g) and growth in cetrimide agar selective for *Pseudomonas.* Submerged culture in Luria-Bertani (LB) medium (LB composition per liter: Triptona, 10 g; yeast extract, 5g; NaCl, 0.5 g; Glycerin 1 mL) were made to produce the biomass used in the biodegradation assays. The minimum salt medium (MSM) used in this work for the nitrophenol compounds degradation was: K_2HPO_4, 0.75 g; KH_2PO_4, 0.2 g; $MgSO_4{\cdot}7H_2O$, 0.09 g; $FeSO_4{\cdot}7H_2O$, 0.06 g; glucose, 0.4 and enriched with the corresponding MNP or PNP (Kulkarni and Chaudhari, 2006).

2.3 Batch equilibrium studies

Equilibrium adsorption isotherms were determined from batch studies done in 250 mL shake flask containing 100 mL of the above MSM with 0.5 g AC and varying amount of initial MNP or PNP concentrations (10, 50 and 140 ppm MNP and 25, 100 and 150 ppm PNP) at different pHs (7.34, 8.34(~pKa) and 9.34±0.01 for MNP and 6.00, 7.00 (~pKa) and 8.00±0.01 for PNP) with constant agitation (0 or 150 rpm in a Branstead labline max 97000 shaker), and temperature (25°C). Samples of 2 ml were taken to measure the

MNP and PNP concentration during the different assays. Adsorption isotherms were analyzed using the Langmuir model (Ec. 1), (Annadurai *et al*., 2000).

$$q = \frac{KaC_e}{1 + aC_e} \quad (1)$$

where q is the quantity of nitrophenol adsorpted in the time (mg g^{-1}), K is the constant related to the adsorption capacity (mg g^{-1}), a is the Langmuir constant (L mg^{-1}) and C_e is the equilibrium concentration of the solute in liquid phase (mg L^{-1}).

2.4 Biodegradation of MNP and PNP

Biodegradation of MNP and PNP was determined separately from batch studies done in 250 mL shake flask containing 100 mL of the above MSM supplemented with 10 ppm of each nitrophenol at the selected pH, and inoculated with 1.7 mg of biomass in dry weight mL^{-1} obtained from LB medium. Once decoloration was achieved 1 mL of each assay was taken to inoculate the respective 50 ppm MNP or PNP solution at the selected pH. After bleaching these solutions, 1 mL was taken to inoculate 100 ppm MNP or PNP solution at respective pH. Samples from the different solutions were taken to determinate optical density (at λ:600 nm) and to monitor MNP or PNP concentration (spectrophotometrical quantification was carried out at the obtained wave length for each pH).

2.5 BAC production and degradation of mononitrophenols

The BAC was produced following the procedure: in 250 mL shake flasks containing 100 mL of MSM adjusted at the selected pH (7.34, 8.34 and 9.34±0.01 for MNP and 6.00, 7.00 and 8.00±0.01 for PNP) were added 0.5 g of autoclaved AC and subsequently were inoculated with 1.0 mL of *Pseudomonas putida* ATCC 700447 adapted previously at 50 ppm MNP or 100 ppm PNP. The growth of the microorganism was permitted for 96 hours at 25ºC and 150 rpm. After culturing, was added the respective nitrophenol to reach concentrations of 50, 100 or 150 ppm in MNP or PNP. The effect of biomass concentration on BAC activity was determinated for PNP degradation; the above procedure was carried out for PNP varying the inoculum to 0.5 mL of the adapted *P. putida* ATCC 700447.

During the decolorization samples of supernatant and few pellets of carbon were taken out to monitor spectrophotometrically the nitrophenol concentration and the microorganism immobilization by scanning electromagnetic microscopy (SEM) and surface area determination.

2.6 MNP and PNP quantification

MNP and PNP were determined spectrophotometrically (Thermospectronic GENESYS 5) at the maximum wave length obtained for MNP and PNP at different pHs according to standard methods of analysis (Shamsipur *et al*., 2005). For MNP λ_{max}:300 nm at pH

7.34 and λ_{max}:310 nm at pH 8.34 and 9.34, for PNP λ_{max}:370 nm at pH 6.00, λ_{max}:436 nm at pH 7.00 and λ_{max}:450 nm at pH 8.00.

3. Results And Discussion

3.1 Physicochemical characterization of the activated carbon

The bituminous activated carbon used was defined as a mesopore solid, with a type II isotherm according to IUPAC classification (Bansal and Goyal, 2005), with a mean pore volume of 0.438 cc g^{-1} and a surface area of 830 m^2 g^{-1}. The activated carbon displayed a basic character (acid groups 0,095 and basic groups 0,115 mmol g^{-1} of carbon), that is in agreement with the point of zero charge obtained (p.z.c: 11), similar results were reported by Navarrete *et al*. (2007). The obtained immersion enthalpy in benzene was Δ_{C6H6} 118,7 J g^{-1}, value that is in the range reported by López-Ramón *et al.* (1999) for activated carbon characterized in benzene (Δ_{C6H6} from 109,7 to 146,0 J g^{-1}).

3.2 Adsorption onto activated carbon

The single-solute adsorption of MNP and PNP at three different pHs and two agitation rates (0 and 150 rpm) were perform using a bituminous activated carbon. Langmuir isotherms of each nitrophenol compound were obtained at different conditions. Table 1 summarizes the Langmuir adsorption constants K and a gotten after the linearization of the isotherms. Adsorption affinity of the two nitrophenolic compounds to the activated carbon was found to be higher when the molecules are ionized (pH > pKa). At the best adsorption conditions for the nitrophenol compounds (150 rpm and pH 9.34 and 8.00 for the MNP and PNP, respectively) a 100% of sorption was obtain within 4.5 h. The basic pH underneath of the activated carbon point of zero charge (p.z.c 11) increases the adsorption due to the affinity between the dissociated molecule and the surface carbon charge (Bansal and Goyal, 2005). The difference in adsorption affinity can be explained mainly by the steric hindrance due to the size and shape of the solute molecules and the reduction of intermolecular hydrogen bonds in aqueous solution by the formation of intramolecular hydrogen bonds between hydroxyl group and the substituent, when nitro substituent is as much as close to hydroxyl group (Pura and Atun, 2005).

Table 1. Langmuir parameters of each nitrophenolic compound on activate carbon

	0 r.p.m		150 r.p.m.	
	K (mg g^{-1})	*a*(L mg^{-1})	*K* (mg g^{-1})	*a* (L mg^{-1})
MNP (pKa 8.34, water solubility 12.4 g L^{-1})				
pH 7.34	9.09	0.410	18.5	0.039
pH 8.34	40.0	0.011	76.9	0.009
pH 9.34	62.5	0.006	100	0.006
PNP (pKa 7.08, water solubility 13.5 g L^{-1})				
pH 6.00	28.6	0.005	29.4	0.196
pH 7.00	35.7	0.002	61.34	0.086
pH 8.00	100	0.001	101	0.028

3.3 MNP and PNP biodegradation

The single-solute biodegradation of MNP and PNP at three different pHs and constant agitation (150 rpm) were perform using *Pseudomonas putida* ATCC 700447. The initial biodegradation rate was determinated at the tested conditions (Table 2). For both concentrations 50 and 100 ppm PNP a 100% degradation was obtained at about 24 and 48h, respectively, without significant differences among the pHs; while for MNP the greatest degradation was obtained during the first 6h (79% of a 100 ppm solution) and 71h (36% of a 50 ppm solution) at pH 9.34 (Fig. 1).

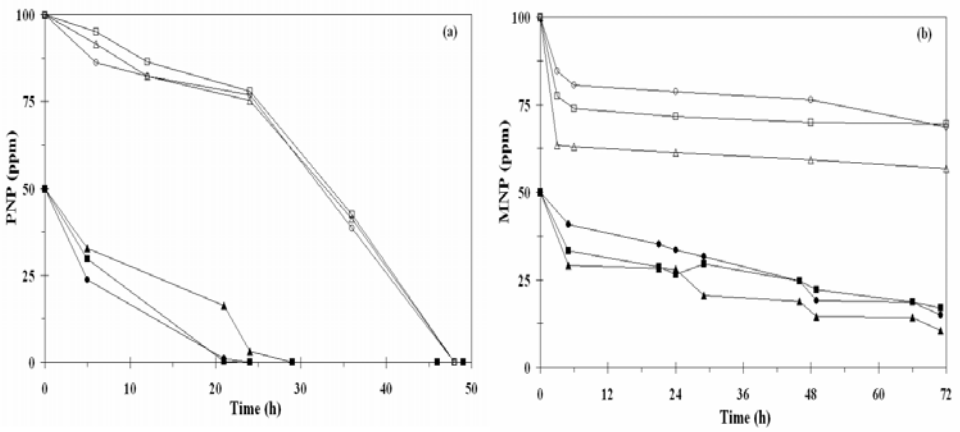

Figure 1. pH and initial concentration effect on PNP (a) and MNP (b) degradation by Pseudomona putida ATCC 700447. Full signs are for 50 ppm initial nitrophenol concentration and empty sign for 100 ppm. pH for PNP degradation were 6.00 (o), 7.00(□) and 8.00 (Δ) and for MNP were 7.34 (o), 8.34(□) and 9.34 (Δ).

Table 2. pH effect on degradation of 50 and 100 ppm MNP and PNP by *P. putida* ATCC 700447

MNP			**PNP**		
	50 ppm	100 ppm		50 ppm	100 ppm
pH	**Initial degradation rate (ppm h^{-1})**		**pH**	**Initial degradation rate (ppm h^{-1})**	
7.34	2.174	7.0440	6.00	5.251	2.549
8.34	4.087	10.682	7.00	4.074	0.816
9.34	5.192	18.284	8.00	3.463	1.413

The results display for both concentrations a higher MNP biodegradation at pH 9.34, although a MNP concentration increment increase the initial degradation rate, possibly by the previous adaptation of the microrganims, the MNP degraded decrease with higher concentrations. Zeyer and Kearney (1984) reported the MNP biodegradation by strain of *P. putida* at pH 6.5 and that toxicity is increased at acidic pH (< pKa), on the contrary alkaline pH (> pKa) reduced it and hence accelerates its metabolism. On the other hand, the greatest PNP biodegradation rate was presented at pH 6.00 but contrary to MNP at 100 ppm this decreased. The completed PNP degradation was obtained at the different pH. Nevertheless, Kulkarni and Chaudhari (2006), reported that PNP

degradation by a native *P. putida* was inhibited in acidic range (pH 4.0 – 6.5), and within 16.5h was degraded 300 ppm PNP at pH 7.5 – 9.5. The pK, of PNP and MNP is 7.03 and 8.34, respectively. At the pHs where a great metabolism occurred, PNP was mainly not charged whereas MNP was dissociated. Hence, metabolism does not seem to be a function of the charge, these results are similar to reported by Zeyer and Kearney (1984) for o-nitrophenol and m-nitrophenol biodegradation by a *P. putida* strain.

3.4 BAC production and degradation of mononitrophenols

The single-solute adsorption and degradation on BAC at the studied pHs and concentrations displayed higher decoloration times than the adsorption process but lower than biodegradation. The time was increased in up to 12h in front of adsorption. This possibly occurred by the biofilm formed on the carbon surface during the microorganism growth, which increase the adsorption resistance and decrease the free sorption area. The decrease in the surface area was evidenced by the SEM micrographs obtained during the culture and the drop in the sorption surface area (Fig. 2). A 100 ppm MNP solution was decolorated in at least a 90% within 16h with no significant difference for the three pHs (Fig 3b). Now that with the suspended microorganism at these conditions was obtained a 36% MNP degraded and a pH effect was observed, this perform of the BAC correlates with literature work that the cells immobilized on the activated carbon can protect the microorganisms from the toxicity of pollutants. Moreover, the suspended-cell systems are more sensitive to the change of pH (Annadurai *et al.*, 2002).

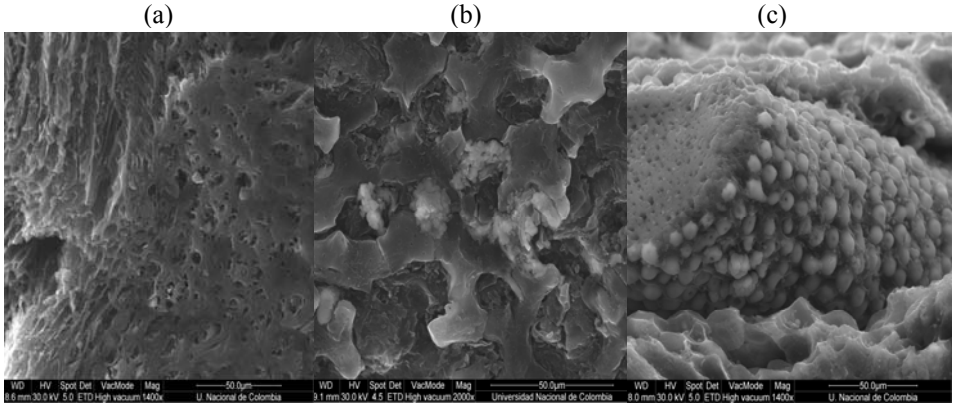

Figure 2. SEM images for (a) original activated carbon and (b-c) immobilized activated carbon.

The inoculum volume effect over the BAC production was evaluated over 150 ppm PNP solutions. The concentration profiles displayed an expected performance; while for a 1.0 mL inoculum the decoloration time was 6.5h, for the 0.5 mL inoculum was 16h (Fig. 3a). Due to the difference in cell concentration was expected a less free adsorption surface area with the 1.0 mL inoculum but a higher biodegradation rate, contrary to the 0.5 mL inoculum. The BAC performance is similar to reported by Li *et al.* (1998) who obtained a completed degradation of 50 ppm PNP within 24h by *P. putida strain* JS444, and Abu-Salah *et al.* (1996), that reported a total time of loss of color of 24 h in a rank

from 100 to 160 ppm using pulverized activated coal as backup of immobilization and a mixture of stumps where the predominant one was a remote native of *Pseudomonas sp.*. As for MNP at the end of the 16 h no significant effect of the pH is observed.

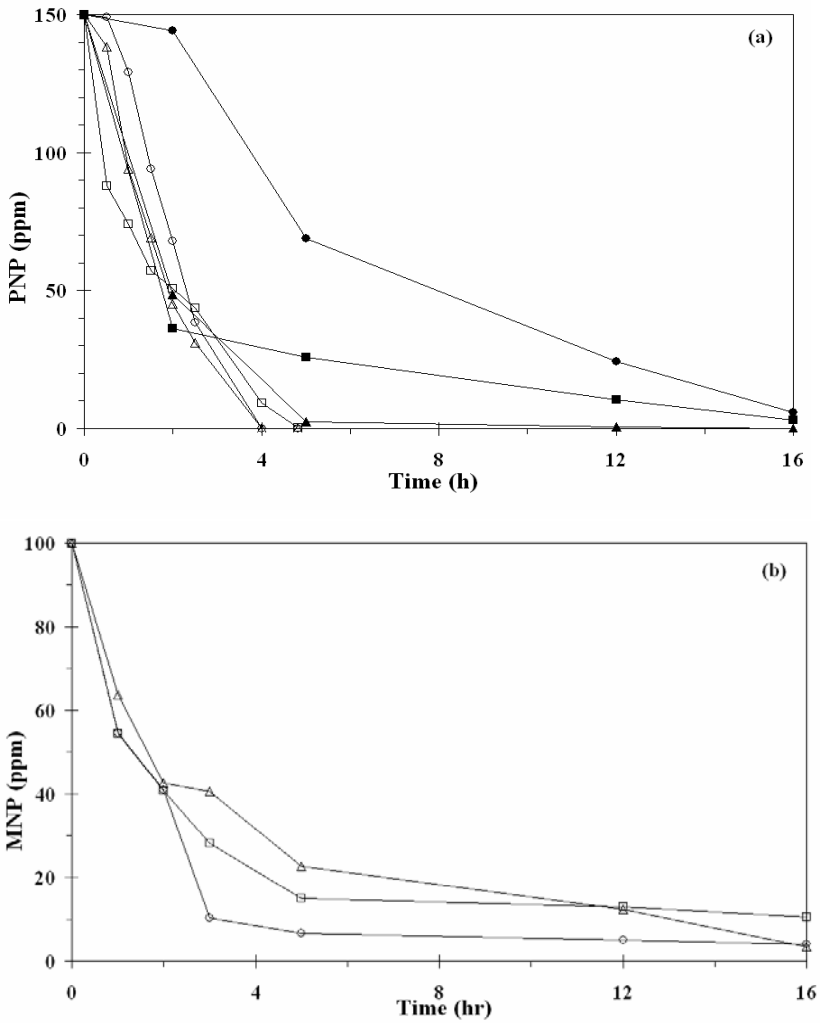

Figure 3. Inoculum volume effect on 150 ppm PNP degradation (a) and 100 ppm MNP (b) degradation by activated carbon with Pseudomona putida ATCC 700447. Full signs are for 0.5 mL inoculum and empty sign for 1.0 mL of inoculum. pH for PNP were 6.00 (o), 7.00(□) and 8.00 (Δ) and for MNP were 7.34 (o), 8.34(□) and 9.34 (Δ).

4. Conclusions

The activated carbon that was a mesopore solid displayed a good capacity of retention of the PNP and MNP, getting a complete adsorption within 4.5 h at 150 rpm for concentrations of about 150 ppm.

Pseudomonas putida ATCC 70047 degrade PNP (in a 100%) and has the capacity of degrade MNP (in up to 79%), nevertheless the degradation rate depends of the solution pH because of the dissociated state of the molecule.

The adsorption and degradation of MNP and PNP in BAC was achieved at the evaluated concentrations 100 and 150 ppm, respectively, with no significant difference among the decoloration time at the evaluated pHs. A main effect caused by the biomass concentration used in the immovilization on the activated carbon was observed on the PNP decoloration rate, possibly because of the increase in the mass transfer resistance by the formed biofilm.

5. References

Abu-Salah, K., G. Shelef, D. Levanoxf, R. Armonb and C.G. Dosoretza, 1996, *J. Biotechnol.*, 51:265-272.

Annadurai, G., R–S. Juang and D–J. Lee, 2002, *J. Environ. Sci. Health.*, A37(6):1133-1146

Annadurai, G., B.S. Rajesh, K.P.O. Mahesh and T. Murugesant, 2000, Bioprocess Eng., 22(6),493-501

Bansal, R.C. and M. Goyal, 2005, Actived Carbon Adsoption. First Edition, Taylor & Francis Group., Florida, EE.UU

Barkauskas, J. and F. S. Cannon, 2003, Potentiometric titrations: Characterize functional groups and adsorbed species on activated carbon, paper presented at Carbon 2003 - An International Conference on Carbon, Oviedo, Spain, July 6 - 10.

EPA., United States Environmental Protection Agency, 1998, Prevention, pesticides and toxic substances. EPA-738-F-97-016. December 2007. http:// www.epa.gov/REDs>.

Gemini, V.L., A. Gallego, C.E. Gómez, E.I. Planes and S.E. Korol, 2006, Biologic processes for p-nitrophenol detoxification, Higiene y Sanidad Ambiental, 6:145-149 (in Spanish)

Kalme, S.D., G.K. Parshetti, S.U. Jadhav and S.P. Govindwar, 2007, *Bioresour. Technol.*, 98(7):1405-1410.

Kulkarni, M. and A.Chaudhari, 2006, Bioresour. Technol., 97(8):982-988

Li, P.H.Y., F.A. Roddick and M.D. Hobday, 1998, *J. Chem. Technol. Biotechnol.*, 73:405–413

Navarrete L., J.C. Moreno-Piraján, L. Giraldo and V. García, 2007, Phenol 3-chlorophenol adsorption on activated carbon by immertion heat, Información tecnologica, 18(3):71-80 (in Spanish)

Pura, S. and G. Atun, 2005, *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 253:137-144

Shamsipur, M., G. Raoof, H. Sharghi and B. Hemmateenejad, 2005, Ann Chim., 95(1-2):63-76

Weber, W.J., M. Pirbazari and G.L. Melson, 1978, *Env. Sci. Tech.* 127:817-819

Zeyer, J. and P.C. Kearney, 1984, *J. Agric. Food. Chem.*, 32:238-242