Anaerobic biodegradation of aniline under different electron accepting conditions

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Abstract
Aromatic amines are important industrial chemicals. Due to their high toxicity and persistency in both water and soils, they are of environmental concern. Aniline is not biodegradable in normal anaerobic condition. A novel approach of anaerobic biodegradation coupled with selective redox mediators, is proposed. Different redox mediators, namely manganese dioxide, Ferric citrate, ferrous chloride and magnetic iron oxide nanoparticle were tested. Fe_3O_4 nanoparticles, with size of 10 nm, were shown as the best, leading to 98 % of aniline removal efficiency within 24 h of operation at 37 °C. Biodegradation of aniline followed a pseudo first order kinetic model. Products of reaction were identified by GC/MS, revealing that the biodegradation occurred via catechol pathway. Methanogenic activity tests suggest that an IC50 of aniline is ~10 mM and that Fe_3O_4 nanoparticles are not toxic on un-adapted biomass.

Keywords
Anaerobic biodegradation; Aromatic amines; Aniline, Electron acceptors; magnetic Fe_3O_4 nanoparticles.

INTRODUCTION

Aromatic amines are generally identified as those chemical compounds having in their molecular structure one or more aromatic rings, bearing one or more amino substituents. They range from the simplest aniline to highly complex molecules with conjugated aromatic or heterocyclic structures and multiple substituents [1].

Aromatic amines, specially aniline, are major sources include several chemical industry sectors such as oil refining, synthetic polymers, dyes, adhesives, rubbers, perfume, pharmaceuticals, pesticides and explosives [2]. They are also the main products of some azo dyes reduction [1] and a by-product of the manufacturing of compounds such as pesticides, pharmaceuticals, semiconductors, explosives, polyurethane foams and dyes.

Discharge of aromatic amines to the environment is of environmental concern as it is toxic to aquatic life and also exerts additional oxygen demand due to autoxidation reaction involved during its biodegradation. Most of them have been found as carcinogenic, mutagenic, and resistant to microbial degradation. Biological treatment of aniline containing wastewaters provides more specific conversions, is relatively inexpensive and usually results in complete mineralization. A drawback of using aerobic treatment for aromatic amines degradation, is that many of them are prone to autoxidation once they are exposed to oxygen. Since autoxidation often involves enlargement of the molecules, their biodegradability may consequently be decreased [3]. The anaerobic degradation of aromatics is a more recently discovered microbial capacity that still awaits a deeper understanding despite the fact that microbial metabolism in the absence of oxygen is the most ancient of all life processes. The mineralization of aromatic compounds by facultative or obligate anaerobic bacteria (and some archaea) can be coupled to anaerobic respiration with a variety of electron acceptors, e.g., nitrate, sulfate, iron(III), manganese(II), and selenate, with each one conserving different yields of energy. Our studies focused on improved aniline biodegradation under anaerobic conditions, testing different electron acceptors ($NaNO_3$, MnO_2, Ferric citrate, $FeCl_2$

and magnetic Fe_3O_4 nanoparticle). In an attempt to identify the final products of anaerobic biodegradation HPLC and GC/MS analysis were performed..

MATERIAL AND METHODS

Syntheisis of biocompatible magnetic Fe_3O_4 nanoparticles: Fe_3O_4 nanoparticles were prepared by a controlled chemical co-precipitation method, according to Sun et al. (2006). Solution I was prepared by dissolving 0.01 mol $FeCl_2 \cdot 4H_2O$ and 0.02 mol $FeCl_3 \cdot 6H_2$ O in 50 mL distilled water and solution II was composed of an aqueous ammonium hydroxide (25–28%, w/w) solution (1.5 mol L^{-1}). The surfactant (sodium oleate) was added to the former solutions as a coating agent for protect the NP, that is useful in strengthening and preserving the parent molecule. Precursor solution I was added into Precursor solution II, drop wise with strong stirring under nitrogen atmosphere. The colour of the solution changed from light brown to black, indicating the formation of magnetic Fe_3O_4 nanoparticles, which were allowed to crystallize completely for another 60 min under rapid stirring. Nanoparticles were characterized by STEM, FTIR and XRD.

Biodegradation of aniline: The primary electron donating substrate was composed of 2 g L^{-1} chemical oxygen demand (COD) of a NaOH-neutralised volatile fatty acids (VFA) mixture, containing acetate, propionate and butyrate in a COD based ratio of 1:10:10. Basal nutrients were also added: NH_4Cl (2.8 g L^{-1}), $CaCl_2$ (0.06 g L^{-1}), KH_2PO_4 (2.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (1 g L^{-1}). Medium was buffered at a pH of 7.3 ± 0.2 with $NaHCO_3$ (2.5 g L^{-1}). Non-adapted anaerobic granular sludge was added to the medium at a concentration of 2.5 ± 0.5 g L^{-1} volatile suspended solids (VSS). Different electron acceptors, $NaNO_3$ (10 mM), MnO_2 (10 mM), Ferric citrate (10 mM), $FeCl_2$ (10 mM), Fe_3O_4 nanoparticle (3 mg), were tested. Aniline concentration was 0.2 mM aniline. Experiments were conducted at ambient temperatures of 30-35°C.

RESULTS AND DISCUSSION

The reactions of aniline biodegradation followed pseudo first order reaction (Figure 1). 97.5 % of aniline biodegradation was achieved when sodium nitrate was used as an electron acceptor (Figure 2A). MnO_2 and Ferric citrate were worse electron acceptors, after 48 h of reaction, aniline biodegradation was only of ~20 % and 29 %, respectively. Non-toxic and biocompatible Fe_3O_4 nanoparticles, were developed, charaterized and tested. FTIR analysis indicate that sodium oleate is successfully bound to the surface of Fe_3O_4 nanoparticles. The XRD diffraction pattern of the nanoparticles matches the pattern for the magnetite. STEM images reveal that synthesized coated particles were mostly spherical in shape, although particles were much aggregated on sheets. Sized was estimated as 8-10 nm (Figure 3). This new material was found to be a good electron acceptor for aniline biodegradation in redox environment, 98 % of degradation was observed within 24 h (Figure 2B). Control tests indicated that the adsorption is negligible (<2 %). Although the good results obtained also with NaNO3, magnetic Fe_3O_4 nanoparticles have the advantages of being easily recover rom the medium, regenerated and re-used. Additionally, low amounts are needed to achieve good catalytic results.

To get the information about the biodegradation pathway, GC/MS analysis were performed and catechol was identified with a mass fraction (*m/z*) of 109.3 (Figure 4), revealing that the biodegradation occurs via catechol pathway.

CONCLUSIONS

The results of this study indicate that magnetic Fe_3O_4 nanoparticle and $NaNO_3$ are very promising electron acceptors for anaerobic biodegradation of aniline. The magnetic Fe_3O_4 nanoparticle has shown more powerful than conventional electron acceptors, with the main advantages of being easily recover from the reaction medium with a magnet. GC/MS analysis showed that the biodegradation occurred via catechol pathway.

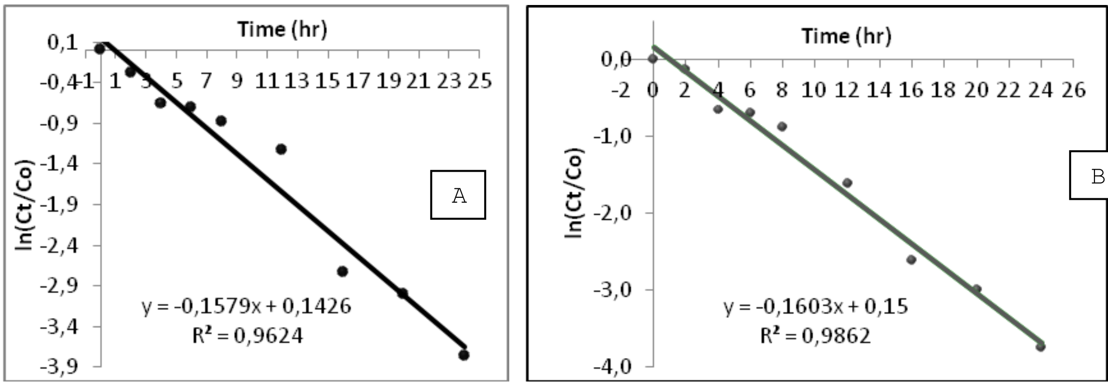

Figure 1. Pseudo first order Kinetics during aniline biodegradation with $NaNO_3$ (A) and Fe_3O_4 nanoparticle (B) as electron acceptor.

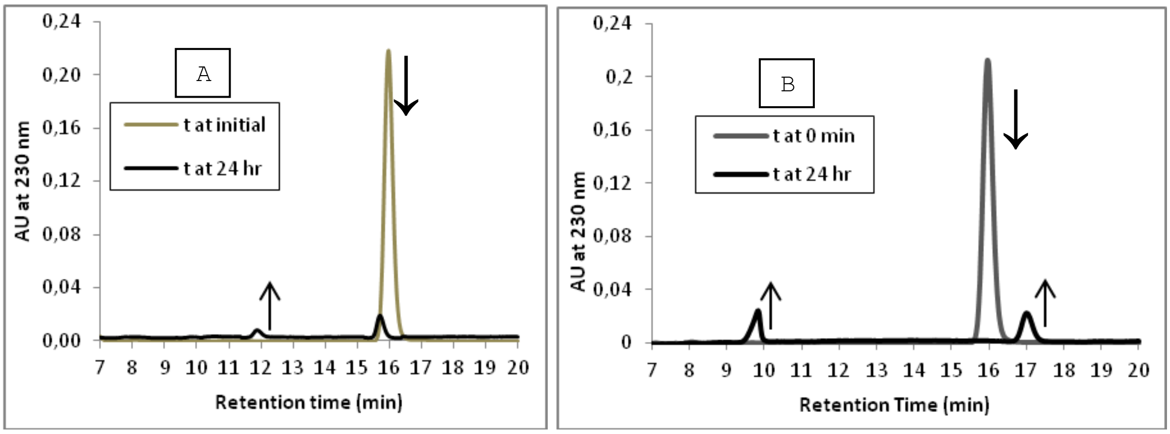

Figure 2. HPLC analysis of 0.2 mM Aniline during biodegradation with $NaNO_3$ (A) and Fe_3O_4 nanoparticle (B) as electron acceptor.

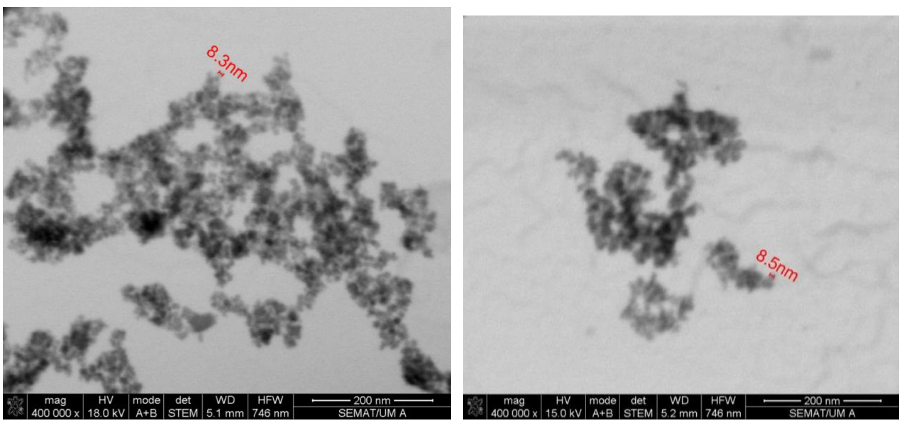

Figure 3. Characterization of coated Magnetic Fe3O4 nanoparticle by Scanning electron microscopy (STEM).

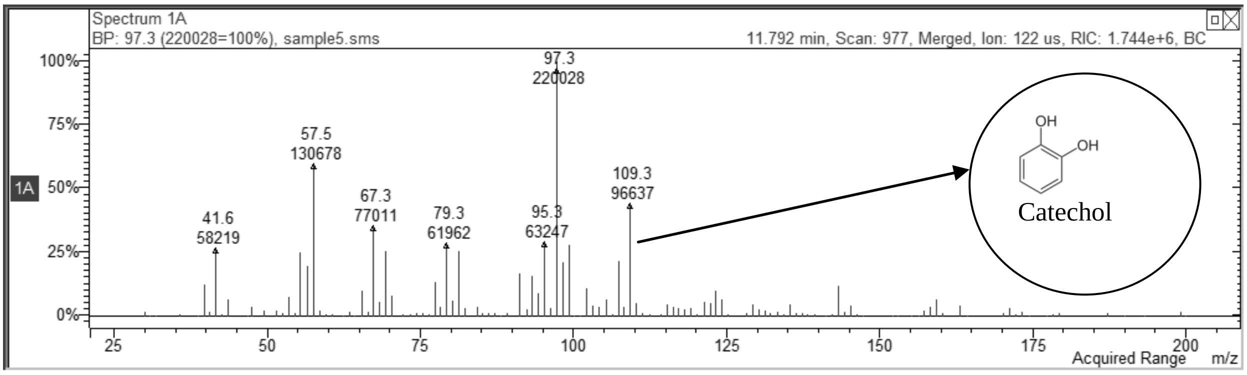

Figure 4. GC/MS analysis of 0.2 mM Aniline after biodegradation. Identification of catechol.

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REFERENCES

[1] Pinheiro, H.M., Touraud, E., Thomas, O., 2004. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes and Pigments.* 61, 121–139.
[2] O'Neill, F.J., Bromley-Challenor, K.C.A., Greenwood, R.J., Knapp, J.S., 2000. Bacterial growth on aniline: implications for the biotreatment of industrial wastewater. *Water Research.* 34 (18), 4397–4409).
[3] Pereira, R., Pereira, L., Van der Zee, F.P., Alves, M.M. 2011. Fate of aniline and sulfanilic acid in UASB bioreactors under denitrifying conditions. *Water Research* 45, 191-200.
[4] Sun, J., Zhou, S. Hou, P., Yang, Y., Weng, J., Li, X., Li, M. 2007. Synthesis and characterization of biocompatible Fe_3O_4nanoparticles. *Journal of Biomedical Materials Research Part A* 80(2):333-41.