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Short Commentary

THE USE OF SOLID MEDIA FOR BACTERIAL GROWTH IN DEGRADATION OF DYES

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ABSTRACT

Dyes are normally difficult to eliminate from effluents by conventional biological wastewater treatments. There are many references in which it is proven a poor removal by activated sludge or other systems in wastewater treatment plants. Their xenobiotic nature and their low concentration in residual streams (20 – 200 mg/l) make them to be difficult substrates for bacterial growth. Actually it is not clear the role they play in biological systems. Under anaerobic environment, it seems they act as electron acceptors in organic matter biodegradation. Under aerobic environment, it seems that a few number of microorganisms are able to use them as electron donors, in the presence of oxygen. Combined anaerobic-aerobic processes have efficiency on dye removal due to the first step in dye degradation, the breakdown of the azo bond, is assumed to be done under anaerobic conditions.

The use of solid supports for bacteria which utilize azo dyes is an attractive alternative for remove them from residual streams. These supports need to have two characteristics: an adequate texture for bacterial growth (particle and porous size) and affinity for dye. Under this situation, dye is adsorb and concentrated on solid surface and bacteria can grow and degrade it on the surface of the solid. The surface of the solid seems to be a good environment for dye degradation, because oxygen concentration can be low inside the porous structure and microaerophilic conditions can be established, therefore cleavage of the azo bond can be performed easily. Several solids can be used on this purpose: activated carbon, bentonite and kaolin, for example. The solid used has to

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be investigated in terms of texture of the surface, growth of microorganisms and dye degradation.

INTRODUCTION

Actually it is assumed textile dyes to be degraded under a combined biological anaerobic-aerobic process (Panswad and Luangdilok, 2000; Tan et al., 2000; Coughlin et al., 2003; Van der Zee and Villaverde, 2005; Lodato et al., 2007). Most of the dyes used in textile industry are from the class azo dyes and they need firstly an anaerobic environment for performance of the reductive cleavage of the azo bond. The hydrolase (azoreductase) involved needs no oxygen present and reductive environment for hydrogenation of the –N=N- bond. On the other hand, aromatic amines formed have to be degraded under aerobic oxidative conditions (Figure 1), otherwise the oxidation will not be complete and some toxic and carcinogenic intermediates can be generated (Coughlin et al., 1999).

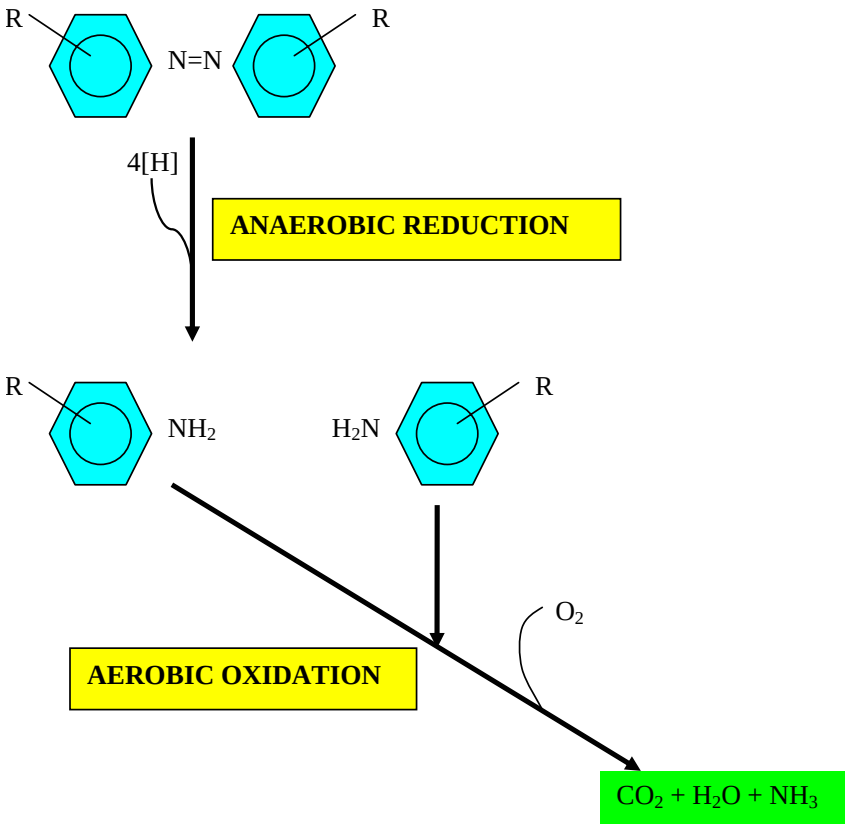

Figure 1. Scheme of azo dye degradation showing the two steps for two environments, anaerobic and aerobic.

Some authors describe azo dyes degradation under aerobic conditions (Coughlin et al., 1997; Coughlin et al., 2002; Coughlin et al., 2003; Buitrón et al., 2004; Davies et al., 2006).

In this case, a high substrate specificity is expected which reduces industrial application of the process.

If oxygen tension can inhibit hydrogenation of the azo bond it does not mean rejection of aerobic degradation of azo dyes. The solution can be a solid support in which azo dye is adsorbed and concentrated under low oxygen concentration. In this "environment" bacteria can grow and degrade it.

In this case the election of the support has to be affected by the property of having two environments for dye degradation. Firstly, dye molecule has to be fixed in a zone in which oxygen concentration is low, promoting bacterial growth on this region. Secondly the products from dye degradation (aromatic amines) have to emigrate to a region with high oxygen concentration, giving the possibility for bacterial degradation of these substrates.

These conditions can be reproduced using a support with a porous structure and with the ability for dye adsorption. Dye is adsorbed and fixed inside the porous structure, where oxygen concentration is low and products formed after bacterial activity are desorbed and conducted to the surface of the solid, where oxygen concentration is much higher (Figure 2).

For the solid selected an adequate relation between particle size and the porous structure is needed because inside the porous structure colonization of bacteria has to be achieved. If it is assumed bacterial size about 1-3 μm, porous size has to be bigger and particle size has to be high enough for supporting a good number of cells.

Finally, the solid used has to be able to adsorb the dye or dyes and conduct them to a low oxygen region, then the support needs high specific surface and porous depth.

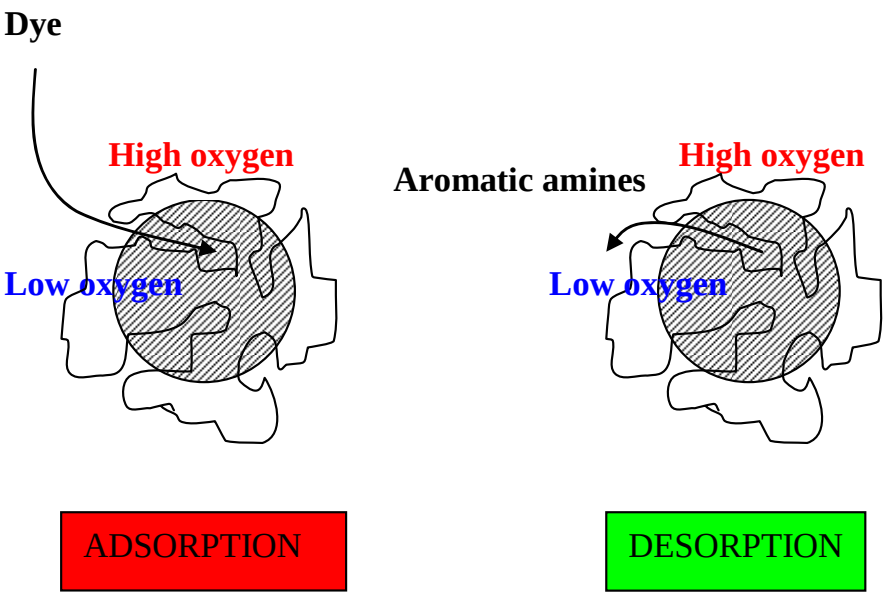

Figure 2. Picture of the support and adsorption of the dye inside the porous structure (low oxygen environment). Products from dye degradation are released out of the porous to the external surface of the solid (high oxygen environment).

SOLID STUDY

Different solids can be studied as supports for azo dyes degradation, natural or artificial made. The best way for investigating texture and porous structure is SEM (Scanning Electron Microscopy), which permits a view of the relief of the solid showing porous structure in high detail. Comparing bacterial size, porous structure and particle size, the best “microniche” for bacterial growth and dye degradation can be selected.

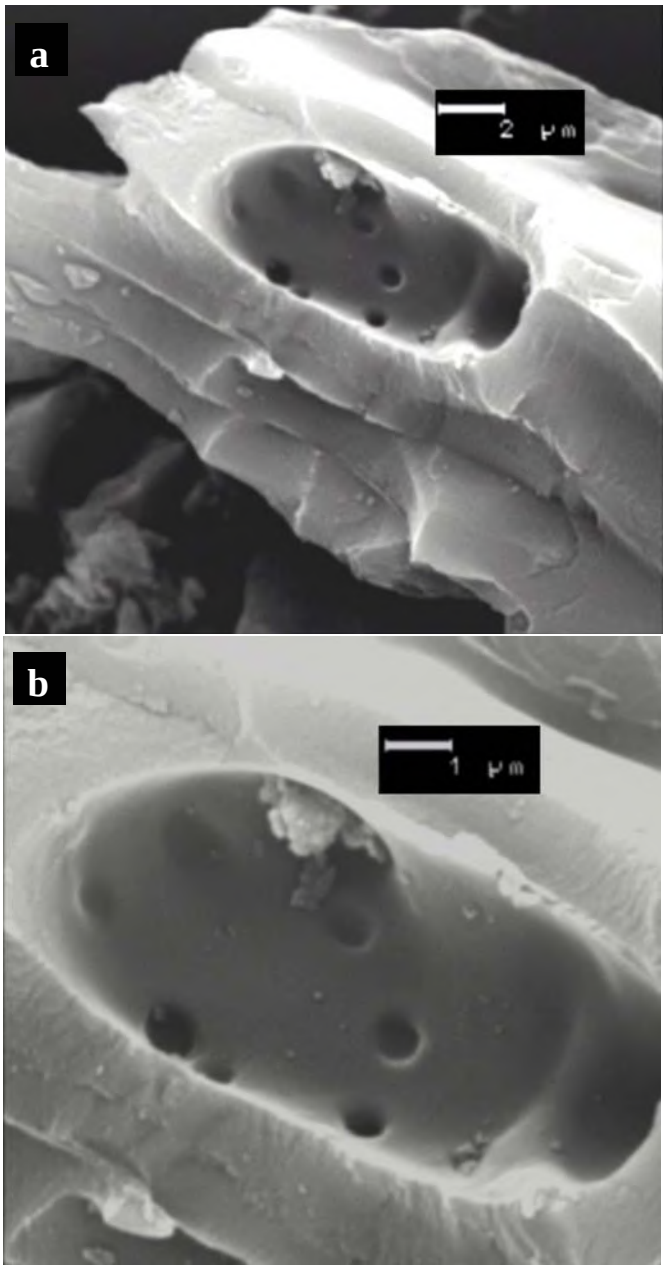

Figure 3. SEM microphotograph of activated carbon. Magnification: 5 000 in *a* and 10 000 in *b*.

In Figure 3 a particle of *activated carbon* is shown by SEM in which porous structure can be observed clearly. In the center of the microphotograph a macroporous with some microporous indicates how can be the microniche for bacteria. Comparing porous size with size bar (2 μm and 1 μm), one can conclude that microporous can not be colonized by bacteria and macroporous have the adequate size and texture for been colonized by bacteria.

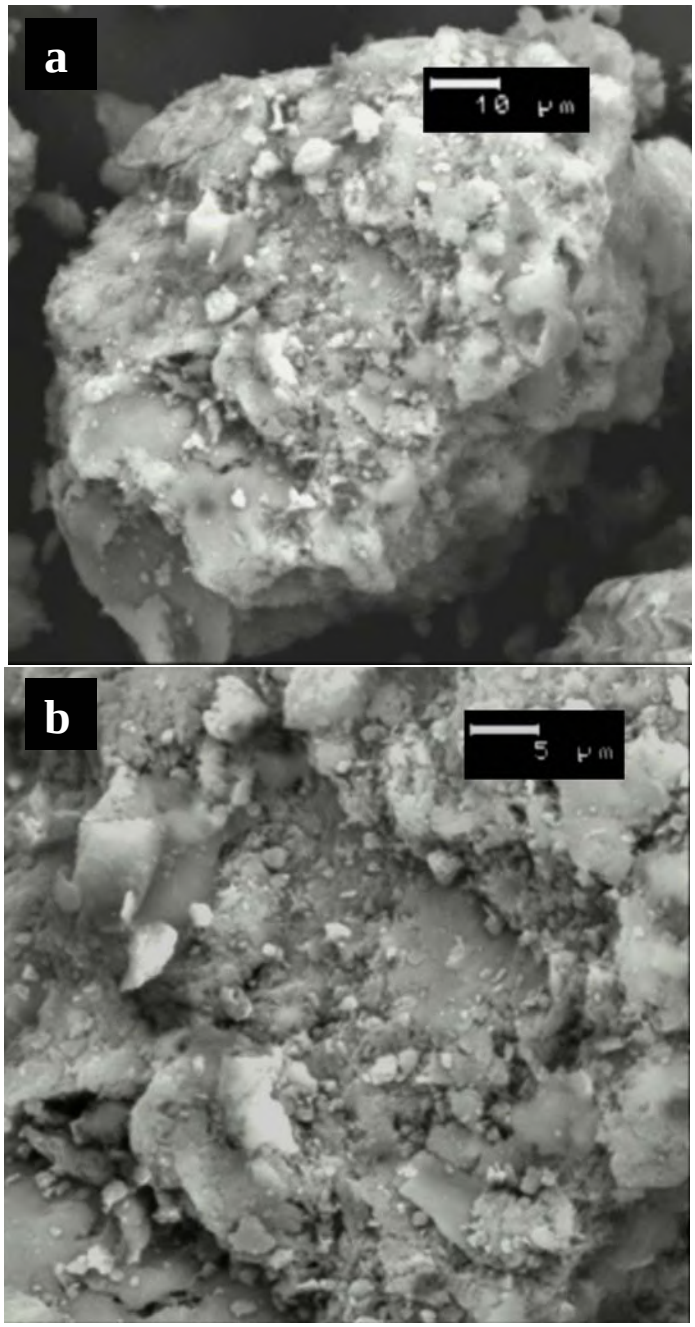

Figure 4. SEM microphotograph of bentonite. Magnification: 1 000 in *a* and 2 000 in *b*.

In Figure 4 a particle of *bentonite* can be observed by SEM. This particle has an adequate size for been colonized by bacteria, but texture of the surface has not a good porous structure for generating a microenvironment with low oxygen concentration. The porous structure is not deep enough for having a lack of oxygen in the inner structure.

Figure 5 is the microscopic study of *kaolin* as a support for microorganisms. In comparison with activated carbon and bentonite, kaolin has a much lower particle size which reduces availability of space for bacterial colonization. The scaly texture of kaolin does not seem to be a good environment for bacteria and porous structure needed for low oxygen ambient is not present.

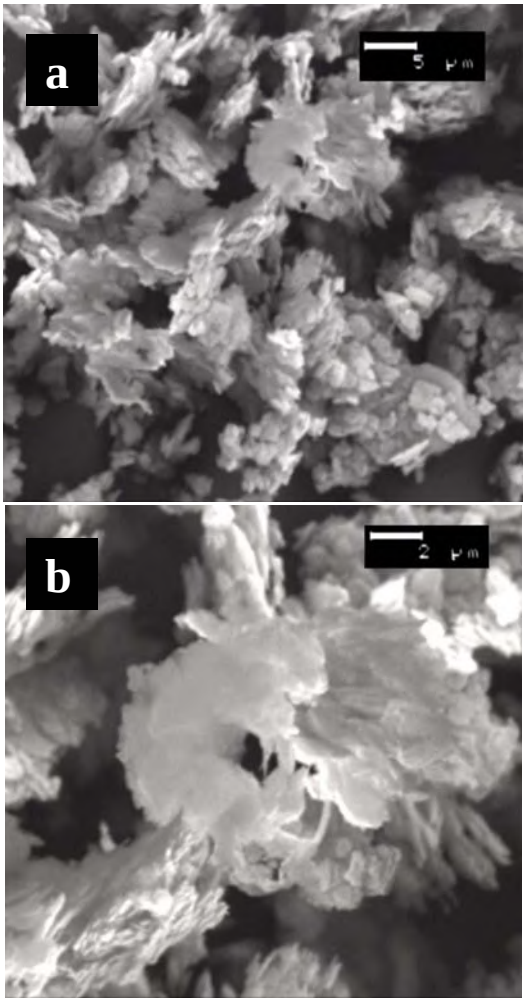

Figure 5. SEM microphotograph of kaolin. Magnification: 2 000 in *a* and 5 000 in *b*.

In view of these results and comparing the three solids studied: *activated carbon*, *bentonite* and *kaolin*, it can be concluded that the adequate solid for bacterial colonization seems to be activated carbon. Particle size is in the range desired (a good number of cells can be "living" in a particle) and macroporous structure of activated carbon seems to be an

adequate microniche for supplying both environments, low and high oxygen, for biodegradation of azo dyes.

BACTERIAL COLONIZATION

Once the support has been selected for the assays, bacterial colonization has to be achieved on the surface of the solid. For this purpose strains that show azo dye degradation activity have to be collected from textile wastewaters or obtained from other sources, for example, enrichments from soil or water which show biodegradation power on azo dyes.

These strains are inoculated on the solid by culture them in liquid media under agitation conditions with the solid and azo dye present. The best way for promoting bacterial growth is using batch cultures (erlenmeyers for example) with a moderate dye concentration (similar to textile wastewaters, 20-200 mg/l, Van der Zee & Villaverde, 2005). The solid used is saturated by azo dye in a continuous system and placed in the medium in which bacteria is inoculated. Saturation of the solid takes two advantages: dye is strongly concentrated in the inner structure of the adsorbent, where oxygen concentration is low and the concentration of dye in the bulk liquid is not been affected by adsorption and biodegradation can be followed by measuring dye concentration in the bulk liquid. The high concentration of the azo dye inside the porous structure promotes bacterial growth and dye degradation. If the environment is adequate and adaptation of bacteria is achieved, a spectacular growth can be observed (Figures 6 and 7).

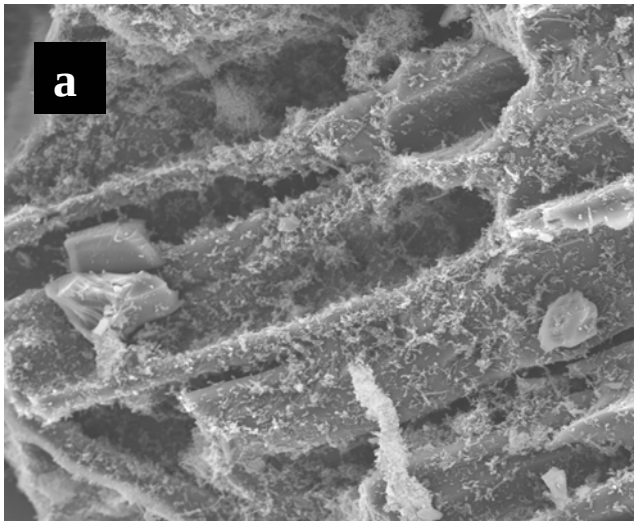

Figure 6. (Continued)

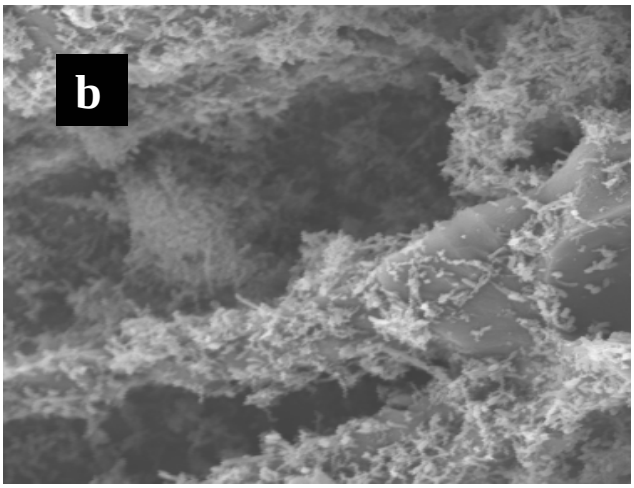

Figure 6. SEM microphotograph of activated carbon colonized by bacteria in a medium in which a textile dye is present (Acid Orange 7, CI 15510). In microphotograph *a* bacteria can be observed fixed on the surface of the adsorbent. In more detail, microphotograph *b* shows bacteria inside the porous structure.

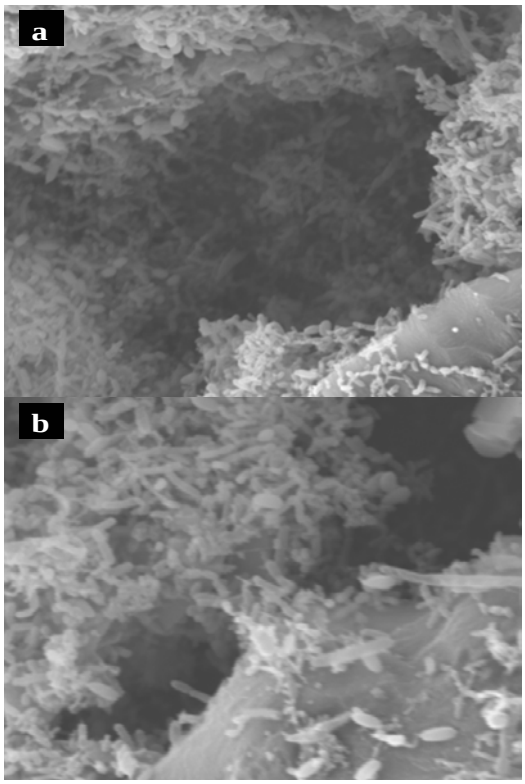

Figure 7. SEM microphotograph of microniches occupied by bacteria in activated carbon structure in a medium in which a textile dye is present (Acid Orange 7, CI 15510). Cells are present in the border of the porous structure (*a* and *b*) and they can be visualized inside the porous structure (*a*), where oxygen concentration is low and the first step in dye degradation can be performed.

DYE DEGRADATION

Visual observation of cells on the surface of the solid is not a proof of a dye degrading population of microorganisms. These microorganisms can prosper by the presence of several carbon sources and dye degradation has to by proven measuring dye concentration in the medium.

In the next figure, two strains (E and P) isolated from textile wastewaters and inoculated on activated carbon, were tested to degrade Acid Orange 7 (CI: 15510):

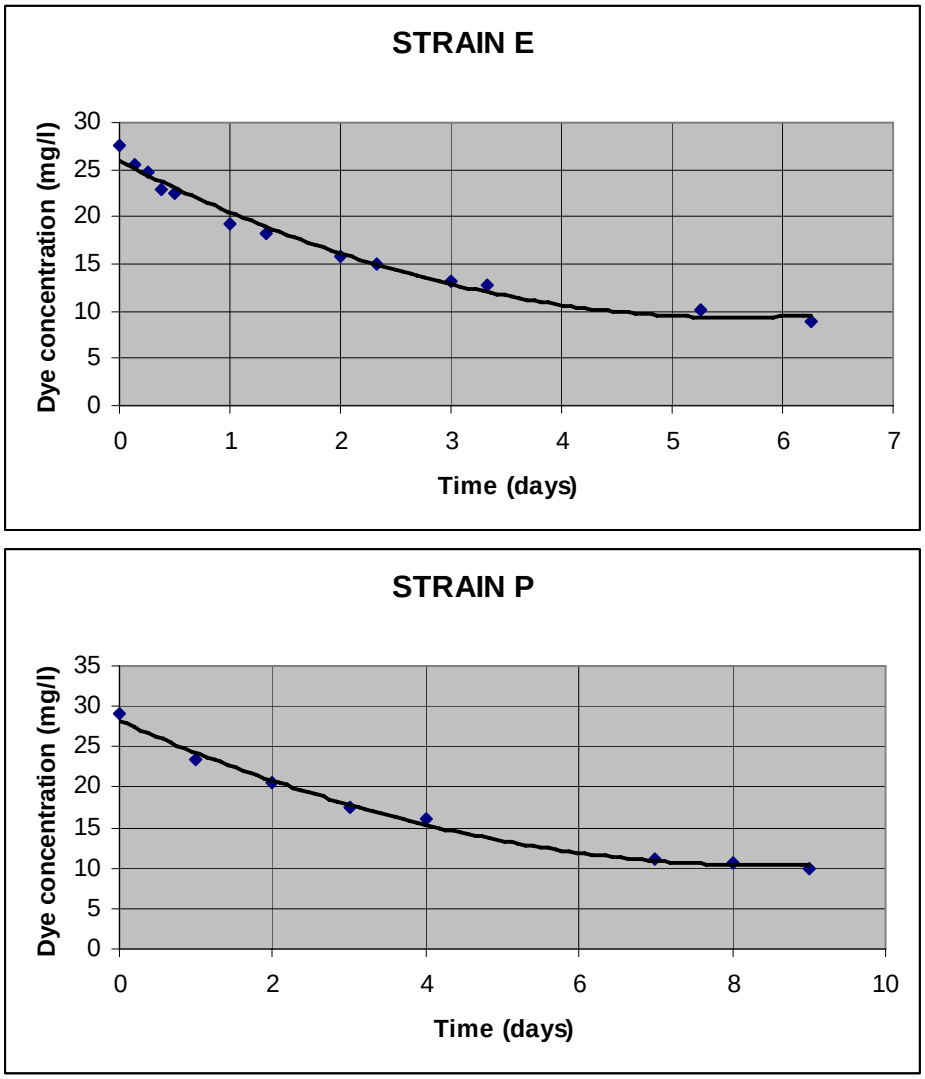

Figure 8. Degradation of Acid Orange 7 (CI: 15510) by two strains isolated from textile wastewaters and inoculated on activated carbon. Experiments were performed by saturation of activated carbon in continuous system and inoculation of the strains on saturated activated carbon in the liquid medium. Time for dye degradation is counted in batch culture.

In a batch system, dye variation in time is only due to substrate utilization rate (dye degradation), because there is not incoming or effluent substrate:

$$\frac{dS}{dt} = r_{su} \tag{1}$$

r_{SU} is the substrate utilization rate, **S** substrate concentration and **t** is the time. Kinetics of dye degradation is often a second order kinetics:

$$r_{su} = -kS^2 \tag{2}$$

k is the kinetic coefficient and mathematical expression for dye degradation can be written:

$$\frac{dS}{dt} = -kS^2 \tag{3}$$

$$\frac{dS}{S^2} = -kdt \tag{4}$$

Integrating the differential equation (4), it can be obtained:

$$\frac{1}{S} = \frac{1}{S_o} + kt \tag{5}$$

Kinetic coefficients can be calculated by linearization of curves in Figure 8 (Barragán *et al.*, 2007):

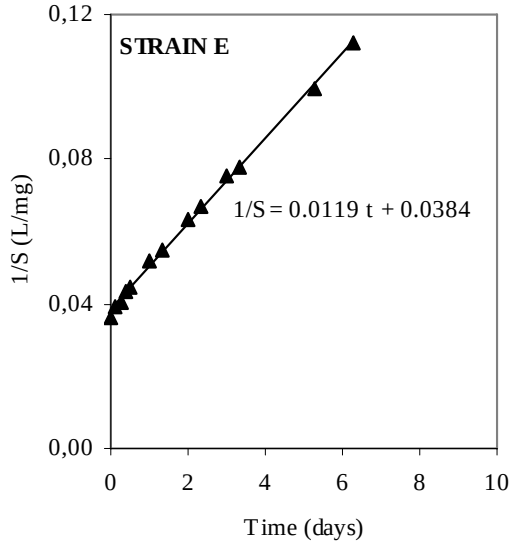

Figure 9. (Continued)

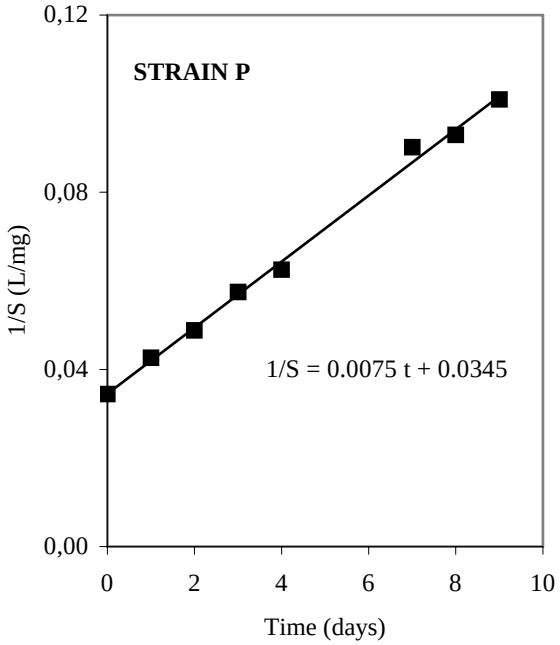

Figure 9. Graphical presentation of dye degradation for strains E and P for a second order kinetics.

STRAIN E ..... k_E = 0.0119 L/(mg days)
STRAIN P ..... k_P = 0.0075 L/(mg days)

k is the kinetic coefficient for strains E ($k_{E)}$ and P (k_P). In view of these results, dye degradation is much faster for strain E than for strain P and it can be a reason for selecting strain E in this environment. Kinetic study has to be done under these conditions because changing bacterial environment can be a motive for modifying kinetics.

CONCLUSIONS

A new technique for dye removal from textile wastewaters is presented in this work. The idea is to inoculate bacteria on an adequate solid which has to play the role of a support for microorganisms and an environment of low-high oxygen. Activated carbon has both characteristics because macroporous structure represents a microniche with low oxygen concentration inside the porous and high oxygen concentration outside, in the outer surface of the adsorbent. By this way, the dye can be adsorb to the inner structure and degraded under low oxygen conditions, needed for the cleavage of the azo bond. Aromatic amines formed, with much lower molecular weight, are desorbed to the outer structure and degraded aerobically.

Kinetics of dye degradation can be studied by a second order model with regards to substrate concentration and kinetic coefficients can be used for selecting the adequate strain for dye degradation.

REFERENCES

Barragán, B.E., Costa, C. & Márquez, M.C. (2007). Biodegradation of azo dyes by bacteria inoculated on solid media. *Dyes and Pigments*, 75, 73-81.

Buitrón, G., Quezada, M. & Moreno, G. (2004). Aerobic degradation of the azo dye acid red 151 in a sequencing batch biofilter. *Bioresource Technology, 92,* 143-149.

Coughlin, M.F., Kinkle, B.K., Tepper, A. & Bishop, P.L. (1997). Characterization of aerobic azo dye-degrading bacteria and their activity in biofilms. *Water Science and Technology, 36 (1),* 215-220.

Coughlin, M.F., Kinkle, B.K. & Bishop, P.L. (1999). Degradation of azo dyes containing aminonaphthol by *Sphingomonas* sp. strain 1CX. *Journal of Industrial Microbiology and Biotechnology, 23,* 341-346.

Coughlin, M.F., Kinkle,. B.K. & Bishop, P.L. (2002). Degradation of acid orange 7 in an aerobic biofilm. *Chemosphere, 46,* 11-19.

Coughlin, M.F., Kinkle, B.K. & Bishop, P.L. (2003). High performance degradation of azo dye Acid Orange 7 and sulfanilic acid in a laboratory scale reactor after seeding with cultured bacterial strains. *Water Research, 37,* 2757-2763.

Davies, L.C., Pedro, I.S., Novais, J.M. & Martins-Dias (2006). Aerobic degradation of acid orange 7 in a vertical-flow constructed wetland. *Water Research, 40,* 2055-2063.

Lodato, A., Alfieri, F., Olivieri, G., Di Donato, A., Marzocchella, A. & Salatino, P. (2007). Azo-dye conversion by means of *Pseudomonas* sp. OX1. *Enzyme and Microbial Technology, 41,* 646-652.

Panswad, T. & Luangdilok, W. (2000). Decolorization of reactive dyes with different molecular structures under different environmental conditions. *Water Research, 34 (17),* 4177-4184.

Tan, N.C.G., Borger, A., Sienders, P., Svitelskaya, A., Lettinga, G. & Field, J.A. (2000). Degradation of azo dye Mordant Yellow 10 in a sequential anaerobic and bioaugmented aerobic bioreactor. *Water Science and Technology, 42 (5-6),* 337-344.

Van der Zee, F.P. & Villaverde, S. (2005). Combined anaerobic-aerobic treatment of azo dyes- A short review of bioreactor studies. *Water Research, 39,* 1425-1440.