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Research Article

Treatment of Dairy Effluents in Biological Fluidized-Bed Reactors using Oyster Shells as Ecological Garnishing

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Abstract: We investigated the effectiveness of a new ecological garnishing of natural origins in MBBR reactors: The shells of oysters, used for the biological treatment of dairy effluents, in the presence of fungi *Aspergillus niger* and *Penicillium chrysogenum*. The abatement performance of organic matter was compared with the same bioreactor design in the presence of a common reference support (Kaldnes K3), conventionally used on an industrial scale. Pollution parameter monitoring results (Chemical Oxygen Demand COD, total nitrogen soluble NKT, phosphorus P, suspended matter SM and biochemical oxygen demand BOD5) obtained using oyster shells as garnishing media, are favorable after only 24 h of biological treatment. Organic matter removal efficiencies are comparable sometimes even better than K3 media. All the results show that oyster shells can provide an ecological support for biofilm colonization in MBBR reactors and allow for a satisfactory pollution abatement rate.

Keywords: Biofilm, biological fluidized-bed reactors, biological treatment, dairy effluents, ecological garnissing, oyster shells

INTRODUCTION

The dairy industries consume large amounts of water daily during their processes, especially to maintain the hygienic and sanitary conditions required (El Jaâfari *et al*., 2015; El Jaafari *et al*., 2016) which generates waste water with a high content of organic matter, as well as waste production and management. These wastewaters are liquid discharges that contain varying amounts of organic pollutants (protein, lipid and osidic nature) and products used for the cleaning and disinfection of specific industrial machines (e.g., nitric acid, detergents based on soda) (Geary and Moore, 1999; Djelal *et al*., 2007; Hazourli *et al*., 2007). Biological treatment is a means of ecological purification that represents an increasing trend and relies on the use of microorganisms capable of degrading organic matter (El Jaâfari *et al*., 2014a). The mobile bed reactors are among the designs that can implement a biological treatment; it has been proven that it effectively reduces the rejections of dairies to the regulations in force before they are discharged in the sewers or in the environment (Djelal *et al*., 2007; El Jaafari *et al*., 2014b; Aitcheikh *et al*., 2014; Bassin and Dezotti, 2018).

Since these bioreactors are a design that require the use of colonization supports, the objectives of this study is to substitute the conventionally used synthetic supports (usually made of plastics) with other natural supports able to ensure optimal colonization of biofilm, in the presence of *Aspergillus niger* and *Penicillium chrysogenum* as biomass, their capacity and resistance in a stressful environment such as the presence of acid or phenolic products has been demonstrated in many studies (Esteban *et al*., 2006; Djelal *et al*., 2007; Wolski *et al*., 2012; Aitcheikh *et al*., 2014; El Jaâfari *et al*., 2014a; El Jaafari *et al*., 2014b; Ladeira Ázar *et al*., 2018; Bassin and Dezotti, 2018).

The purpose of this work is to study the use of a new packing as a support for colonization in the MBBR reactor, these are Oyster Shells (OS). Apart from their use as decorative objects, some research proposes their uses as a component in the proposed poultry feed formula by recovering the limestone and calcium. Or, in

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the form of powder to replace the coagulant/flocculant during the physicochemical treatment of effluents while others propose their use after sorting, drying, grinding and sieving, this calcium carbonate naturally enriched with mineral salts and marine trace elements, or they conserve for the calcium modification of agricultural land and the fight against soil acidification (Meunier *et al*., 2002; Stepnowski *et al*., 2004; Crini, 2006; Marie *et al*., 2008; Ani *et al*., 2011; Jatto *et al*., 2013; Kassuwi *et al*., 2013; Anwar *et al*., 2017).

Other authors followed the same approach with waste from the forest or agro-food industry, such as Meunier *et al*. (2002) who studied the adsorption of metals such as lead by adsorption on cocoa shells. that have proven effective as a biosorbent; Crini (2006) studied the use of bark sawdust as unconventional adsorbents for dye removal; Stepnowski *et al*. (2004) used fish scales as the natural adsorbent of a carotenoid pigment in seafood wastewater; Kassuwi *et al*. (2013) studied them as a biofilm carrier in anaerobic digestion.

Our preliminary work focused on the use of fish scales of different natures and sizes (SarGros, ombrines, sardines) as supports for biofilm colonization in MBBR reactors (El Jaâfari *et al*., 2014a; El Jaafari *et al*., 2014b; Aitcheikh *et al*., 2014). They have shown that these supports significantly improve the effluent purification performance of dairies (El Jaâfari *et al*., 2014a; El Jaafari *et al*., 2014b) by a fungal biomass. The present study aims to study these new supports by comparing them with those used on an industrial scale, as well as to study their surfaces after the biological treatment to confirm the presence of biofilm at the end of the treatment. This approach contributes to the value of oyster shells and to offer an alternative to the materials conventionally used as media facing the problem of depletion of the non-renewable natural resources of which they are constituted.

MATERIALS AND METHODS

Preparation of biomass: Reference strains of *Aspergillus niger* (*11G323A*) and *Penicillium chrysogenum* (*I00393*), are used in this study. They are chosen for their resistance under stressful conditions (e.g., presence of acid or base, detergents, phenolic products…) and for their proven efficiency in the degradation of the pollution, more specifically the dairy effluents ones (Owen and Jonson, 1955; Esteban *et al*., 2006; Djelal and Amrane, 2013; El Jaâfari *et al*., 2014a; El Jaafari *et al*., 2014b; Ladeira Ázar *et al*., 2018).

The growth of these fungi was insured in a liquid culture medium LB followed by a 3 day of incubation at 27°C for *Aspergillus niger* and 8 days for *Penicillium chrysogenum* (Owen and Jonson, 1955; Esteban *et al*., 2006; Djelal and Amrane, 2013; El Jaâfari *et al*., 2014a; El Jaafari *et al*., 2014b; Ladeira Ázar *et al*., 2018). The fungi cells are later separated by centrifugation (4800 g, 20 min).

Fungi cells recovered are added to the bioreactor containing synthetic effluent, after going through 3 successive operations of washing/centrifugations (4800 g, 20 min) with the artificial effluent to eliminate as much as possible of the traces of the culture medium.

Table 1: The characteristics of the synthetic effluent (values averaging 10 measurements)

Parameter	Interval of variability
COD (mg/L)	4265±60
BOD5 (mg/L)	1280±10
SEM (g/L)	0.93±0.02
TNK (mg/L)	3920±50
Phosphorus (mg/L)	64±0.8

Table 2: Realized tests

Garnishing	*Aspergillus niger* (AN)	*Penicillium chrysogenum* (P)
None (Control)	+	+
Oyster Shells (OS)	+	+
Kaldness K3 (K3)	+	+

+: Tests realized

Preparation and characterization of the effluent: A synthetic effluent of a stable and controllable composition is used in this study; he is prepared from UHT milk that was diluted 50 times with distilled water. The average characteristics of effluent model are grouped in Table 1.

COD/BOD5 ratio is around 3, indicating its biodegradability. So, biologic degradation can be used this effluent.

Garnishing used new ecological garnishing: The oyster shells of the Crassostrea gigas species bivalve filibranche mollusk belonging to the family Ostreidae (Anwar *et al*., 2017) and the genus Crassostrea, were used to conduct this study.

Before each test, the shells are washed with hot distilled water and finally dried.

Industrial garnishing: The commercial Kaldnes K3 (standard support), is used as a reference support to compare the abatement performance of organic matter with the ecological supports using.

Biological bioreactor and tests carried out: The bioreactor used consists of a simple mobile-type glass tank (MBBR) with a total volume of 60 L. The oxygen supply is provided by fine aerators bubble from the base of the reactor, homogenization and agitation of the effluent are provided by water pumps (Fig. 1).

Several experiments were conducted, in presence and absence of the garnishing of oyster shells and in the presence of Kaldness. All the experiments were conducted in the presence of *A. niger*, or *P. chrysogenum*. The realized tests are summarized in Table 2.

Performance monitoring of biological purification: Several monitoring tests are conducted at the start of

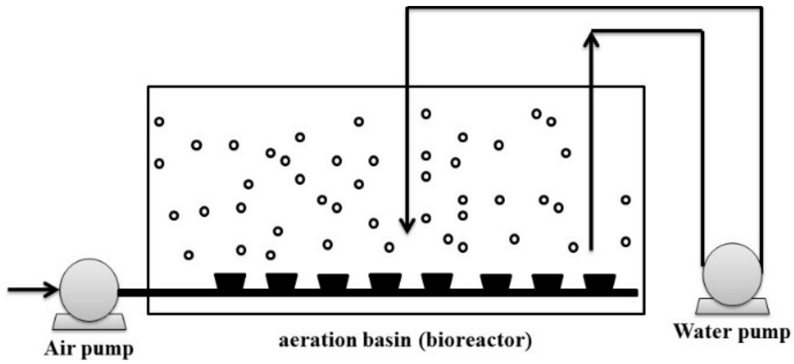

Fig. 1: Schematic representation of the bioreactor

the tests, then after every 6 h up to 24 h: The total nitrogen is determined by the Kjeldahl method according to the French standard NF EN 25663:1997. COD is determined according to the French standard NFT90-101: 2001. Phosphorus was monitored by a colorimetric method using the phosphomolybdic complex according to the German standard DIN 38405-D11-1 OPO43:1993. Suspended matter (SM) is determined by filtering a volume of the effluent through a filter paper (0.45 µm) (Rodier, 2016). The BOD5 is measured by BOD meter HACH LQV166.98.00001.

A microscopic observation in SEM (Philips, XL30 Model) was also conducted to assess the colonization of microorganisms on the garnishing.

RESULTS AND DISCUSSION

Evolution of COD: Figure 2, represents the evolution of the abatement of the COD for the two fungi in the presence of oyster shell OS, support K3 and in the absence of garnishing.

The abatement of COD increases with time because of the use of organic compounds by microorganisms, we noted that the presence of supports promotes biodegradation; a difference of more than 30% in the COD abatement is recorded between the processes using a garnishing and the process without garnishing.

For *Aspergillus niger* the maximum abatement is recorded for shells, it is almost 95% in 24 h, compared to 84% for kaldnes supports, a quite significant difference.

Apart from this recording, no difference in the COD abatement between the two supports is considered significant (<5%). We could then say that the use of oyster shells offers an almost similar treatment of pollution obtained by using Kaldnes supports, sometimes even better in the case of *A. niger* after 24 h of treatment.

Abatement of BOD5: BOD5 is defined by the amount of oxygen consumed by microorganisms for 5 days to biologically degrade organic matter. This measure gives an approximation of the biodegradable organic matter load in a waste. The ratio COD/BOD5 gives a first estimate of the biodegradability of the organic matter of a given effluent (Akkaoui *et al*., 2017).

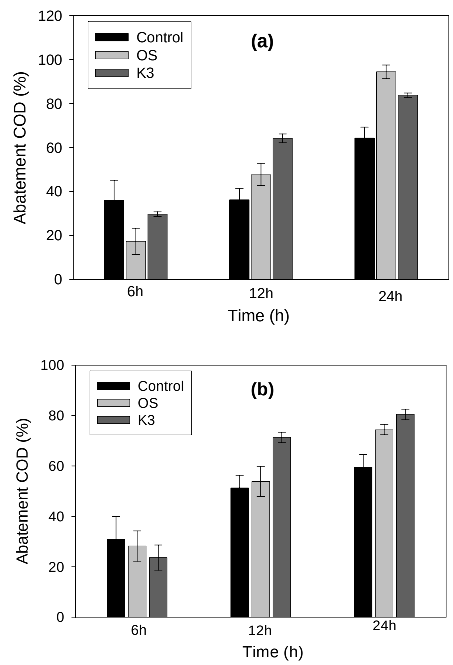

Fig. 2: Evolution of COD; (a): Using *Aspirgilus niger*; (b): Using *Penicillium chrysogenum*

The results of evolution of BOD5 (Fig. 3) show that the abatement of BOD5 in the presence of the supports is better compared to the witness (significant difference> 20%). The abatement of BOD5 is also almost similar for both strains, regardless of the garnishing used (difference not significant <5%).

Evolution of phosphorus: Microorganisms use phosphorus as an essential nutrient for the construction of their genetic material and energy molecules such as ATP and membrane phospholipids, thus orthophosphates dissolved in water can be used in the growth of these microorganisms because they are bioavailable (Wang *et al*., 2002; Behera and Varma,

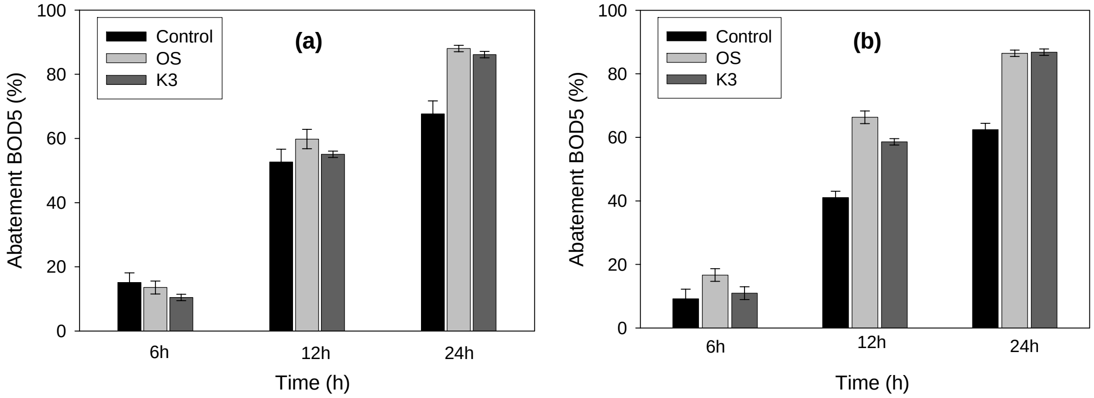

Fig. 3: Evolution of BOD5; (a): Using *Aspirgilus niger*; (b): Using *Penicillium chrysogenum*

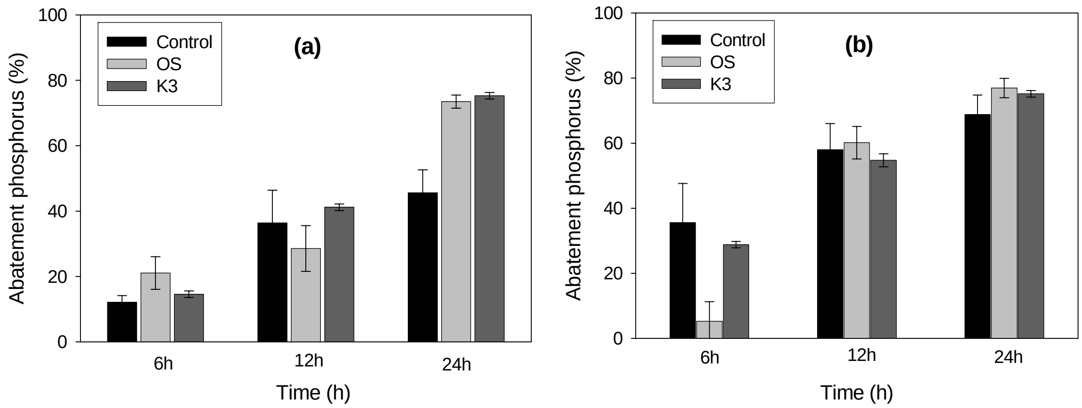

Fig. 4: Evolution of phosphorus; (a): Using *Aspirgilus niger*; (b): Using *Penicillium chrysogenum*

2017). During the decomposition of the phosphorus organic matter, the microorganisms present in the water transform it into dissolved mineral phosphates according to the process of mineralization (Behera and Varma, 2017).

During the biological treatment, the bound phosphorus is transformed under the effect of the acids produced by these fungi in soluble mineral format which is none other than the orthophosphates.

An interesting percentage of phosphorus abatement is also recorded around 24 h of treatment.

According to Fig. 4, the two supports have almost the same performance in the abatement of phosphorus, on the other hand it is noted that the presence of a garnishing in the bioreactor can improve the treatment.

Evolution of TNK: Figure 5, presents the evolution of total nitrogen as a function of time.

We note that the reduction of total nitrogen in the presence of the supports is better compared to the witness (significant difference >10%). We also noted that this is almost similar for both strains, regardless of the garnishing used (difference not significant <5%).

In general, nitrogen undergoes different transformations during a biological treatment (passage from the ammoniacal form to the nitrous one then nitric form and then return to the gaseous form); the decrease observed in the graph is due to the incorporation of nitrogen into the new cells of the fungi produced. These fungi provide the treatment of the organic pollutant load they need to make their metabolisms, as well as many chemical elements, of which nitrogen is the most important, since it is a significant component of the fungal cell and represents about 5 % of dry matter (Mendes *et al*., 2017).

Evolution of suspended matter (SM): Figure 6, represents the evolution of the amount of suspended matter as a function of time.

We note that the reduction of the suspended matter in the presence of the supports is better compared to the witness (significant difference> 10%). This is almost similar for both strains, regardless of the garnishing used (difference not significant <5%).

The suspended material increases at a given time during the treatment for the witness to decrease at the end, this could be explained by the detachment of the fungi (following agitation) that adhere to the inner wall of the bioreactor to form a biofilm, (El Jaâfari *et al*.,

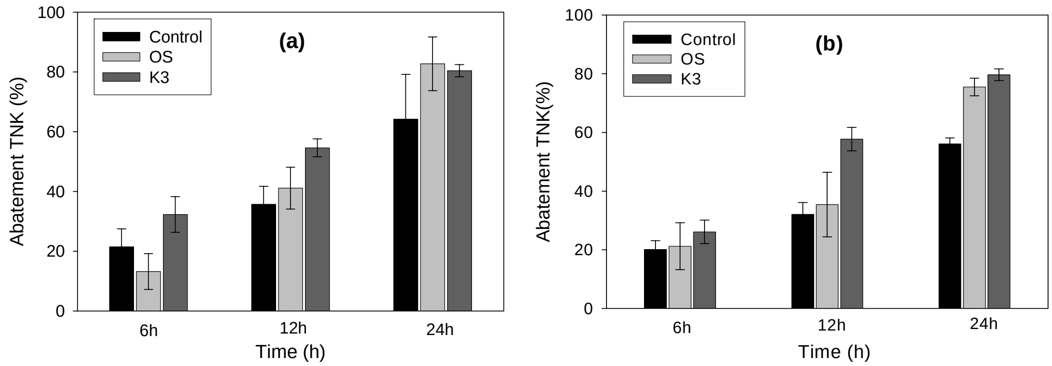

Fig. 5: Evolution of TNK; (a): Using *Aspirgilus niger*; (b): Using *Penicillium chrysogenum*

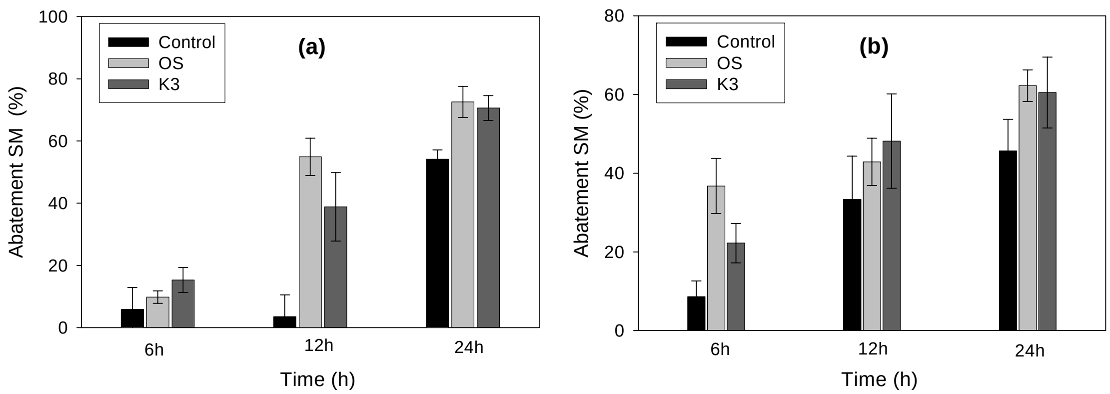

Fig. 6: Evolution of the SM; (a): Using *Aspirgilus niger*; (b): Using *Penicillium chrysogenum*

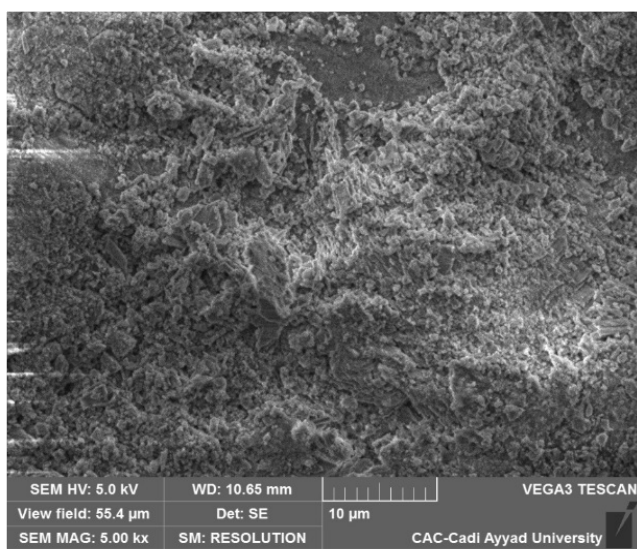

Fig. 7: SEM surface of the oysters colonized by *A. niger* (5K magnification)

2014a; El Jaafari *et al.*, 2014b; Behera and Varma, 2017). The absence of this increase shows that fungi adhere perfectly to snail shells.

Our study focused on evaluating the effect of the use of the new ecological and originally natural (OS) garnishing on the biodegradation of dairy effluents in the presence of *Aspergillus niger* and *Penicillium chrysogenum*. This is highlighted by the different analyzes carried out during our experience. A similar treatment was carried out with supports used in MBBR with Kaldnes industrial supports (K3) to be able to compare the performance of the organic depollution.

The study was conducted using two different designs: a bioreactor using a mobile bed held in suspension, on which biomass colonization will be performed (MBBR) and a bioreactor involving free fungal biomass.

For all the quality control parameters of the depollution (BOD5, COD, SEM, TNK, P), we noticed that the first design was always better and more efficient.

Previous research (Boutaleb *et al*., 2008; Kubota *et al*., 2008; Aitcheikh *et al*., 2014; El Jaâfari *et al*., 2014a; El Jaafari *et al*., 2014b) has shown that microorganisms grow better in the presence of a colonization support and that the sessile state and their development according to a biofilm allow them to withstand environmental stresses. Figure 7 shows the ability of oyster shells to develop a fungal biofilm.

Pal Shailesh *et al*. (2016) showed efficacy of removal of organic matter in MBBR on a dairy effluent, same for Andreottola *et al*. (2002) who proved that MBBR is effective in removing organic and nitrogenous matter and can be used to improve activated sludge systems; Djelal *et al*. (2007) used a set of fungi amplified for 24 h for a biodegradation treatment of whey in batch culture and obtained a 70% percentage after 142 h (38% in 40 h); Another study by Mannan *et al*. (2005), using activated sludge treatment in the presence of *Aspergillus niger*, recorded an 86% COD abatement for 120 h of treatment. This goes in line with the new results obtained, which show that *A. niger* and *P. chrysogenum* were more efficient in the degradation of organic effluent pollution in the presence of an adhesion surface.

The free culture system has a problem with the amount of excess sludge that is deposited which weakens the removal efficiency (Bae *et al*., 2003), hence the need to improve this treatment by using the biomass adsorption media purifier.

In this study we used two fungi to compare their efficiency in terms of organic matter removal since they have proven an important use in the field of treatment of dairy effluents. A recent study by Louaste *et al*. (2014) on a dairy effluent enhances the performance of fungi use during biological treatment and thus achieves an 85% COD abatement at the end of treatment. *Aspergillus niger* recorded a performance in elimination of the carbon and nitrogenous matter and in suspended matter, the percentages obtained are respectively, 95%, 82% and 97%. As for *Penicillium chrysogenum* its effectiveness has been demonstrated in terms of biodegradation of phosphorus pollution with a percentage of 76%.

To compare the proposed media, we used K3 industrial supports. According to the results obtained, the two supports showed comparable results apart from the COD and in the case of *A. niger* (there is probably a synergistic effect between the strain and the support).

Broch-Due *et al*. (1997), used the same type of treatment to treat wastewater from paper mills using conventional industrial supports and obtained after 2 days of reactor preparation a percentage of 65 to 75% for COD 85 at 95% BOD5 after a retention time of 5 h same as Shin *et al*. (2006) who used MBBR to treat wastewater from textile industries and obtained 85% COD in 44 h

By comparing our results with these, it was possible to valorize the treatment using oyster shells to obtain first: A more effective treatment in terms of pollution parameters especially that the percentages obtained are of the same importance to those obtained. by the K3; And second: It was possible to reduce the treatment time either compared to our first study carried out in 48 h of treatment (Aitcheikh *et al*., 2014) or compared to that carried out by Djelal *et al*. (2007) of 40 h while it took us 24 h in this study.

CONCLUSION

The biological treatment using oyster shells (purely naturel garnishing) as moving bed is satisfactory. For MBBR designs using these natural supports, the best results obtained after 24 h using *A. niger*, abatement of the pollution has made effectively. The DOC could be reduced up to 95%, BDO5 abatement reached 90%, phosphorus 78% and total nitrogen about 80%. This naturel support may be available as industrial waste, proved then its effectiveness in developing the colonization of an efficient biofilm.

Finally, this ecological and economic way of valorization no need any previous preparation of the naturel garnishing, contributes to sustainable development.

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