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SNAIL SHELLS: A NEW ECOLOGICAL PACKING FOR MOVING BED REACTOR MBBR IN THE BIOLOGICAL TREATMENT OF DAIRY EFFLUENT

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ABSTRACT
Chemical colonization supports are a critical component of moving bed reactors. In this study, we developed a new colonization support for MBBR reactors based on snails shells and tested its effectiveness during the biological treatments of dairy effluent in the presence of fungi *Aspergillus Niger* and *Penicillium chrysogenum*. We assessed Chemical Oxygen Demand (COD), Total Nitrogen TNK, soluble phosphorus (P), and Suspended Matter (SM) as parameters indicator of pollution. We found that the use of snail shells as colonization support promote an elimination yields of COD as high as 85% for *Aspergillus Niger* and 74% for *Penicillium chrysogenum* after 24 hours of treatment, which indicate that these shells can constitute a perfect colonization support for biofilm formation.

KEYWORDS: dairy effluent, biofilm, biomaterial, biological treatment.

INTRODUCTION

Dairy Wastewater is a liquid discharges that consists of varying amounts of organic pollutants (protein, lipid and glucoside) and industrial chemicals used for cleaning and disinfection purposes (*e.g.* nitric acid based detergents soda) (Burgaud 1969, Geary *et al*., 1999, Djelal *et al*., 2007). The biological treatments of dairy discharges include an ecological purification step based on the use of microorganisms capable of degrading the organic pollutants (El Jaafari *et al*., 2014a). These treatments are often executed in the moving bed reactors. While, these reactors have been shown to effectively reduce pollutants from dairies wastewaters to acceptable regulatory levels before they are discharged in the drain or in the environment (Moletta and Torrijos, 1991, Djelal *et al*., 2007, Djelal and Amrane, 2013, Aitheikh *et al*., 2014, El jaafari *et al*., 2014b), they are also well suited for implementation of new, efficient and cost effective biological treatments.

An important component in the design of the moving bed reactors is the colonization supports. These solid supports usually of synthetic materials allow biofilm formation, a step necessary to intensify the anaerobic digestion. Due to their critical importance, the objective of this study is to develop new colonization support based on natural materials instead of the conventionally used synthetic one. These materials will be chosen for their specific surface area and their capacity to ensure optimal colonization of biofilm by fungi *Aspergillus Niger* and the *Penicillium Chrysogenum.* Our attention turn to snail shells (EC), they are routinely used in the manufacturing of the buttons, jewelry and art collections. Previous studies have suggested that snail shells powder can be used to replace the coagulant /flocculent during the physico-chemical treatment (processing) of effluents, or even as component in the feed formula proposed in poultry (Guillaume, 2004, Ani *et al*., 2011, Houndonougbo *et al*., 2013, Jatto *et al*., 2013), however, to our knowledge they have never been tested as colonization supports. Therefore, the aim of this study is to investigate the utility of this new biomaterial as a support of colonization in the MBBR reactors.

MATERIALS & METHODS

Biological Model and preparation of the effluent

For reasons of sampling difficulties, and to work with a stable and controllable composition of effluent, a model of synthetic rejection imitating discharges of dairy effluent was prepared based on UHT milk diluted 50 times with distilled water. In the study we used *Aspergillus Niger* (11G323A) and *Penicillium chrysogenum* as the purifying biomass. The use of these two fungi is justified by their increased capacity and resistance in stress environments caused for instance by the presence of acids, bases, or phenols (Guest and smith, 2006, Tabuc, 2007, Coulibaly *et al*., 2008, Djelal *et al*., 2009, El Jaafari *et al*., 2014a, El jaafari *et al*., 2014b), as well as their proven efficiency in the degradation of pollutants, especially ones found in dairy effluent (Owen and Jonson, 1955). Briefly, *Aspergillus Niger* or *Penicillium chrysogenum* are inoculated in Luri-Bertani (LB) broth and incubated at 27 °C for 3 days or 8 days respectively (Muler, 1774, Tabuc, 2007, Coulibaly *et al*., 2008, Djelal *et al*., 2009, , Wolski *et al*., 2011, El Jaafari *et al*., 2014a, El jaafari *et al*., 2014b). Then, the fungi are recovered by centrifugation of the resulting culture media at 4800G for 20min. Thereafter, we have performed three successive operations

of washing /centrifugation (4800G, 20 min) by the artificial effluent to remove any traces of the culture environments before eventually adding the recovered microbial populations to the bioreactor containing the synthetic rejection,

Garnishing and Preparation

The shells of the Thebapisanaspecies (Muler, 1774) of the family Helicidae were used in this study. The gastropod mollusk measures 9-20 mm high and 12-25 mm in diameter and has a slightly depressed spherical shell containing 5.5 to 6 white towers (Muler, 1774, Kerny and Cameroun, 1979, Ani et al., 2011, Houndonougbo et al., 2013, Owen and Janson, 2013). The chemical composition of the shell consists essentially of calcium carbonate (89 to 99%). The remaining components include tricalcium phosphate, silicon, calcium sulfate, magnesium carbonate, iron sesquioxide, phosphoric anhydride and sometimes cesium and rubidium. This mixture of elements are altogether cemented by a compact organic matrix consisting essentially of conchiolin, sclera protein chitinoïdale similar to what is found in snails shell used in this study or to beetles cuticles (Castillo, 2005). To prepare our sample, shells obtained from snails were vigorously cleaned and washed with hot water. Shells were washed again with distilled water, dried in the sun, and perforated by a millimeter in diameter to promote a circulation flow of the effluent inside, as shown in the picture (Fig. 1).

FIGURE 1. The snail shells used in biological treatment processes

Bioreactor and experimental conditions

The bioreactor used in this study consists of a simple moving bed type of glass tank (MBBR) with a total volume of 70 liters, the oxygen is provided by fine bubble aeration from the base of the reactor, homogenization and agitation of the effluent is provided by water pumps (Fig. 2).

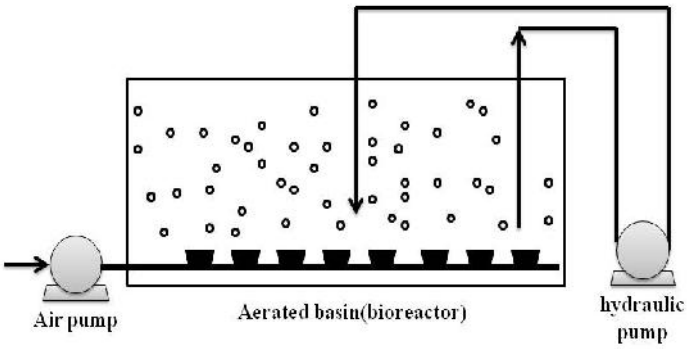

FIGURE 2. Schematic representation of the bioreactor

Experimental tests were conducted in the presence of snail shells trimming (the amount of snail shells used was 527 ± 0.5 grams) and/or in the presence of *A. Niger* or *P. chrysogenum* according to condition listed in the table 1

Table. 1. Tests preformed

	Shell Presence (yes/no)	Strain
Test 1	No	*A. niger*
Test 2	Yes	*A. niger*
Test 3	No	*P. chrysogenum*
Test 4	Yes	*P. chrysogenum*

N.B: Each test was repeated three times.

Analytical Method

Parameters listed below were assessed by sampling every 6 hours after the initiation of the experiments. Phosphorus (P) was measured by a colorimetric method using the phosphomolibdique complex (DIN 38405-D11-1, 1993), Total Nitrogen (NKT) is determined by the Kjeldahl method according to a standard method previously described (NF EN 25663, 1997), Chemical Oxygen

Demand (COD) is determined according to the French standard method (AFNOR, NFT90-101, 2001), and The Suspended Matter (SM) is determined by filtering a volume of the effluent through a filter paper (0.45 µm), according to standard method previously described (Taiek *et al*., 2014).

RESULTS & DISCUSSIONS

The evolution of phosphorus

Phosphorus is an essential nutrient used microorganism to build genetic material, energy molecules (ATP) and membrane phospholipids (Hamdi, 1991). According to the mineralization process the phosphorus organic matter present in the water is transformed by microorganisms into dissolved inorganic phosphates (orthophosphate) used in the growth of microorganisms due to its bioavailability for microorganisms) (Jernejc *et al*., 1995), the same case during biological treatment, phosphorus related turns under the effect of acid produced by these fungi to soluble mineral format which is none other than orthophosphates (Vallissilev *et al*., 1995).

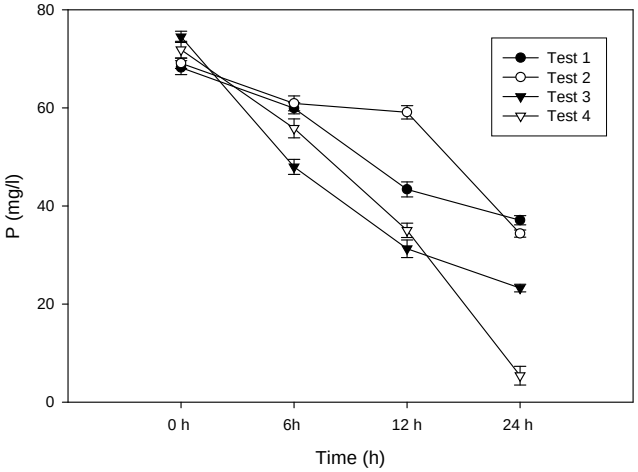

FIGURE 3. Evolution of consumption of phosphorus

Our results shown in figure 3 indicate that *Penicilium chrysogenum and Aspergillus niger* in presence or absence of snails shell consumed phosphate significantly. *Penicillium Chrysogenum* prompted a reduction of 93% in 24 hours comparing 50% of *Aspergillus Niger*, and also more importantly against a witness contains the same strain. We also note that the *Penicillium chrysogenum* recorded a reduction of 93% in 24 hours against 50% of *Aspergillus Niger*, and also more importantly against a witness contains the same strain with the absence of biomaterial and which marked a percentage of about 69%, those results shows that the presence of snails shell have an important impact on the consumption of phosphorus when it's compared to a witness in absence of biomaterials.

The evolution of the COD

Figure 4 shows changes in COD of biomaterial for both fungi.

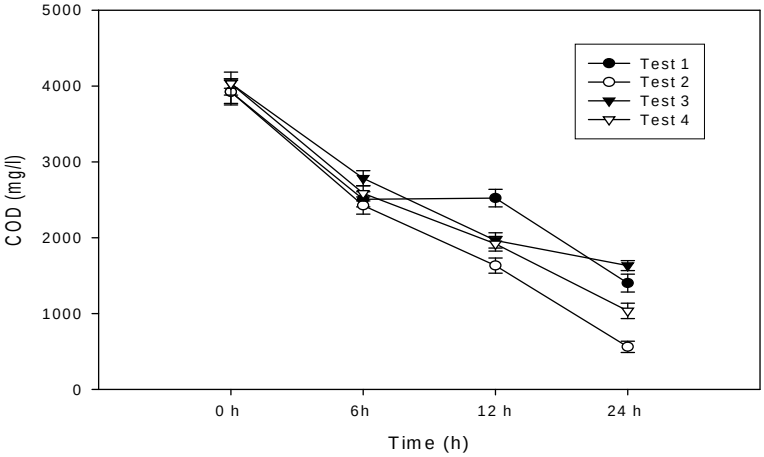

FIGURE 4. The evolution of the COD

Our Data in figure 4 indicated the degradation of the organic matter increases with time, and may vary depending on the strain used. The maximum reduction of COD is recorded for *Aspergillus Niger* which is 85, 71% in 24 hours, against a percentage of 74,33% pour *Penicillium Chrysogenum*. The chemical oxygen demand increases over time due to the use of organic compound by the *A. Niger* and *P. Chrysogenum*, the presence of the shells promotes the biodegradation.

The development of consumption of NKT

Figure 5 shows the evolution of the total nitrogen in function of time.

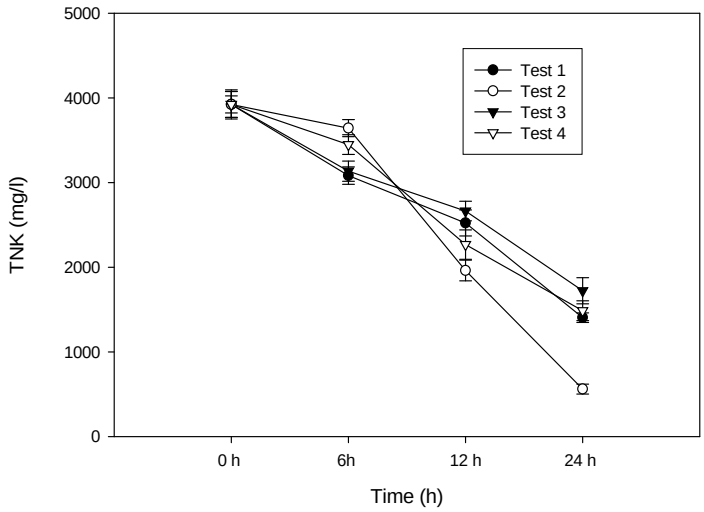

FIGURE 5. The evolution of consumption TKN

We notice that the elimination of the nitrogen is important for both fungi but much more remarkable using *Aspergillus Niger* (~86%) than *Penicillium Chrysogenum* (62.1%) during 24 hours of treatment. Generally nitrogen undergoes various transformations during biological treatment (passage of the ammonium form to nitrous form then nitric, then back to gaseous form); the decrease observed in the graph is due to the incorporation of nitrogen in the new cells produced Fungi. These fungi provide treatment of organic pollution load they need for the metabolism of many chemical elements, including nitrogen, which ranks first, since it is an important component of the fungal cell and which represents about 5% of its dry matter (Valissilev *et al*., 1995).

The evolution of suspended matter (SM)

Figure 6 illustrates the evolution in the amount of suspended matter in function of time.

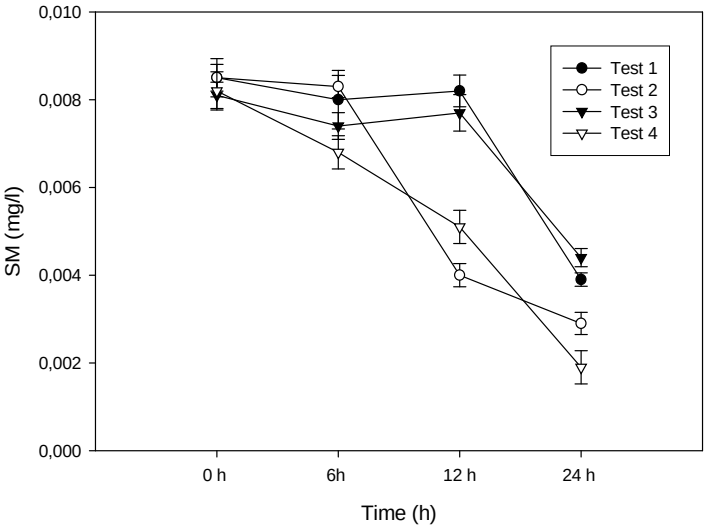

FIGURE 6. Evolution of degradation of the (SM)

It is noted that for both fungi suspended matter decreases considerably with time and reaches a percentage of 65.88% for *A. Niger* and 79.34% for *P. chrysogenum* during 24 hours of treatment. Suspended matter increases at some point treatment so that it decreases in the end; this could be explained probably the detachment of Fungi (after agitation) that adhere to the inner wall of the bioreactor to form a biofilm (Castillo, 2005, El Jaafari *et al*., 2014a). The absence of this increase shows that the Fungi adhere to snail shells perfectly.

DISCUSSION

Our study focused on evaluating the effectiveness of the new environmentally ecological packing and natural biodegradation of effluent dairies, in the presence of *Aspergillus Niger* and *Penicillium Chrysogenum*. First of all, the agitation and aeration of the bioreactors lead in one hand increasing the rate of biodegradation of the organic compounds present in the effluent (Deronzier *et al*., 2001), and in the other hand a Fungi detachment has acceded to the bioreactor therefore generating an increase in SM during treatment for the controls used. Comparing the two fungi we note the COD does not register a significant difference after 24 hours of treatment see Table 2.

TABLE 2. Comparison of the percentages of abatement percentages in A. Niger and P. chrysogenum after 24 hours of treatment in presence of snail shells.

	With *A. Niger*	With *P. chrysogenum*
COD	85,71%	74,33%
NKT	85,7%	62,1%
P	50,24%	92,48%
SPM	65,88%	79,34%

For the reduction of COD, nitrogen and phosphorus (Table 2), we note that *Aspergillus Niger* is efficient in terms of carbonaceous matter and nitrogen removal while the *Penicillium Chrysogenum* achieved remarkable results for biodegradation of the phosphorus matter. Our previous research studies on biomaterial like fish scales have shown that fungi and specifically *Aspergillus Niger* grow better in the presence of these biomaterials and gave very satisfying results (Aitcheikh *et al*., 2014, El Jaafari *et al*., 2014a, El jaafari *et al*., 2014b). These results show that the fungi need an adhesion surface to optimize their biodegradation activities of this pollution in the effluent, and can organize themselves to form a biofilm, it is then adhesion results development of micro-organisms on all surfaces exposed to humid and non-sterile environment (La revue trimestrielle du réseau Ecrin, 2005, Bombenet, 2007, Boutaleb, 2007, Boutaleb *et al*., 2008a, Boutaleb *et al*., 2008b). A degradation treatment by a set of fungi on the whey is carried out in batch culture Djellal and Perrot, 2007 after amplification of fungi for 24 hours, the reduction of the COD to 70% for 142 hours (38% in 40 hours). Another study (Mannan *et al*., 2005), using an activated sludge treatment in the presence of *Aspergillus Niger*, gave a reduction in COD of 86% for 120 hours of treatment. Thus an effluent treatment made by agri-food industries (Hamdani *et al*., 2004), which uses the coagulation-sedimentation of physico-chemical treatment and which gave the results of phosphorus abatement of 89% and 94% SM. By comparing these results, it was possible to enhance treatment with the use of snail shells to obtain first a more effective treatment in terms of P and SM, especially as the percentages obtained in secondary treatment are also important than those obtained in primary processing performed by Hamdani *et al*., 2004; and second, we could reduce the evening processing time compared to our first study carried out in 48 hours of treatment, or in relation to that directed by (Djelal and Perrot, 1991) 40 hours 24 hours face of this study. Finally this study shows that in the presence of material (the snail shells), the processing results are satisfying, from the point of view of processing time and rate of degradation of organic matter.

CONCLUSION

The biological treatment in a moving bed by a purely biological lining proved to be very efficient for having ecological and economical point. Fungal growth showed very favorable results which are observed by the high rate of degradation of organic matter recorded during the study. The treatment option is an innovative aspect since this is the first time these shells will be used as adhesion support for microorganisms in general for biological treatment.

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