

Dairy Wastewater Treatment in Moving Bed Biofilm Reactor using Sardine's Scales as Biomass Support

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Abstract-This study consists on the application of moving bed biofilm reactor system (MBBR) using *Aspergillus Niger* fungi and fish sardine's scales, to reduce the quantity of organic biodegradable matters. The Tests are made on synthetic wastewater, prepared from complete milk UHT miming dairy effluent. Chemical oxygen demand (COD) and other physicochemical parameters are following-up in time. And sardine's scales ratio is optimizing for ecological and economic reasons. In the term of this work we demonstrated the effect of sardine's scale in biological aerobic treatment.

Keywords- *Dairy wastewater, biologic epuration, Aspergillusniger, sardine's scales.*

I. INTRODUCTION

The management of wastewater coming from manufacturing processes is a central point for the dairy industries. The effluents, rejected by the company before treatment, have a high pH as well as a high biochemical oxygen demand (BOD), because of the detergents and milk [1].

The treatment of dairy wastewater by biological purification process has many advantages in terms of capital investment, operating costs and efficiency compared to other process[1-2]. Therefore, the use of a specific microbial biomass comes to be the most economic and efficient solution to reduce excess sludge [2]. A viable alternative is bio-augmentation strategies, such as the addition of external microorganisms with high capacity for the specific degradation of a target substrate [2].

Preliminary work had shown the positive effect of the presence of scales in the moving bed bioreactors, as a support of colonization and biofilm formation [3]. This study aims to confirm this effect by using the scales of sardines and seek the optimal volume mass ratio to introduce for a better result.

A. Biological model

During manipulation, *Aspergillus niger* fungi (11G323A) are used. Choice is justified by its resistance to the organic pollutants such as detergents [4], and they are widely used in the treatment of effluents of the agri-food industries [5-6].

The manipulation of fungi was performed through the preparation of a culture Luria Bertaniliquid(LBL), followed by incubation for 72 hours at 27 °C. The cells were then recovered by centrifugation (4800 g, 20 min), washed three times with the artificial effluent contained in the bioreactor, diluted in a small volume of the effluent, and finally added back to the bioreactor.

B. Biomaterial

Fish scales (industrial waste) of *Sardinapilchardus* species is used in this study as biomaterial of biofilm formation in laboratory pilot of moving bed biofilm reactor MBBR. Sardine's scales were washed several times with hot water, dried overnight at 60 °C and stocked at ambient temperature[7].

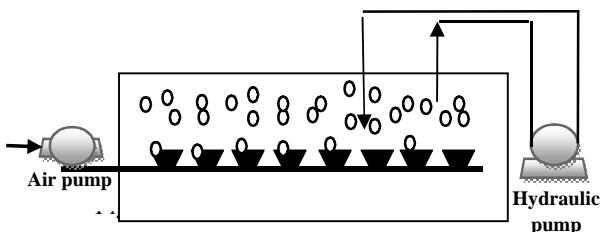
C. Effluent

In order to present the waste water generated by the dairy industries, a model effluent has been prepared with similar physicochemical properties from UHT milk diluted 50 times with distilled water[1].

The use of diluted synthetic effluent justified on the one hand by the difficulties of sampling and transport of samples, and on the other hand, by the experimental need to work with effluent composition stable controllable.

D. Bioreactor

The bioreactor used consists of a simple glass tank based on the principle of a moving bed biofilm reactor (MBBR), its total volume was about 50 liter, equipped with an air pump to inject filtered air continually, and hydraulic pump to ensure the homogenization and agitation of the effluent with.



E. Analytical methods

Temperature and conductivity were determined directly by sampling using conductivity meter (HANNA instruments, EC215); pH was measured using a pH meter (Fisher Scientific, Basic AB15); Phosphorus was determined by colorimetric method with complex phosphomolybdc [8, 9];Suspended matter (SM) was determined by filtering a volume of waste water on cellulosic filter (0.45 μm) [1]; COD was determined by oxidation in acid medium by excess potassium dichromate in the presence of silver sulfate as a catalyst and mercury sulfate [10]; total nitrogen (NTK) was determined according to Kjeldahl method [11].

II. RESULTS AND INTERPRETATION

Table 1 show the evolution of the pollution parameters in the effluent, with and without presence of sardine's scales.

A. Evolution of pH

Figure 2 shows the evolution of pH for the different quantities of biomaterial used.

We note that the pH decrease through time to achieve a value 5, this acidity differs depend the quantity of used scales.

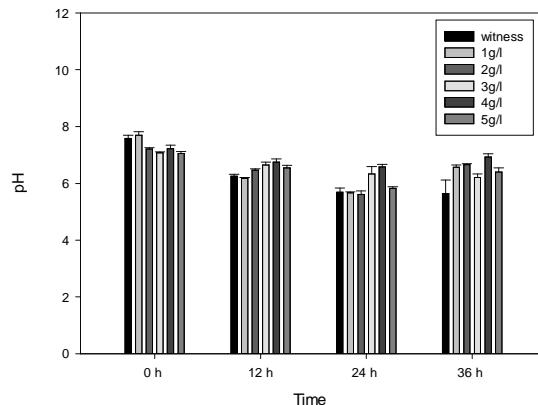


Fig. 2.Evolution of pH

Aspergillus niger producing acids such as citric acid and glucuronic acid degrading sugars present in the environment, these acids are responsible for the acidification of the medium. Three phases explain the three variations of the pH, an exponential growth phase (phase I) in which a rapid increase in the mass of fungi occurred. The consumption of organic compounds during this phase was very important, however the pH decreased slightly.

The phase of disruption of the growth (phase II) defined a decrease in the rates of growth of the mycelium and consumption of organic compounds, this would be due the disruption of the primary metabolism of *Aspergillus niger* resulting from the depletion of the medium in phosphorus and nitrogen [12].

The reduction of nucleic acid synthesis stimulates the accumulation of citrate and the pH dropped so sharply indicating the accumulation of citric acid. The pH return to neutrality due to catabolizing the citric acid by *Aspergillus niger* which mark the stationary phase (phase III). [13].

Table 1. Evolution of the pollution parameters in the effluent.

| Sardine's scales (g/l) (test name) | Time (hours) | T (°C) | pH | COD (mg/l) | TNK (mg/l) | Phosphorus (mg/l) | Suspended Matter (SM)(mg/l) |
|------------------------------------|--------------|--------|-----|------------|------------|-------------------|-----------------------------|
| 0g/l (Witness) | 0 | 17 | 7.6 | 4128 | 1401 | 68.75 | 0.88 |
| | 12 | 22 | 6.3 | 2654 | 630.45 | 52.83 | 0.54 |
| | 24 | 22 | 5.7 | 2476.8 | 490.35 | 48.12 | 0.69 |
| | 36 | 22 | 5.6 | 1494 | 280.2 | 42.5 | 0.51 |
| | 48 | 21 | 6.8 | 148608 | 210 | 33.8 | 0.23 |
| 1g/l (Test N°1) | 0 | 18 | 7.7 | 4128 | 1471.07 | 70.55 | 0.99 |
| | 12 | 24 | 6.2 | 2592 | 770.55 | 43.38 | 0.55 |
| | 24 | 23 | 5.7 | 2400 | 700.5 | 39.12 | 0.71 |
| | 36 | 23 | 6.6 | 2016 | 210.15 | 39.1 | 0.34 |
| | 48 | 20 | 6.8 | 1632 | 140.1 | 25.2 | 0.32 |
| 2g/l (Test N°2) | 0 | 18 | 7.2 | 4128 | 1471.07 | 67.6 | 0.82 |
| | 12 | 23 | 6.5 | 2592 | 770.55 | 24.11 | 0.35 |
| | 24 | 22 | 5.6 | 1728 | 560.4 | 33.5 | 0.9 |
| | 36 | 22 | 6.7 | 96 | 280.2 | 12.33 | 0.52 |
| | 48 | 22 | 7.2 | 48 | 70.05 | 8.67 | 0.44 |
| 3g/l (Test N°3) | 0 | 17 | 7.1 | 4128 | 1401 | 72.84 | 0.95 |
| | 12 | 21 | 6.7 | 3360 | 840.6 | 43.38 | 0.63 |
| | 24 | 22 | 6.3 | 2880 | 700.5 | 35.35 | 0.47 |
| | 36 | 22 | 6.2 | 1282 | 350.25 | 36.6 | 0.41 |
| | 48 | 23 | 7.2 | 672 | 210.15 | 27.17 | 0.33 |
| 4g/l (Test N°4) | 0 | 18 | 7.2 | 4128 | 1471.05 | 67.77 | 0.78 |
| | 12 | 19 | 6.8 | 2993 | 980.7 | 32.57 | 0.36 |
| | 24 | 19 | 6.6 | 1754 | 700.5 | 48.2 | 0.62 |
| | 36 | 20 | 6.9 | 310 | 420.3 | 16.53 | 0.85 |
| | 48 | 19 | 7.4 | 248 | 140.1 | 11.78 | 0.6 |
| 5g/l (Test N°5) | 0 | 17 | 7.1 | 4128 | 1401 | 73.01 | 0.92 |
| | 12 | 21 | 6.6 | 2496 | 700.5 | 40.27 | 0.6 |
| | 24 | 22 | 5.8 | 2304 | 490.35 | 27.99 | 0.38 |
| | 36 | 23 | 6.4 | 1236.3 | 420 | 32.5 | 0.39 |
| | 48 | 22 | 7.1 | 768 | 350.5 | 32.08 | 0.39 |

B. Evolution of the suspended matter (SM)

Figure 3 shows the evolution of the quantity of suspended matter.

We note for all tests, suspended matter decreases through time, but increase between 24 and 36 hours, at the end experience the SM decrease with a percentage of 45%.

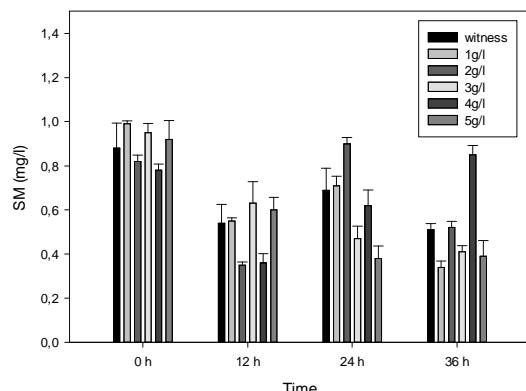


Fig. 3.Evolution of suspended matter

In the biological treatment, suspended matter should decrease over time. Here, the increase of SM can be explained by the fact that fungi used during this experience adhere to the wall of the bioreactor, form a biofilm which, after grubbing, causes an increase of the material in suspension [1].

C. Evolution of phosphorus

Figure 4 represent the evolution of phosphorus removal. Phosphorus decreases in the first twelve hours, increase in 24 h, and then decreases to achieve a value of 80% (2 to 4 g/l).

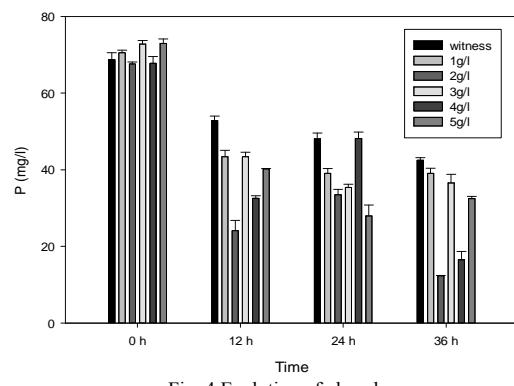


Fig. 4.Evolution of phosphorus

Microorganisms use orthophosphates, in their bioavailable form to build their genetic material, their membrane and for having energy [14] that explains decrease of phosphorus. During the biological treatment, bound phosphorus is transformed to a soluble format (orthophosphates) by the mineralization process [15], under the effect of citric acids produced by *A. niger* [16] which explain the increase in orthophosphates.

D. Evolution of COD

Figure 5 shows the evolution of COD for the different tests in this study.

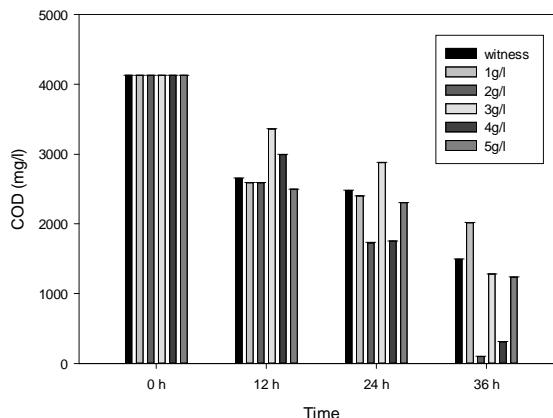


Fig. 5.Evolution of COD

The biodegradation of organic matter increases through time varies depending on the amount of scales; the maximum reduction of COD noted was 99% (using 2 g/l of scales). The COD increases due to the use of the organic compounds by *Aspergillus niger*, this biodegradation and most important for quantities ranging between 2 and 4 g/l.

E. The evolution of NKT

Figure 6 represents the evolution of total nitrogen over time. We note that elimination of nitrogen was important in almost all the quantities used. The maximum value obtained was 80.95% for test using 2g/l of scales.

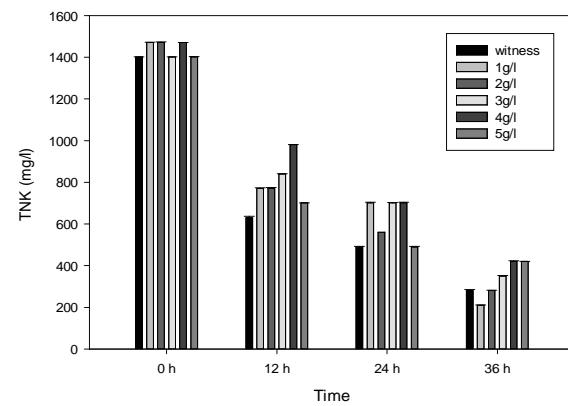


Fig. 6.Evolution of total nitrogen

In general the nitrogen undergoes various transformations during biological treatment (passage of the ammoniac form to the acid form then nitric and back to gaseous form): the decrease observed in the fig. 6 above is due to the incorporation of nitrogen into the new cells of *Aspergillus niger* produced. These fungi need for their metabolisms many chemical elements, including nitrogen which ranks first, because it is an important component of the fungal cell and represents approximately 5% of its dry matter [17].

III. DISCUSSION

Decrease in pH is a due to the production of acids during the depletion of phosphorus and nitrogen [12-13], The decrease was optimal at 36 h of treatment.

Based on this term, following table shows percentage of organic degradation observed in the witness and the test using 2 g/l of scale which gave the most satisfactory results (test N°2 with 2g/l of scales), within only 36 hours of treatment.

Table 2. Summary of the best results obtained of COD, TNK, P and MS reductions using sardine's scales:

| | DCO (%) | TNK (%) | P (%) | MS (%) |
|---------------------|---------|---------|--------|--------|
| Witness | -64 | -85 | -50.8 | -44.95 |
| Test N°2 (36 hours) | -97.67 | -80.95 | -81.76 | -39.02 |

Note: + positive evolution (increase) -: negative evolution (reduction)

Compared with other studies, the experiments of Djelal and Perrot, giving reduction of 70% in COD but after 142 hours and with inoculation with amplified fungi (during 24 h) [5];

Another study, of Mannan et al. (2005) using a treatment by activated sludge in the presence of *Aspergillus niger*, consist of COD reducing by 86% in 120 hours of treatment [18].

Scales surfaces optimize biodegradative activities of fungi. Compared to the other quantities, 2g/l represent the mass/volume ration most suitable for adhesion. The results showed that this mode of treatment, allows reduction of 97.67% in COD, 80.95% in nitrogen and 81.76% in phosphorus.

IV. CONCLUSION

V.

The evolution of the parameters of this pollution is depending on two actions, the first is linked on the presence of BM (biomaterial) and the second is in relation with the quantities of sardine's scales used.

The presence of sardine scales actually improves the properties of biodegradability; Study factor ratio mass volume shows that the optimal amount of scales to introduce is 2 g/l.

REFERENCES

- [1] S. Castillo, Etude d'un procédé compact de traitement biologique aérobie d'effluents laitiers. Thèse de doctorat au INSA de Toulouse, France, 2005.
- [2] H. Djelal, M. Rigail, L. Boyer, (s.d.), Les effluents industriels et leur traitement. http://www.cairn.info/zen.php?ID_ARTICLE=MAV_020_0275.
- [3] A. El Jaafari, N. Boutaleb, B. Bahlaouan, T. Taiek, M. Bennani, S. Lazar et Said El antri, Use of Three Types of Fish Scales as Biomass Support in Moving Bed Biofilm Reactor

for Biological Treatment of Dairy Wastewater. International Journal of Engineering Research & Technology.. 2014. N°3, 1636-1639.

- [4] L. Coulibaly, G. Gourène, N. Spiros, Agathos, Sélection d'un champignon filamenteux pour l'épuration des eaux usées: le phénol comme inhibiteur modèle de discrimination entre *Aspergillus niger* et *A. oryzae*, Sciences & Nature. 2008, N°2, 111-119.
- [5] H. Djelal, M. Perrot, Utilisation de champignons spécifiques pour la biodégradation d'effluents industriels, l'eau l'industrie les nuisances. 2007, N°306, 85-90.
- [6] M. Hamdi, Nouvelle conception d'un procédé de dépollution biologique des margines, effluents liquides de l'extraction de l'huile d'olive. Thèse doctorat en Biologie cellulaire et Microbiologie. Marseille, 1991.
- [7] P. Stepniewski, G. Olafsson, H. Helgason, & B. Jastorff, Recovery of astaxanthin from seafood wastewater utilizing fish scales waste. *Chemosphere*. 2003.
- [8] DIN 38405-D11-1 OPO43, Dosage des composés phosphoriques méthodes photométrique par phosphomolybdique, 1993.
- [9] T. Taiek, N. Boutaleb, B. Bahlaouan, A. El jaafari, H. Khrouz, S. Lazar, S. El antri, Production of New Bio Fertilizer from Waste of Halieutic Activities, Brewing Industry and Brandy Distilleries in MoroccoInternational, Journal of Engineering Research & Technology. Vol. 3 - Issue 8.August – 2014, 1599-1603.
- [10] NFT90-101, Détermination de la demande chimique en oxygène (DCO), Février, 2001.
- [11] NF EN 25663, ISO 5663, Janvier 94, Indice de classement T90-110; Norme Afnor Année 1997 Tome 2.
- [12] A. Chmiel, Kinetic Studies of Citric acid by *Aspergillus niger* : Phase Mycelium Grown and Product Formation. *Acta Microbiologica Polonica*, 1975. 185-193.
- [13] O. Siboukeur, M-D. Ould El Hdj & F. Zargat, Contribution à l'étude de la production d'acide citrique par *Aspergillus niger* cultivé sur Moût de Dtes de la variété Ghars. *Rev. Ener. Ren: Production et Valorisation*, 2001. 93-96.
- [14] K. Jernejc, M. Vendramin and A. Cimerman, Galactose Inhibition *Aspergillus niger* Lipid Composition of Citric Acid Accumulating Condition Enzyme, *Microbiol. Technol.* 1989, N°11, pp. 452-456.
- [15] D. BANAS, J-C. LATA, Les phosphates, Laboratoire d'Ecologie, Systématique et Evolution. Université Paris-Sud. <http://sylvie.tarantino.pagesperso-orange.fr/LB%20polluant%20habitat/PhosphateLB.pdf>.
- [16] N. Valissilev, M-T. Baca, M. Vassileva, I. Franco & R. Azcon, Rock phosphate solubilization by *Aspergillus niger* on sugar-beet wste medium. *Appl Microbial Biotechnol*, 1995. 546-549.
- [17] G. Deronzier, S. Schétrite, Y. Racault, J-P. Canler, A. Liénard, A. Hédout, Traitement de l'azote dans les stations d'épuration biologiques des petites collectivités. Ministère de l'Agriculture et de la pêche Document technique FNDZE, 2001. n°25.
- [18] S. Mannan, A. Razia&M-Z.Alam, Use of fungi to improve bioconversion of activated sludge. *water Research* 39, 2005. 2935-2943.