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Filamentous fungi and natural supports as a carrier in moving bed biofilm reactors for ecological treatment of halieutic industrial effluent

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ABSTRACT

In this work, a biological treatment of fishing industry wastewater has been studied in a fluidized biofilm bed reactor moving bed biofilm reactor (MBBR) using natural media to insure biofilm colonization. Sardine's scales, drum's scales and snail's shells are agro-industrial waste available used in this work as a support for colonization in presence of *Penicillium chrysogenum* and *Aspergillus niger*, for the objective of optimizing the yields of the depollution and to make it more economical and ecological. For all pollution parameters, results show that this new MBBR biological treatment design offers comparable performance over the conventional design, which engages conventional Kaldnes carriers made of plastic. The study also highlights the effect of the nature of the support as well as of the biomass on biological treatment. The use of Sardine's scales allows better treatment of the chemical oxygen demand, on the other hand, the use of the drum's scales and snail's shells allows better treatment of the suspended matter and total nitrogen. The correlation between the pollution parameters as well as the intrinsic properties of the various supports in a multidimensional statistical analysis, highlighted the implication of the hydrophobicity and the specific surface to improve the quality of the biological treatment, by the relation that they have to explain the biofilm formation.

Keywords: Moving bed biofilm reactor; Fishing industry; Biological treatment; Biofilm; Organic pollution; Bio-carriers; Biomass

1. Introduction

In the last few years, population increase and industrialization have caused a deterioration in water quality. Wastewater coming from food processing plants, dairies, breweries, tanneries and pharmaceutical industries contains high levels of pollutants. This wastewater is one of the main sources of surface and groundwater contamination. Effective treatment strategies to reduce or eliminate pollutants in wastewater are being adopted by industry and scientists to attain standards of discharge that are becoming increasingly stringent. Pollutants that need to be controlled include suspended solids, biochemical oxygen demand (BOD_5), total nitrogen (TN) and total phosphorus (TP).

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To face this important issue, physicochemical treatment and biological treatment are the present treatment methods [1]. Activated sludge treatment is one of the most commonly adopted biological treatment processes that requires the recirculation of a fraction of the sludge produced. This process reduces BOD_5, performs nitrification, denitrification and biological dephosphatation [2]. The search for a technology with the advantages of activated sludge processes and biofiltration has led to the creation of a new, more compact process called a biofilm reactor. Under the biofilm growth mode, the microbial cells are stabilized in a layer of extracellular polymeric substances.

The biofilm formation in this technology can reduce both installation and operating costs compared to processes with planktonic biomass, because of its lower sludge volume, higher operational efficiencies, better mechanical stability, improved hydraulic retention time (HRT), and potential for microbial reuse [3]. However, there are several variants of biofilm processes, but the moving bed biofilm reactor (MBBR) technology is a specially promising solution applied to effluent treatment. It integrates the best characteristics of processes with suspended and adherent biomass growth [4].

Conventional biofilm carriers in MBBR are inert surfaces (e.g., plastic, gravel, sand) that provide sufficient solid surface for the development of biofilm [3–5].

Multiple natural supports have been tested in the literature as biosorbent and also showed excellent results such as the scales of *Oreochromis niloticus* [6], babassu coconut epicarp [7], cupuassu shell [8], rice biomass [9] and Bengal gram shell [10]. Since the cost and availability of the material used are an important factor in the implementation of a compact and industrially applicable process, the aim of this work is therefore the conception and study of a biological process using natural supports, at the same time industrial waste, in a MBBR to increase the efficiency of the treatment of halieutic effluent. Fish scales and snails shells are tested to ensure carrier in the MBBR reactor, using two different filamentous fungi: *Aspergillus niger* and *Penicillium chrysogenum*. This choice is aligned with the principles of circular economy and sustainable development and offers an alternative to plastic supports made from petroleum.

2. Material and method

2.1. Bioreactor

The bioreactor used is a glass reservoir of a total volume of 20 L, oxygenation is ensured by air insufflated through fine aerators making bubbles in the reactor base. Homogenization and agitation of the effluent are ensured by a mechanical agitator (Fig. 1).

2.2. Fungal cell culture

Reference strains of *P. chrysogenum* (100393) and *A. niger* (11G323A) were used in this study. The culture medium used was PDA media for solid cultures of the fungi. Concerning the cultures in a liquid medium, a malt broth was sterilized by autoclaving at 120°C for 20 min, then inoculated and finally incubated 72 h at 30°C.

The fungal cells were then separated by centrifugation (4,800 g, 20 min), recuperated and added to a volume of the effluent for a contact time between them and the synthetic effluent.

This contact is ensured by a slight agitation for a minimum of 30 min and then at the end, this mixture is added to the bioreactor which contains the synthetic effluent.

2.3. Preparation of synthetic fisheries effluent

We opted to use synthetic sewage with similar composition compared to an industrial one. The artificial effluent was prepared using 160 g of heads, viscera, bones and tails of fresh sardines (*Sardinella* sp.) and 400 mL of distilled water. The mixture was mixed in a blender and then sieved to obtain a concentrate. The concentrate was then diluted with distilled water to the desired concentration [11].

2.4. Natural carriers and physical–chemical properties

The scales of *Sardina pilchardus* (sardines scales), *Umbrina roncador* (drums scales) and the shells of the family Helicidae (snails shells) were used for this study. These scales were collected, washed several times with

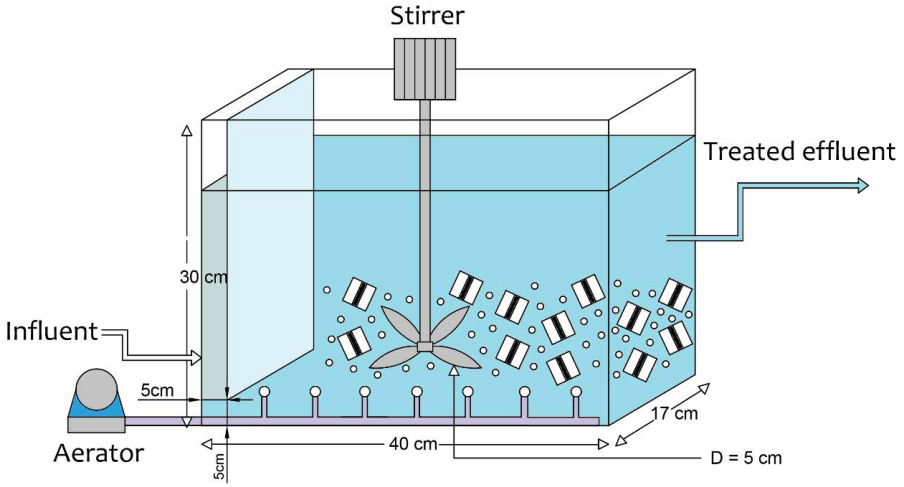

Fig. 1. Schematic representation of the bioreactor used during this study.

demineralized hot water, dried for 24 h at 60°C and stored at room temperature [12]. In order to prepare our sample, the shells obtained from snails were then cleaned with hot water. The shells were washed again with distilled water, dried in the sun and perforated to a diameter of one millimeter in order to promote the diffusion of the effluent inside.

Kaldnes K3 supports are used as biomass support in MBBR reactors. This type of support has a specific surface area of 500 m^2/m^3 [13]. The specific surface area of our biomaterials was evaluated according to the study of nitrogen adsorption–desorption isotherms using the Brunauer–Emmett–Teller method. This technique consists in determining the volume of a monolayer of gaseous molecules adsorbed on the solid of interest [14].

The contact angle informs about hydrophobicity. For these parameters, three measurements are made for each sample. A 2 μL drop is formed at the end of a syringe to be automatically deposited on the surface of the sample to be tested. A digital image is immediately acquired using a CCD camera placed on a goniometer (Visiodrop-MCAT of GBX SC Instruments, France).

2.5. Analytical method for measuring purification performance

After collecting the samples, they were directly analyzed. Many assays were carried out at the beginning 6, 12 and 24 h. Total nitrogen was determined by catalytic thermal oxidation with a high-temperature TOC analyzer (Model: TOC-L Shimadzu, Japan). The chemical oxygen demand (COD) was assessed through the use of the Thermoreactors ECO6 VELP Scientifica.

Phosphorus was measured by a colorimetric method using the phosphomolybdic complex according to the German Standard [15]. Suspended matter (SM) was obtained by filtration of a volume from the effluent on filter paper (0.45 μm) according to the method described by [16]. A microscopic observation in scanning electron microscopy (SEM) was also conducted to study the colonization of microorganisms on supports. The common glutaraldehyde binding protocol is then performed. After metallization, each surface is examined by observation (average of 3 counts) by SEM scanning electron microscopy Philips, Model XL30.

2.6. Data analysis

The statistical technique of principal component analysis (PCA), was used to evaluate the existence of relationships between pollution parameters and their removal efficiencies, as well as the intrinsic properties of the different media (specific surface and hydrophobicity). The statistical analysis was performed using the data analysis software STATISTICA 10.

3. Results and discussion

3.1. Effect of HRT on treatment quality

The HRT refers to the average time that the wastewater to be treated remains in the reactor, and is one of the most important parameters of biological wastewater treatment. It is the average reaction time of wastewater and microorganisms in the bioreactor [17]. Adequate HRT allows sufficient contact between microbial biofilm and wastewater, resulting in an organic pollutant removal efficiency [18]. Table 1 shows the results of the abatement of the various parameters of pollution using the various natural supports and fungal biomass. These tests have as control assays with indigenous flora and Kaldnes media.

It was observed in Table 1 that an increase in the duration of HRT (from 6 to 24 h) had a positive effect on the effectiveness of treatment. Whatever the support and the presence or not of the biomass. The total nitrogen removal was not significant (not exceeding 40%) except in the case of using Kaldnes supports in the presence of *P. chrysogenum*, the removal was almost 70%, but it required 24 h of HRT. For the other parameters, regardless of the choice of biomass and support, the interesting percentage of pollutant removal (>70%) requires a minimum HRT of 24 h. Nutrient and organic matter removal increase with HRT. By comparing with other authors (Table 2), it can be observed comparable depollution performance in shorter times [19–22].

3.2. Chemical oxygen demand

Table 1 shows that the COD treatment varies depending on the nature of the support used and is also strongly

Fig. 2. Image of media specification of snail shells; (a) species *Theba pisana* belonging to the family of Helicidae, (b) sardine scales (species *Sardina pilchardus*), (c) drum scales (species *Umbrina roncador*), and (d) industrial supports type Kaldnes K3.

Table 1
Evolution of different pollution removal depending on hydraulic retention time (HRT)

Time (h)	Media	Biomass	SM (%)	COD (%)	P (%)	TN (%)
6	Kaldnes (K)	Indigenous flora (IF)	9.78 (±1.30)	25.77 (±2.10)	45.50 (±1.41)	0.00 (±0.00)
12	Kaldnes (K)	Indigenous flora (IF)	14.13 (±2.70)	34.22 (±3.19)	43.75 (±1.09)	3.28 (±0.26)
24	Kaldnes (K)	Indigenous flora (IF)	28.26 (±2.14)	47.62 (±2.35)	42.77 (±0.40)	8.33 (±0.14)
6	Kaldnes (K)	*Aspergillus niger*	16.22 (±4.50)	90.00 (±1.50)	64.71 (±1.50)	21.07 (±2.00)
12	Kaldnes (K)	*Aspergillus niger*	2.70 (±6.50)	93.96 (±2.00)	77.57 (±1.50)	23.79 (±4.00)
24	Kaldnes (K)	*Aspergillus niger*	35.14 (±4.00)	98.00 (±0.50)	85.88 (±0.50)	32.25 (±8.00)
6	Drum scales (U)	*Aspergillus niger*	73.58 (±7.00)	44.12 (±5.00)	56.18 (±5.50)	7.69 (±3.00)
12	Drum scales (U)	*Aspergillus niger*	86.79 (±7.00)	41.18 (±2.00)	64.54 (±2.00)	30.77 (±3.00)
24	Drum scales (U)	*Aspergillus niger*	83.02 (±3.00)	61.76 (±10.00)	74.30 (±3.00)	38.46 (±7.00)
6	Snail shells (H)	*Aspergillus niger*	70.18 (±2.00)	29.41 (±6.00)	63.50 (±3.00)	6.66 (±1.50)
12	Snail shells (H)	*Aspergillus niger*	78.95 (±6.00)	47.06 (±5.50)	58.65 (±10.00)	15.57 (±4.00)
24	Snail shells (H)	*Aspergillus niger*	71.93 (±5.00)	44.12 (±2.50)	73.42 (±6.00)	28.89 (±2.00)
6	Sardine scales (S)	*Aspergillus niger*	22.22 (±4.00)	90.00 (±6.00)	58.41 (±5.00)	3.38 (±2.00)
12	Sardine scales (S)	*Aspergillus niger*	51.85 (±11.00)	98.00 (±1.00)	63.86 (±0.50)	7.77 (±3.00)
24	Sardine scales (S)	*Aspergillus niger*	48.15 (±4.00)	98.00 (±2.00)	64.56 (±4.00)	13.19 (±5.00)
6	Kaldnes (K)	*Penicillium chrysogenum*	2.27 (±0.10)	53.92 (±0.30)	53.21 (±2.00)	16.71 (±0.20)
12	Kaldnes (K)	*Penicillium chrysogenum*	20.70 (±0.10)	57.76 (±1.00)	61.13 (±1.90)	36.82 (±0.60)
24	Kaldnes (K)	*Penicillium chrysogenum*	90.90 (±0.20)	73.12 (±2.00)	79.95 (±3.00)	69.90 (±2.90)
6	Drum scales (U)	*Penicillium chrysogenum*	39.18 (±2.00)	37.14 (±2.00)	22.14 (±0.70)	3.09 (±0.15)
12	Drum scales (U)	*Penicillium chrysogenum*	83.78 (±3.00)	45.71 (±1.00)	29.52 (±0.40)	4.98 (±0.90)
24	Drum scales (U)	*Penicillium chrysogenum*	97.29 (±3.90)	54.28 (±0.70)	44.04 (±2.00)	9.01 (±0.40)
6	Snail shells (H)	*Penicillium chrysogenum*	33.33 (±3.00)	63.29 (±1.10)	28.57 (±1.10)	12.18 (±2.00)
12	Snail shells (H)	*Penicillium chrysogenum*	57.77 (±3.00)	77.41 (±4.00)	37.50 (±0.80)	20.11 (±0.50)
24	Snail shells (H)	*Penicillium chrysogenum*	91.11 (±0.50)	98.00 (±0.00)	42.65 (±0.13)	33.65 (±0.80)
6	Sardine scales (S)	*Penicillium chrysogenum*	0.00 (±0.00)	29.64 (±0.50)	20.90 (±0.40)	1.34 (±0.02)
12	Sardine scales (S)	*Penicillium chrysogenum*	24.32 (±1.20)	57.00± (0.80)	29.76 (±0.20)	2.58 (±0.10)
24	Sardine scales (S)	*Penicillium chrysogenum*	37.83 (±2.00)	77.85 (±4.00)	87.20 (±0.36)	17.47 (±0.70)

Table 2
Performance comparison of various treatment technologies

	Effluent type	Influent pollutant concentrations (mg/L)	HRT (h)	COD (%)	TN (%)	P (%)	Reference
MBBR	Palm oil mill	COD: 1,500.00; TN: 115.00; P: 126.50	72	56.60	94.40	ND	[19]
MBBR	–	COD: 231.38; TN: 80.84; P: 9.68	6	79.78	63.21	41.98	[20]
MBBR	–	COD: 223.08; TN: 67.47; P: 8.31	9.5	80.91	71.81	50.06	[21]
MBBR	Terephthalic acid	COD: 2,500.00	24	68.00	ND	ND	[17]
SBR	Poultry slaughterhouse	Not mentioned	48*24**	97.00	98.00	ND	[22]
MBBR	Halieutic	COD: 2,882.00; TN: 85.32; P: 4.30	24	98.00	69.90	87.20	This study

*: anaerobic; **: aerobic

influenced by the presence or absence of fungal biomass. The maximum removal with a percentage of 98% is related to snails shells (H) using *P. chrysogenum* from 24 h, sardines scales and Kaldnes using *A. niger* fungus from only 12 h. Essays conducted with indigenous flora, showed a very moderate COD abatement of 47.62% even after 24 h of treatment, which proves that the presence of fungal biomass is required for a high percentage abatement of COD. COD is frequently measured as a time-saving marker of the organic pollutant in water. The efficiency of the purification process is given as a proportion of the organic matter that has been purified during the treatment cycle [23]. In the literature, the interesting percentage of COD treatment in a MBBR system are evoked [23–29], have even reached a percentage of 99% in the case of the biological elimination of phenol from saline wastewater in MBBR [28]. A similar

study using snail shells in the MBBR system scores a 75.34% of reduction in the COD of municipal effluent [27].

3.3. Suspended matter

For assays with *A. niger* fungus best result is showed when using drum scales (U), SM abatement was 83.02%. In the case of the use of *P. chrysogenum* as biomass, the best result is showed when using drum scales (U) with the highest percentages of 97.29% and by using snail shells with percentages of 91.11%. These results are comparable to when using Kaldnes supports and in which we recorded a percentage of 90.90%.

For tests using only indigenous flora, the percentages of SM do not exceed 28%. The increase of the percentage of removal of SM over time can be explained by the formation of a thick, robust biofilm. Any decrease in this percentage can be explained by the detachment of the biomass from the support [30], or in the use of natural supports, it can be associated with the deterioration and loss of the support itself [31]. This is observed (but only slightly) in some cases of treatment using *A. niger* when going from 12 to 24 h. By comparing with other authors, Aitcheikh et al. [32] demonstrated a 79.34% SEM removal percentage using snail shells as biomass support in the MBBR system, this percentage remains interesting and comparable to that reached in this study.

3.4. Phosphorus

There are three forms of phosphorus present in wastewater: the soluble form of phosphate (also called orthophosphate), polyphosphate, or phosphorus bound to organic compounds. Biological phosphorus removal consists of incorporating the phosphorus present in the influent into the cell biomass, which is then removed from the process [30]. Phosphate can be removed in two different ways: assimilation and enhanced biological phosphorus removal (EBPR).

Assimilation takes place only during the growth phases, EBPR occurs when the wastewater goes through aerobic and anaerobic zones alternately. Most of the removal of TP is attributed to the assimilation mechanism [33].

A significant reduction of 84% in TP was observed by Yang et al. [34] and Brown et al. [35]. Both studies were conducted on synthetic wastewater using an anoxic anaerobic MBBR system with a particular interest in phosphorus removal.

In this study, for *A. niger*, an interesting percentage of phosphorus removal is recorded towards the end of the treatment, with percentages of abatement of 85.88% for Kaldnes supports, 74.30% for drum scales, 73.42% for snail shells and 64.56% for sardine scales (Table 1). For *P. chrysogenum*, the best phosphorus removal is ensured when using sardines shells and Kaldnes with percentages of 87.20% and 79.95% respectively (Table 1).

For *A. niger*, an interesting percentage of phosphorus removal is recorded towards the end of the treatment, with percentages of abatement of 85.88% for Kaldnes supports, 74.30% for drums scales, 73.42% for sardine scales and 64.56% for snails shells (Table 1).

For *P. chrysogenum*, the efficiency of the MBBR system in terms of phosphorus removal is not high for drums scales and snails shells with a respective percentage of 44.04% and 42.65%, in contrast to sardines shells and Kaldnes with rates of 87.20% and 79.95% (Table 1). We notice that the treatment of the phosphorus pollution is more significant for drums scales using *A. niger*, and for sardines scales using *P. chrysogenum*. The natural supports tested have the same abatement performances as Kaldnes supports. Compared to previous similar studies, the phosphorus removal efficiency of our system is not high for drum's scales and snail's shells. A possible explanation might be the presence of a high nitrate concentration in our saline effluent. Indeed, the presence of nitrate is associated with the capacity of phosphorus removal. Such interference happens due to increased redox potential and the incapacity to produce the fatty acids needed to liberate phosphorus.

3.5. Total nitrogen

According to the results presented in Table 1, the best configuration, which allowed a relatively better treatment of the nitrogen, is that constituted by the Kaldnes media and *P. chrysogenum* in which the percentage of almost 70% is marked at 24 h. For the other cases, nitrogen removal was low and less than 40%. Compared to data in the literature, higher percentages (greater than 94%) were obtained using the same MBBR system but with an anaerobic–aerobic arrangement [24].

3.6. Specific surface area and hydrophobicity

Specific surface area can be described simply as the external surface of a solid object, including the surface area attributable to pores. Surface adsorption occurs according to two mechanisms: physisorption and chemisorption, differentiated by the nature of the intermolecular interactions between the molecule and the surface, the first one being a physical interaction while the second one interacts by chemical bonding [36]. A high specific surface area of the carrier medium, providing very high concentrations of biofilm in a small reactor volume, determines the system's performance.

A previous study revealed that the scales of *Oreochromis niloticus* had a specific surface area of 2.6 m^2/g [6]. Adsorbents as babassu coconut epicarp [7], cupuassu shell [8], rice biomass [9] and Bengal gram shell [10] have been reported, respectively with values of 1.5, 1.2, 0.5 and 1.4 m^2/g. Comparing the specific surface area of our natural substrates with these agro-industrial wastes, it can be seen that natural media used have higher specific surface areas than those mentioned (Table 3).

According to the literature, several factors are able to facilitate the establishment of biofilms on the materials in contact with water or being of a wet environment, the most important is [38–42].

The hydraulic regime and its variations (water retention time, the flow velocity); the environmental conditions (temperature, physicochemical composition, ionic strength); the nature and concentration of nutrients (organic matter,

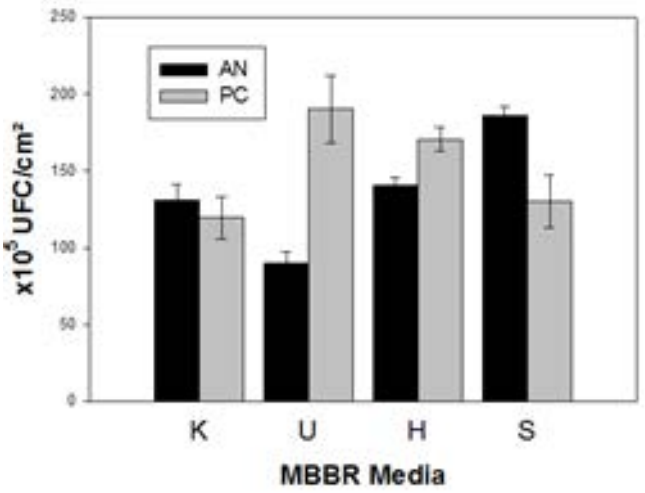

Fig. 3. Enumeration per unit area was observed on different biofilm beds studied after 24 h of contact in the bioreactor.

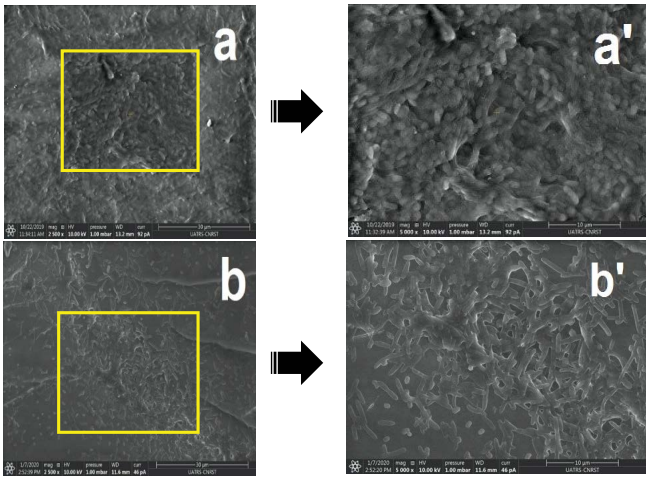

Fig. 4. Scanning electron microscopy (SEM) photos of sardine scales (*Sardina pilchardus*) colonized by (a) *Aspergillus niger* (2,500 × magnification) and (b) *Penicillium chrysogenum* (2,500 × magnification). (a′) and (b′) consists of a double magnification of the yellow frame of (a) and (b) respectively.

inorganic carbon, iron, phosphates); the presence or not of a chlorine residual; the density and nature of bacteria in the water source that has been treated or not and finally the nature of the material used.

Moreover, it is generally accepted that the interactions involved in the initial adhesion of planktonic bacteria to support are subdivided into specific and non-specific interactions (e.g., Lifshitz-van der Waals, electrostatic and acid–base interactions). For the second category, several authors mentioned that hydrophobicity is the one that most affects adhesion and consequently biofilm formation [20,38–42]. According to Table 3, the following order of hydrophobicity is observed: S > H > U > K. Observations in SEM after 24 h of contact between the supports and the inoculated effluent will confirm or not the concordance in this parameter and the colonization by biofilms.

3.7. Colonization of media by biofilms

Fig. 3 shows the results of the estimation of colonization of the different biofilm beds studied after 24 h. It is important to mention that at the beginning of each treatment the supports were clean and did not present any colonization after being washed and dried as described in material and method.

According to Fig. 3, the order of hydrophobicity for *A. niger* is the following: S > H > K > U and for *P. chrysogenum* the order is: U > H > S > K. The colonization by MBBR system, is therefore dependent on both kind of media as well as biomass. The colonization results for *A. niger* seem to agree better with the results for hydrophobicity. Fig. 4 shows SEM photos of colonization of *Sardina pilchardus* by *A. niger* (a) and *P. chrysogenum*. It shows clearly that the colonization by *A. niger* is much larger compared to *P. chrysogenum* on sardine scales (*Sardina pilchardus*) after 24 h of contact in the bioreactor.

To compare the degree of similarities (or differences) between biofilm colonization and the role of hydrophobicity and/or specific area of media in reducing pollution in its different forms, PCA analysis is then generated.

3.8. Statistical analysis

Fig. 5 presents the PCA of data matrix constitute of 9 configurations of MBBR treatment, using 4 types of media (K, H, S, U), 3 types of strains (*A. niger*, indigenous flora, *P. chrysogenum*), and an HRT set at 24 h. The treatment using the native flora and the Kaldnes supports was not subjected to a colonization test, the missing data concerning the enumeration and the formation of biofilm was managed by the option "mean substitution". This case was considered to have pertinent data for all other variables (pollution treatment and specific surface area, etc.).

The mean substitution option will allow it to be projected on the biplot without misleading the information about the "biofilm" variable and its relationship with the other variable to explain a group of individuals.

From the biplot in Fig. 5a, we can distinguish three 3 different groups.

Table 3
Specific surface area and hydrophobicity expressed by contact angle with water (°) of the natural's carriers

	Drum scales	Sardine scales	Snail shells	Kaldnes K3
Specific area (m^2/g)	4	–	5	5
Hydrophobicity (°)	75.0	89.0	82.0	71.5

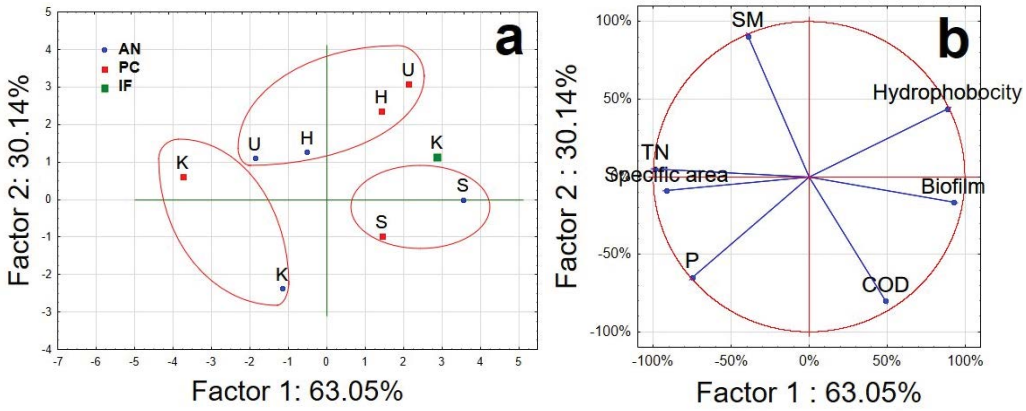

Fig. 5. Principal component analysis (a) the biplot and (b) correlation circle between factors and parameters.

Kaldnes media with *A. niger* and *P. chrysogenum* constitutes a distinct group different from the others. This group presents according to the correlation circles (Fig. 5b) a great performance in the reduction of nitrogen and phosphorus. This group can be well explained by a larger specific area according to the correlation circles presented in Fig. 5b; a group constituted by the sardine scales (Fig. 5a), can be explained by an optimal reduction of the COD (Fig. 5b). This group can be well explained by hydrophobicity and COD variables; the last group constituted by both the drum's scales and snail's shells of a rather comparable abatement quality. This group presents according to the correlation circles (Fig. 5b) a great performance in the reduction of suspended matter and located between specific areas (cases formed by *A. niger*) and hydrophobicity variables (cases formed by *P. chrysogenum*).

Fig. 5b highlights the positive correlation between total nitrogen, phosphorus, suspended matter and the specific area, and also the positive correlation between hydrophobicity, biofilm formation and COD. The treatment then depends both on the nature of the strain and also on the nature of the media.

4. Conclusions

The evaluation of classical parameters demonstrates an improvement in the properties and quality of the clearance by the introduction of natural supports, providing a potential for the colonization of microorganisms and the formation of biofilm on the surface. Biological treatment in a moving bed with a purely biological bio-carrier has proved to be highly effective, both economically and ecologically. The use of sardine scales provides a better treatment of COD with a yield of 98% within a retention time of 24 h, on the other side using drum scales and snail shells with *A. niger* and *P. chrysogenum* allows a higher treatment of suspended matter and total nitrogen with respective percentages up to 97.29% and 69.90%.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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