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Full Length Research Paper

Aerobic treatment of lipid-rich wastewater by a bacterial consortium

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Lipids raise handicap in the treatment of wastewaters in the purification stations. For that, we studied the aerobic biological treatment of greases of synthetic wastewater and restaurant wastewater by selected and optimized bacteria consortium. The results of this work highlighted the beneficial effect of the adaptation of bacteria which was evaluated by the study of chemical oxygen demand (COD) abatement and greases removal in the synthetic medium by using an adapted and not adapted biomass. The adapted biomass allowed a rate of abatement of 84.28 and 93.3% of the COD and greases removal respectively against 51.88 and 66.6% by the not adapted biomass. The biodegradation of the lipid-rich wastewater of restaurant by adapted bacteria gave satisfactory results. Indeed, we recorded an elimination of 83.3% of the COD and 81% of greases. The results obtained also revealed the importance of our bacterial consortium in the biodegradation of greases.

Key words: Greases, biodegradation, lipid-rich wastewater, aerobic treatment and chemical oxygen demand (COD).

INTRODUCTION

The fat content is declined under several families of lipids which in common have their insolubility in water and a density lower than 1. The most significant components of the lipids in environmental technology are the glycerides, these none charged molecules, are esters of glycerol and fatty acids (not very soluble in water) (Touitou, 2006). The general structure of the fatty acids defines their physical characteristics and how they will be biodegraded (Beture-Cerec, 1996). The lipid-rich wastewater is the worn water produced by the various activities of the restoration and of agroalimentary manufactory, it's evacuation without any preliminary treatment in the sewers or the natural environment causes severs environmental problems.

In wastewater evacuation, the solidification of greases at ambient temperature causes the filling of the conduits (Vidal, 2000; Canler, 2001; Gabarda-oliva, 2001; Mauvieux, 2001). Due to their high percentage of fatty acids, greases constitute the favorable medium for the development of the sulfato-reductors micro-organisms (*desulfivibrio, desulfotomaculum, desulfotomanas)* which are responsible for fermentation and formation of hydrogen sulfide (H_2S) which reaction with water leads to the acid sulfuric formation (H_2SO_4) highly corrosive (Grulois et al., 1993; Vidal et al., 2000).

On the rivers, greases form an impermeable layer on the surface which prevents algae and plant's photo-synthesis and causes the asphyxiation of the organisms (Wiyada and Saovanee, 2002). The oxygen over consumption during their degradation by the micro-organisms induces the proliferation of the least oxygen demanding micro-organisms what imbalances fauna and flora (Adam et al., 2000). According to international norms, surface water should not contain more than 0.05 mg/L of fats (Vidal, 2000).

On the ground, greasy waste is not a valuable source

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of energy because of its low content of nitrogen and phosphorus and its low biodegradation in soil. In more, it can involve a certain number of disadvantages such as asphyxiating grounds (Beture-Cerec, 1996) and inhibition of germination by the fatty acids (Hélaine, 1995).

In the purification stations, lipids represent most of the organic pollution of raw waters with 35% of the total COD to treat (Canler, 2001). Their presence harm the correct operation of the treatment on the aerobic basin, by reducing the transfer of oxygen to two different levels: (i) On the level of the floc by adsorption on this one (creation of a lipidic film which reduces the transfer of oxygen dissolved between water and the floc), (ii) On the level of the surface of the basin by the constitution of a film between the wither and water (Canler, 2001). This surfacing is transformed into foam at the time of aeration (Adam et al., 2000) what causes an overflow of aerobic basin (Bridoux et al., 1995). Moreover, these greases inhibit the activity of several purifying micro-organisms (Angilidaki and Ahring, 1992; Hanaki et al., 1981) especially positive Gram bacteria (Pauli, 2001), this problem increases with the number of the double connections of the carbonaceous chain. The presence of the greases in high concentrations in muds affects the performances of dehydration what also penalize the phase of thickening (Canler, 2001). In more the presence of greases is generally associated to production of bad smells (Vidal, 2000; Eckenfelder, 2000).

To cure the harmful effects of wastewater fats, three solutions arised: (i) Incineration, (ii) Hiding in discharge and (ii) Recovery. However, the first and the third solutions are very expensive, whereas the second causes many environmental problems. The treatment upstream in the greases separators, consists in retaining difference in density part of greases, produced before their rejection in the sewers remains, is the most used. This simple physical retention of grease is done on two steps, the first receives the effluents called sluice, the second compartment which is the separator itself is used for flotation of greases on the surface, but this retention does not exceed 20%.

This work constitutes one of the first studies being interested to the biodegradation of the lipid-rich wastewater in Morocco. The goal of this study is to evaluate the effectiveness of the biodegradation of greases, and to highlight performances of micro-organisms responsible for the possible self-purification.

MATERIALS AND METHODS

Preparation of the biomass inoculated

A preculture is prepared with stocks bacteria freeze-dried in a medium LB incubated at 30°C during 24 h under agitation.

*The first type of biomass (not adapted): 5% of preculture in a medium LB incubated during 24 h at 30°C under agitation.

*Second type of biomass (adapted): 5% of preculture in medium WIKIRHAM, with 0.5% of oleic acid incubated during 24 h at 30°C under agitation.

Optimization of the minimum medium

In Erlenmeyer's of 250 ml we have prepared 100 ml of various minima mediums: WIKIRHAM medium (Wickerham and Burton, 1948), MURAY medium (Maury, 1987) and HF medium (0.065% of K_2HPO_4, 0.35% of $(NH_4)\ _2SO_4$). After sterilization, we added 5% of oleic acid and we inoculated the three mediums by 5% of preculture, after incubation during 48 h under agitation at 30°C we carried out reading of the optical density at 610 nm.

Optimization of the concentration of the oleic acid

In Erlenmeyer's of 200 ml, we prepared 50 ml of minimum medium of Wikerham, we added 0.5% of the silicone oil, after sterilization we added filtration in each Erlenmeye, a concentration following of oleic acid 0.1, 0.25, 0.5, 1, 5%, after sowing and incubation during 48 h under agitation at 30°C, we carried out the reading of the optical density at 610 nm.

Optimization of the concentration of the silicone oil

In Erlenmeyer's of 250 ml we prepared 50 ml minimum medium of Wikerham, then we added the silicone oil in each Erlenmeyer's at one of the following concentrations: 0.1, 0.2, 0.3, 0.4 and 0.5%, after sterilization we added in each Erlenmeyer 0.5% of oleic acid, and we inoculated with 5% of preculture. Erlenmeyers were then incubated at 30°C under horizontal agitation during 48 to 72 h, controls were not inoculated erlemeyers incubated under the same conditions. A reading of the optical density was carried out using a spectrophotometer at a wavelength of 610 nm.

Optimization of the standard state of the stock

In Erlenmeyers of 250 ml we prepared 50 ml of minimum medium of Wikerham, we then added 0.5% of the silicone oil, after sterilization we added 0.5% of oleic acid then we inoculated one lot of the erlemeyers by 5% of preculture and the other by the same percentage of the freeze-dried bacteria. After incubation of the Erlenmeyers at 30°C under horizontal agitation during 48 to 72 h, a reading of the optical density of the 2 mediums is carried out at 610 nm.

Synthetic waste processing

In five Erlenmeyer of 500 ml, we prepared 300 ml of a synthetic medium whose composition is: Wikerham Medium (150 ml), Acid oleic (15 ml), Oil silicone (15 ml), Detergent (3 ml), tap water (87 ml). The biomass is added only after sterilization of the mediums. Two Erlenmeyers are inoculated with 10% of preculture of the adapted biomass (v/v) and two others with 10% of preculture of the not adapted biomass. A control is prepared in parallel with the same composition without inoculation. All the Erlenmeyers are incubated at 37°C under agitation during six days. A sample is taken from Erlenmeyers once a day. A following-up of the evolution of the optical density (OD), the pH, the chemical oxygen demand (COD), the manufacturing execution systems (MES) and the lipase activity is carried out daily. The proportioning of greases is

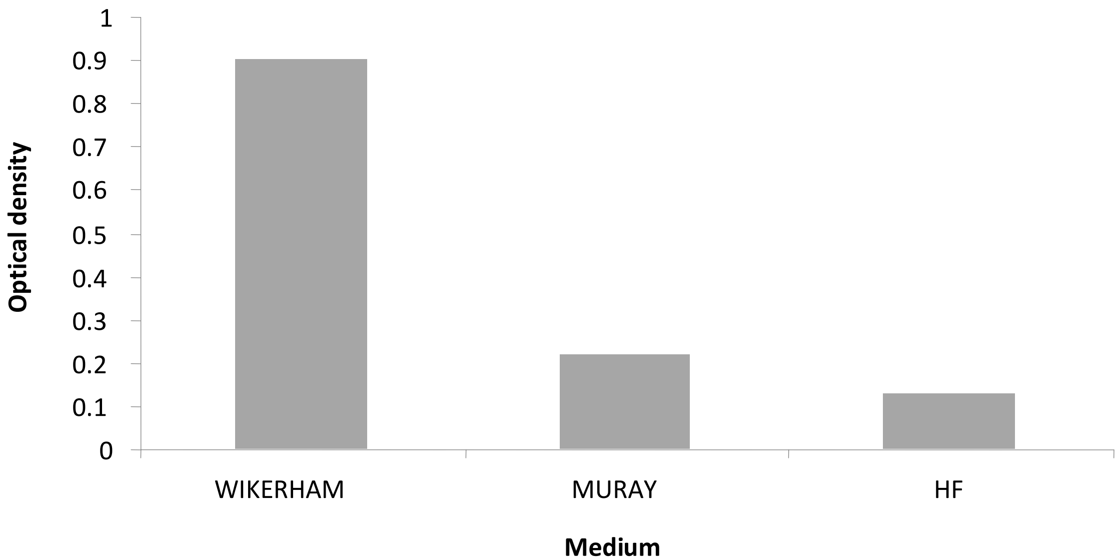

Figure 1. Bacterial growth in three types of different minimum mediums.

determined at the beginning and the end of treatment.

Natural wastewater processing

With an aim of studying the biological treatment of the greases-rich wastewater, we brought natural wastewater samples from a collective restaurant located at Fez city-Morocco, and serving nearly 200 meals a day, with much diversified menus (roasted chickens, chips, peas roasted sounds, salads… etc). Monthly, the restaurant water consumption is between 500 m^3 to 1000 m^3, the quantity of oil used daily is nearly 10 liters, whereas the quantity of detergent used daily is about 1 kg.

The effluent sample was taken from the wastewater tank collector having a volume of 5 liters. In a Erlenmeyer of 500 ml, 300 ml of effluent were incubated at 30°C under horizontal agitation during 12 days. Optical density (OD), pH, chemical oxygen demand (COD) and the content of greases in the effluent were followed.

Analytical methods

The pH was measured using a standard pH-meter CHECKER® model HI. Chemical oxygen demand (COD). The determination of the COD was carried out by the method of potassium dichromate. Biochemical oxygen demand (BOD_5) was determined by oxitop method. Total phosphorus was determined by hot digestion in acid medium. Total nitrogen was evaluated by the method of pressure-sealing. The methods used for determining DCO, the DBO_5, total Phosphorus, total nitrogen, are those described by Rodier (1996). Fat Content was determined by chloroform/methanol solvent extraction method, (Aissam, 2003). The optical density (OD) was measured using a UV/Visible spectrophotometer at 610 nm. The follow-up of OD expresses the evolution of the biomass during the waste processing. The total aerobic mesophilic flora (FMAT) abundances were determined using Agar nutritive medium (Marchal et al., 1982; Maury, 1987). After homogenization of the sample, dilutions in sterile physiological water were carried out in NaCl 0.9%. A volume of 0.1 ml of suitable dilution was spread out over Petri dishes containing the nutritive media (pH 7.0) in triplicate. The inoculated dishes were incubated at 30°C during 24 h.

The lipolytic activity was determined by the colorimetric method based on cleavage of p-nitrophenyl palmitate (p-NPP) at pH 8.0 (Lotrakul and Dharmsthi, 1997).

RESULTS

Optimization of the conditions of bacterial culture

Optimization of the minimum medium

The culture of the stock in the three minima mediums (Wikirham medium, Muray medium and medium HF) allowed us to notice a strong growth on Wikirham medium with, followed by Muray medium whereas the medium HF occupies the third place (Figure 1). We noted that our bacteria found in Wikirham medium the most favorable medium for a good growth what could be due to the alternative composition of this medium in comparison with the two others.

Optimization of the concentration in oleic acid

We notice that except for the 0.1% who knows a weak growth all the other percentages allowed a very good bacterial growth (Figure 2).

Optimization of the silicone oil concentration

We notice that according to the increase in the percentage of silicone oil, the bacterial growth increases to reach a maximum at 0.5% accepted the concentration

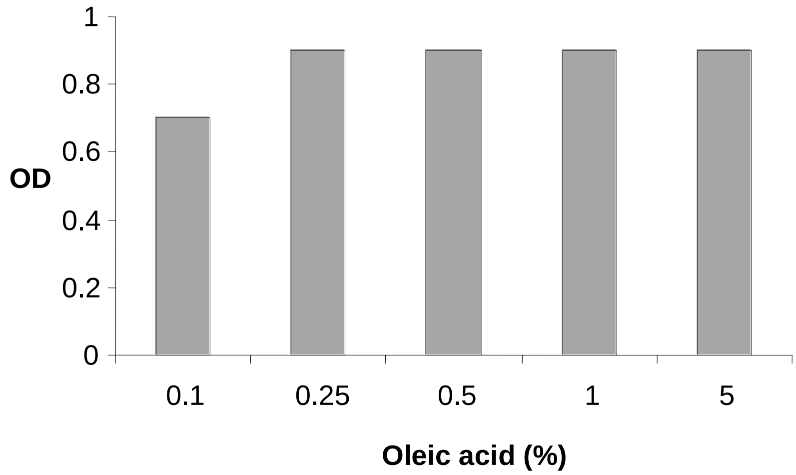

Figure 2. Bacterial growth according to the percentage in oleic acid.

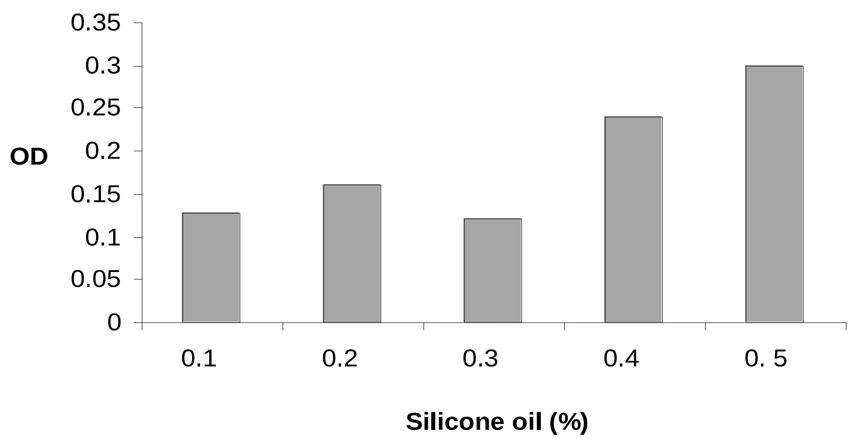

Figure 3. Bacterial growth according to the percentage out of silicone oil.

of 0.3% which knows a weak growth which should be due to an incorrect handling (Figure 3).

Biological treatment of the synthetic effluent by an adapted and not adapted biomass

Chemical oxygen demand (COD)

The results of the evolution of the COD during 6 days show that in absence of micro-organisms (control), the degradation of the organic matter expressed in COD (Figure 4) suggests that the organic compounds hardly undergo a chemical degradation. The adapted biomass showed a considerable degradation of the COD during the 6 days of treatment. The initial COD is 114 g L^{-1} and pass to 18 g L^{-1} at the 6th day what corresponds to a rate of abatement of 84.28% of the COD (Figure 4).

Optical density

The analysis of the results shows that OD increases considerably during the first 4 days as well for the consortium adapted and for not adapted, what returns to an intense activity of the bacteria, which corresponds to the exponential phase of growth. From the 4th day of the treatment, the OD remains constant during approximately 24 h (stationary phase). Then we noted a fall of OD what corresponds to the phase of decline. In the case of the adapted biomass we noted that the biomass was higher in comparison with not adapted conditions (Figure 5).

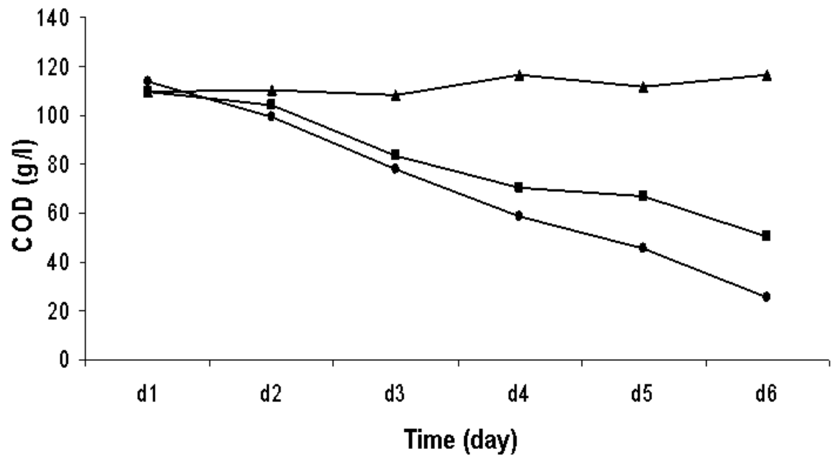

Figure 4. Temporal variation of the chemical oxygen demand (COD) for an adapted biomass (●) and a not adapted biomass (■) by comparison with the witness (▲) during the treatment of a synthetic affluent.

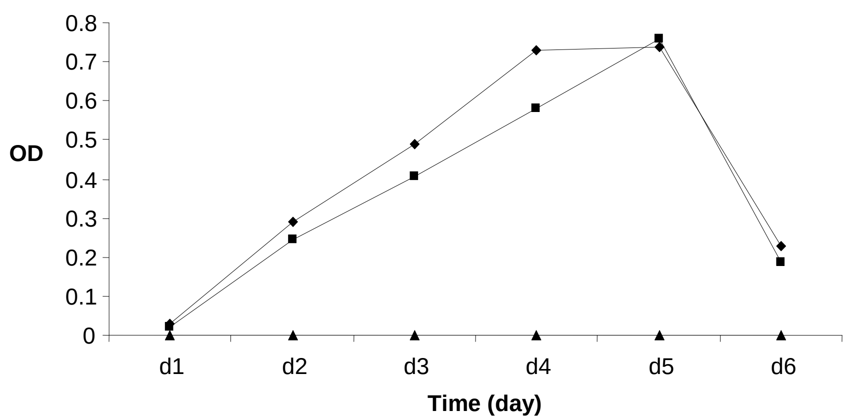

Figure 5. Temporal variation of the optical Density (OD) for an adapted biomass (●) and a not adapted biomass (■) by comparison with the witness (▲) (the sample of calibration) during the treatment of a synthetic affluent.

pH

The variation of the pH in the two types of studied cultures is illustrated in Figure 6. The results show a light reduction in the pH which does not pass under 6.84 in both cases during the first 4 days. From the 5th day, we noted a remarkable reduction in the pH. It reaches 6.28 and 6.5 in the adapted and not adapted biomass, respectively.

Content of greases

The content of greases was studied at the beginning and at the end of the kinetics. The results are presented in Figure 7. We notice that the content of greases strongly decreased in the two types of culture. The adapted biomass allows a rate of abatement of greases of about 93.3%. On the other hand, the percentage of reduction of greases at the end of the treatment by the not adapted biomass does not exceed 66.6%.

Biological treatment of the effluent of a collective restaurant

Physico-chemical and microbiological characterization of the wastewater effluent

The physicochemical and microbiological characteristics of the effluent are presented in Table 1.

This effluent is characterized by a slightly acid pH with

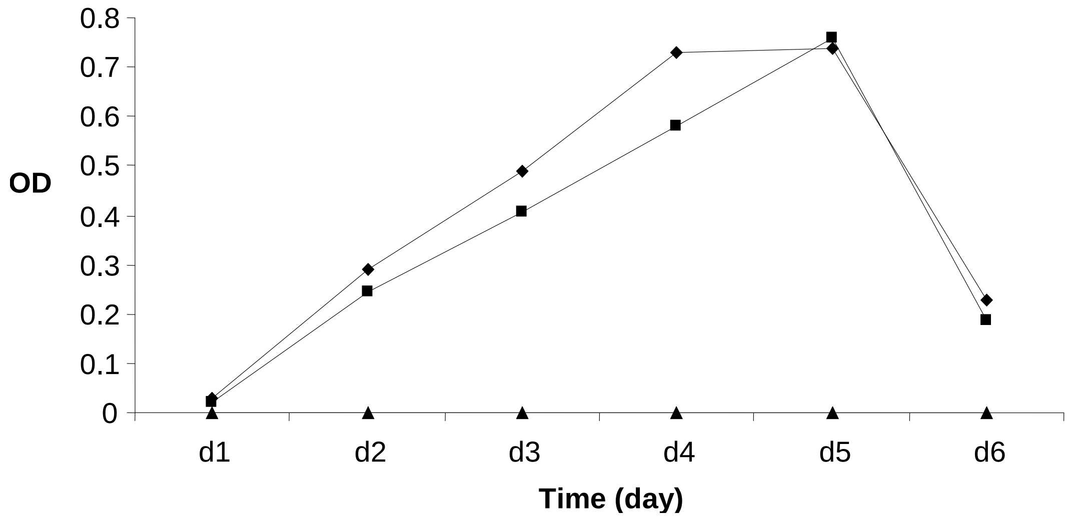

Figure 6. Temporal variation of the pH for an adapted biomass (●) and a not adapted biomass (■) by comparison with the witness (▲) during the treatment of a synthetic affluent

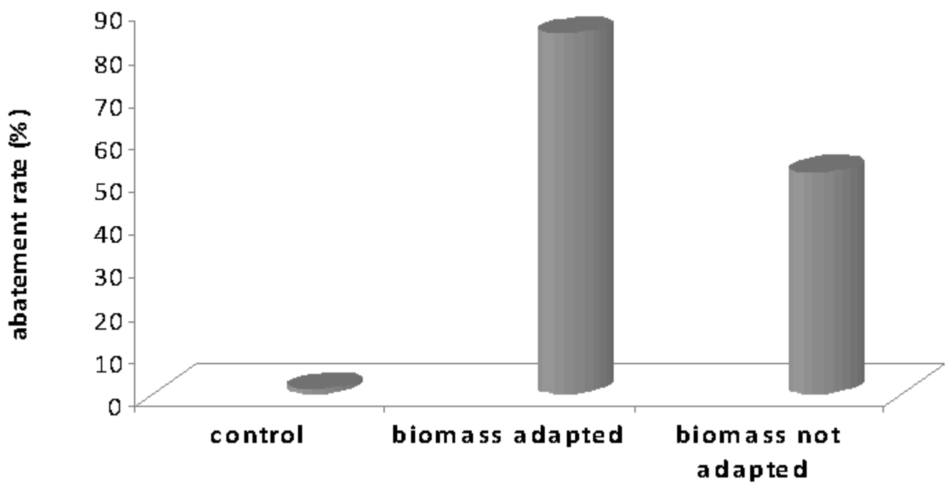

Figure 7. Rate of abatement of greases for the biomass adapted and not adapted during the treatment of a synthetic affluent.

Table 1. Evolution of the enzymatic activity during the synthetic waste processing.

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
LIPASE (LipU)	0.5	1.45	4.02	3.94	3.65	3.01

LipU: LIPASE UNIT

strong organic matter load which is about 1200 mg L^{-1} of COD. According to the standard norms, the indirect COD of the rejections of the domestic effluents should not exceed 200 mg L^{-1}. This high concentration of organic matter is due to grease of the organic waste coming from crockery. The ratio COD/BOD_5 is about 1, 4 what characterizes an effluent with dominant organic pollution which could be treated by biological ways. The total phosphorus concentration is also very high (7.4 mg L^{-1}), it largely exceeds the Moroccan standards of the indirect

Table 2. Physicochemical and microbiological characterization of the synthetic effluent.

Parameter	pH	COD (mg L^{-1})	BOD_5 (mg L^{-1})	Total phosphorus (mg L^{-1})	Total nitrogen (mg L^{-1})	FMAT (UFC ml^{-1})
Value	6.66±1	1200±10	500±20	7.4±0.5	1.05±0.1	$(3 \pm 0.1)\times 10^3$

COD: Chemical oxygen demand, BOD_5: Biochemical oxygen demand, FMAT: Total aerobic mesophilic flora, UFC: Unit formed colony.

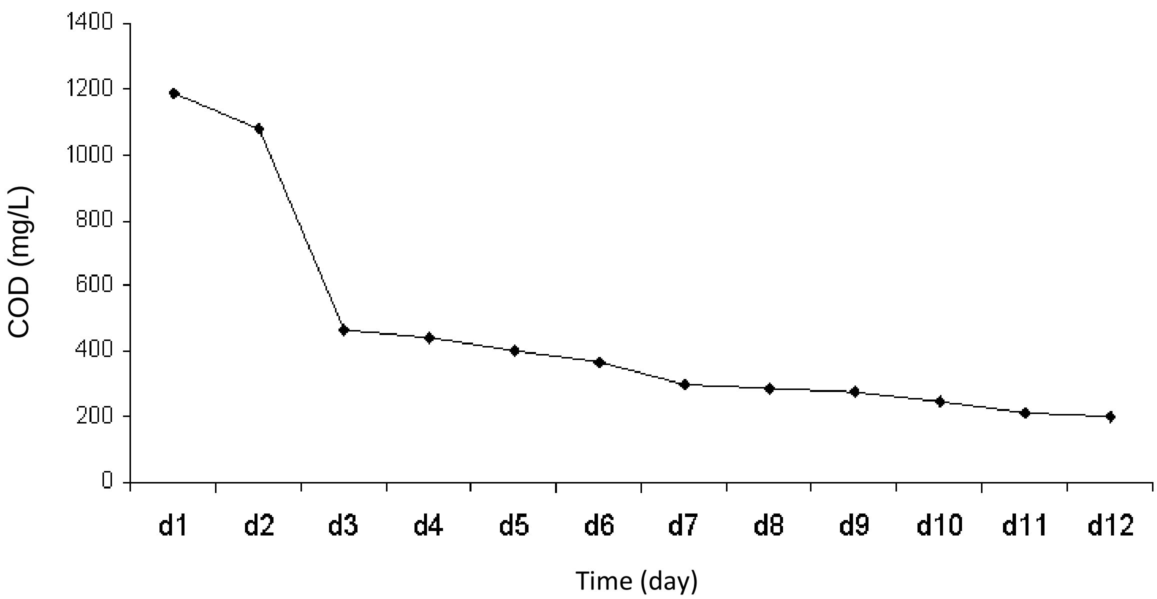

Figure 8. Temporal variation of the chemical oxygen demand (COD) during the waste processing of the restaurant.

rejections, which authorize a maximum of 1.2 mg l^{-1}. This could be explained by the use of the detergents concentrated out of phosphorus in the crockery and the cleaning of the ground. The studied effluent is a mixture of gray water coming from the kitchens and also black water resulting from the toilets what explains the high number of the FMAT obtained during the microbiological characterization (3×10^3 UFC/ml) (Table 2). According to this physicochemical and microbiological characterization, we considered it useful to study the biological treatment of this effluent by adapted bacterial consortium during 12 days. A daily follow-up of OD, pH and COD were carried out. The content of greases was evaluated before and after the treatment of the wastewater effluent.

Chemical demand for oxygen

After one day of treatment, we obtained a reduction of COD which does not exceed 9% (Figure 8). The reduction ratio of COD was amply improved the second day and was about 62.5% what would correspond to a maximum activity of the bacteria, This significant degradation could be primarily due to the performance of the bacteria which are adapted in a medium containing the oleic acid as carbon source. Then, we noted a progressive reduction in the COD from the 3^{th} day until the 11^{th} day where the rate of abatement was about 83.3%.

Optical density

We noticed that OD increases the first days gradually, what corresponds to the exponential phase of bacterial growth, then reaches a maximum at the 7^{th} day and starts to decrease until the 12^{th} day (Figure 9).

pH

We noticed that after two days of treatment, the pH decreases from 6.7 to 6.3. Then, the pH increases gradually during the following days until the day 12 when it reaches 7.7 (Figure10).

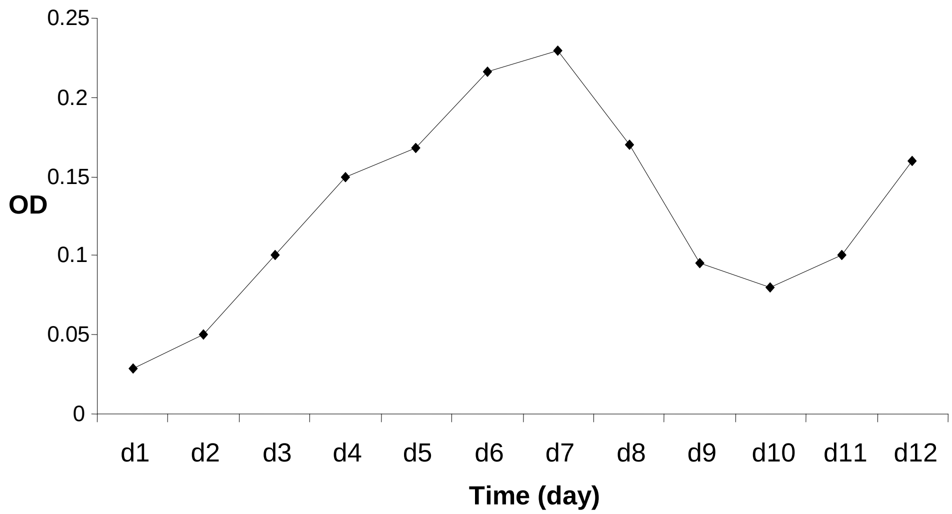

Figure 9. Temporal variation of the optical density (OD) during the waste processing of the restaurant.

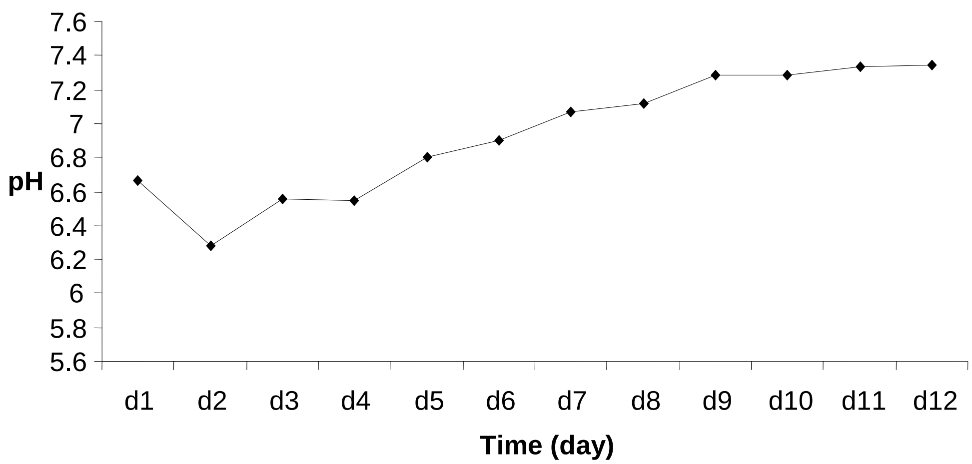

Figure 10. Temporal variation of the pH during the waste processing of the restaurant.

Content of greases

The content of greases decreased of 81% at the end of the 12 days of treatment.

DISCUSSION

Optimization of the conditions of bacterial culture

In optimization of the minimum medium, we noted that our bacteria found in Wikirham medium is the most favorable medium for a good growth that could be due to the alternative composition of this medium in comparison with the two other minima mediums. The optimization of the silicone oil concentration the progressive growth depending on the concentration of this oil is quite logical due to the effect twisted with this substance like emulsifier increasing the contact of the bacteria with the carbon source.

Biological treatment of the synthetic effluent by an adapted and not adapted biomass

During the treatment by the not adapted biomass, the percentage of reduction of the chemical oxygen demand (COD) does not exceed 51.88% what returns to the fact that the enzymatic equipment of the cells is already stimulated and ready to degrade other greases again. Moreover the number of cells is high in the case of the adapted cells, allowing better reducing of organic matter and of producing a new biomass what is in agreement with the bibliography (El Fantroussi, 2005; Thompson et al., 2005) suggesting that to have better degradations of any pollutant, the stock have to be preadapted in medium containing the pollutant so that bacteria are adapted and more competitive.

The result of variation of the pH is similar to that found by Loperana and et al (2007). This pH reduction could be explained by the metabolism of greases which induces the release of the fatty acids and the ions H^+ in the medium via the cycle of Krebs.

The adaptation of the bacteria in a preculture where the source of carbon is the oleic acid allowed the activation of the enzymatic equipment and the induction of a very effective alternative metabolism. Under these physiological conditions, the cells synthesize specific and nonspecific enzymes involving the hydrolysis of greases even to high concentrations what allows growth of the bacteria on the organic matter coming from the catabolism of greases. Indeed, some authors announced the significant role played by the metabolic induction of the micro-organisms to improve the treatment (El Fantroussi, 2005). The enzymatic activity of lipases increases significantly during the treatment (Table 1), what lets to say that these enzymes are responsible mainly for the degradation of the organic matter and especially greases.

Biological treatment of the effluent of a collective restaurant

The results of degradation organic matters are slightly higher than those found by Mondoza-Espinoza and Stephenson (1996); Wakelin and Forster (1997); Loperena et al. (2007, 2009) who compared the greasy liquid waste processing by the commercial inoculums, by an isolated consortium and by an acclimatized endogenous stock. After the 11^{th} day, the COD remains stable what would be explained by an assimilable organic matter deficiency in the medium. We can conclude that the duration of the treatment of greases could be completed in six days.

The content of greases decreased of 81% at the end of the 12 days of treatment. This significant ratio of reduction is higher in comparison with results found by Loperena and et al. (2009) in an essay of liquid waste processing using a bacterial consortium (75%) and even with the use of an inoculum at 38%. Our results could be explained by metabolic induction. Indeed, the oleic acid present in the MMW of preculture would have induced the activation of the enzymatic system responsible for the degradation of greases.

The absence of the phase of latency on the curve showed in OD is probably due to:

(i)The absence of a taking away during first hours when it took place.
(ii) The effluent was inoculated by preculture containing bacteria in exponential phase. These bacteria did not need a phase of latency to prepare the enzymes necessary to the degradation of the organic matter. Indeed, the bacteria have undergoes an adaptation in a minimum medium where the carbon source is the oleic acid. Indeed, the increase in OD during first days shows that the bacteria found in the effluent the organic matter necessary to their growth. When the medium becomes low in carbon source, the bacteria start to be degraded what explains the fall of OD during the last days of the treatment.

The fall of the pH observed in the results could be due to a degradation of greases to fatty acids. This reduction in pH should be due to the release of the ions H^+ by the cells to the reactional medium. Release is carried out during antiport transport of organic compounds of the culture medium towards the cells. The cellular activity thus appears by a fall of the pH, whereas its increase could indicate that the cells are under physiological stress. Then, the pH increases gradually during the following days until the day 12 when it reaches 7.7 (Figure 10). This could be explained by hydrolysis of the fatty acids and organic acids having groupings COO^- and OH^-. The disappearance of these compounds involves a rise in the pH, moreover; the microbial activity involves a degradation of proteins by releasing the ions NH_4^+ which causes the increase in the pH.

Conclusion

Biological treatment of greases by selected and optimized bacteria was evaluated by the study of the degradation of organic matter (COD) and greases of a synthetic medium, with approximately fat content 5%, by using an adapted and not adapted biomass.

The adapted biomass allowed a rate of abatement of 84.28 and 93.3% of the COD and greases respectively against 51.88 and 66.6% for the not adapted biomass.
The biological treatment of restaurant wastewater by adapted bacteria gave satisfactory results. Indeed, we recorded a removal of 83.3% of COD and 81% of greases.

The synthetic waste processing during 6 days and the restaurant processing during 12 days by an adapted preculture showed that the duration of the treatment of greases could be completed in six days. The whole of the results obtained also revealed the importance of our bacterial consortium in processing the liquid waste charged in greases. These bacteria resist to the toxicity of greases well and allow a good level of degradation.

For optimization, it will be interesting to study the impact of stocks immobilization on greases biodegradation. As well as metabolic induction could play a significant role in the improvement of the treatment of greases by these stocks. The addition of the inductors in the culture medium could play a significant role in microbial greases degradation.

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