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Article abstract

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RSE

SIMULTANEOUS NITRATE AND ORGANIC MATTER REMOVAL FROM A DAIRY EFFLUENT BY BIODENITRIFICATION

Abattement des nitrates et de la matière organique contenus dans un effluent laitier par biodénitrification

Ahmed HAMDANI[1, 2, 3], Mohammed MOUNTADAR[3], Omar ASSOBHEI[1]*

[1]Laboratory of Marine Biotechnology and Environment (BIOMARE), Faculty of Science, Chouaib Doukkali University, PO Box 20, El Jadida, Morocco
[2]Department of Life and Earth Sciences (SVT), Regional Center for the professions in Education and Training (CRMEF), Casa/Settat, PO Box 291, Morocco
[3]Laboratory of Water and Environment, Faculty of Science, Chouaib Doukkali University, PO Box 20, El Jadida, Morocco

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ABSTRACT

In order to study the simultaneous removal of nitrate and organic matter from a dairy effluent containing 670 mg·L^{-1} of nitrate (NO_3^--N) and 5 760 mg·L^{-1} of dissolved chemical oxygen demand (CODd), denitrification in a laboratory scale bioreactor consisting of an immersed bacterial bed colonized by an heterotrophic denitrifying flora (HDF) selected for NO_3^- reduction, COD consumption and adapted to grow on an effluent produced by a dairy industry was investigated. The obtained results indicated that at the optimal conditions of temperature (30°C), pH (7), COD/NO_3^--N ratio (5), the operation lasted 108h with total reduction of nitrate in 72h, no nitrite accumulation, and 92% of soluble COD removal in 96h. This indicates that the biodenitrification was accompanied with a high efficiency of matter organic removal as an electron donor, and thereby satisfies the applicable standards.

Key words: dairy effluent, nitrates, chemical oxygen demand, biodenitrification, heterotrophic denitrifying flora, immersed bacterial bed.

RÉSUMÉ

Dans le but d'étudier la biodénitrification d'un effluent industriel laitier à fortes concentrations en nitrates (670 mg·L^{-1} de N-NO_3^-) et en matière organique biodégradable (5 760 mg·L^{-1} de DCO soluble), un procédé de type lit bactérien immergé (fonctionnant en mode continu et colonisé par une flore hétérotrophe dénitrifiante) a été mis en œuvre au laboratoire BIOMARE de la Faculté des Sciences de l'Université Chouaib Doukkali d'El Jadida (Maroc). Lorsque le procédé fonctionne dans des conditions optimales (pH 7, température = 30 °C, rapport massique DCO/N-NO_3^- = 5), des taux d'abattement des nitrates de l'ordre de 100 % sans accumulation de nitrites, couplés à l'enlèvement de 92 % de la DCO soluble ont été obtenus au bout de 72 h et 96 h respectivement, ce qui permet de respecter les normes en vigueur pour ces deux paramètres.

Mots-clés : effluent laitier, nitrates, demande chimique en oxygène, biodénitrification, flore hétérotrophe dénitrifiante, lit bactérien immergé.

*Corresponding author:
Phone: 212 05 23 34 27 68
Email: a_hamdani04@hotmail.com

1. INTRODUCTION

Nitrate concentration in ground and wastewater has greatly increased over the past decades, thereby becoming an environmental and human health issue in both developed and developing countries (DELLA ROCCA *et al.*, 2005; UNEP, 2008; COOKE, 2014). The problems of water contamination by nitrate are many, diversified and complex: eutrophication, intoxication of the aquatic fauna, methemoglobinemia in infants; the formation of nitrosamines from reduced nitrate in the stomach has been suspected to cause cancer (WHO, 1995; GULIS *et al.*, 2001; BHARATI and SHINKAR, 2013).

To avoid adverse impact on human health and the marine environment, it would seem necessary to choose an economical treatment process capable of significantly reducing the nitrate and the nitrite concentrations to acceptable limits (OMS, 2000; CCME, 2012). Actually, nitrate elimination is generally achieved by different methods, but biological denitrification is considered the most effective, economic, environment-friendly and technically promising approach being studied for nitrate removal (BREISHA, 2010; LIU *et al.*, 2014).

The biodenitrification is a convenient way to reduce nitrate from wastewaters by microorganisms that can be either assimilatory or dissimilatory (PARK and YOO 2009; WANG and CHU, 2016). Assimilatory nitrate reduction converts NO_3^--N to ammonia nitrogen by bacterial cells for the biosynthesis (assimilation) of new cellular material when NO_3^--N is the only form of nitrogen available, but dissimilatory nitrate reduction converts NO_3^--N to nitrogen gas by heterotrophic bacteria. Heterotrophic biological denitrification discovered in the late 19th century (GARCIA, 1975) is defined as a reduction of nitrate by many species of bacteria in the presence of a carbon substrate. The nitrate is used instead of oxygen as a terminal electron acceptor. This type of denitrification has been used with success in the treatment of domestic or industrial wastewater. Therefore, the denitrifying biomass needs a source of organic carbon as an electron donor for their respiration. This carbon can initially be present in the wastewater or spring from an external bring.

Several studies of biodenitrification of effluents containing high nitrate concentrations were conducted by the use of an external carbon source such as sugar, alcohol, compost, organic acid or natural materials (POLGE DE COMBRET, 2009; HEALY *et al.*, 2012; RAMIREZ-GODINEZ *et al.*, 2015). However, the cost of the carbon source required is a drawback. When the effluent is rich in both of nitrate and organic compounds, the biodenitrification is not only the best way to remove nitrate but can also contribute to remove organic pollution. Recently, the exploitation of the biodegradable organic matter present either in effluents or in sludge resulting from the wastewater treatment plants is privileged (BERNET *et al.*, 1996; ZAYED and WINTER, 1998; GIUSTINIANOVICH *et al.*, 2015).

Concerning dairy effluents, they are generally treated by means of mechanical, physico-chemical and/or biological methods. However, the physico-chemical techniques are not suitable to this type of wastewater and are less efficient as well as those biological ones (ROTEREAU, 1969; MOLETTA and TORRIJOS, 1999; HAMDANI *et al.*, 2005). Biological treatment can be divided into three categories: aerobic (activated sludge, aerobic bioreactors, biological filters, aeration lagoons, etc.); anaerobic (anaerobic lagoons, anaerobic bioreactors, etc.) and combined aero-anaerobic process. The aerobic and anaerobic treatments are employed to remove soluble organic matter, nutrients, and other specific pollutants by biological agents with the presence or absence of oxygen as appropriate. Compared to aerobic methods, anaerobic treatment, which is conducted under special conditions, is often reported to be a favourable way to treat dairy wastewater (NADAIS *et al.*, 2010; BHARATI and SHINKAR, 2013). Biodenitrification is an important anaerobic or anoxic process performed by specific bacteria that use nitrate as an electron acceptor, take place where sufficient quantities of nitrate are present and require an organic carbon source for synthesis.

The current study is part of this context and aims to evaluate the performances of an immobilized HDF selected for NO_3^- reduction, COD consumption and perfectly adapted to grow on an effluent produced by a dairy factory that uses a great quantity of nitric acid in the washing operation and is released in the coast of El Jadida City (Morocco) without any treatment (HAMDANI, 2002). The objective is to meet the studied wastewater discharge standards required and, therefore, reduce or even eliminate negative environmental and health impacts.

2. MATERIAL AND METHODS

2.1 Effluent sample and analytical methods

Samples of effluent were taken from the drain that receives the total liquid ejected by the dairy unit. Treatment tests were carried out on an average sample of 100 L, representative of 24h of ejected effluent according to the flow (100 L per fraction of x L per y m^3).

Physico-chemical analyses of dairy effluent before and after treatment were performed according to the methods described in the French water standard Methods, AFNOR (1986). The following parameters were measured: temperature, pH,

suspended matter (SM), volatile suspended solids (VSS), total and soluble chemical oxygen demand (CODt, CODs), total and soluble biochemical oxygen demand in five days (BOD_5t, BOD_5s), nitrate (NO_3^--N), nitrite (NO_2^--N), total Kjeldahl nitrogen (TKN-N) and total phosphorus (TP-P).

Bacteriological analyses comprised the enumeration of:

i. Heterotrophic denitrifying flora: by the method of the most probable number after culture on nutrient broth (Difco) supplemented with 1 g of KNO_3 at 30°C.
ii. Anaerobic heterotrophic flora: on Mossel medium used by BLECON (1985) and incubation at 30°C in anaerobic jars under atmosphere H_2/N_2.
iii. Aerobic heterotrophic flora: after incubation at 30°C on Petri dishes containing the Tryptone-glucose Yeast Agar (Bio Mérieux).
iv. Total aerobic flora: after incubation for 24h at 37°C on Petri dishes containing the Plate count agar (Biokar, France).

For the aerobic and anaerobic heterotrophic flora and the total aerobic flora, the results were expressed in number of Colony Forming Unit (CFU) per 100 mL of sample. Only the Petri dishes containing between 30 and 300 colonies were counted.

2.2 Operating conditions

The tests were carried out by using a vertical column manufactured in glass and filled with plastic garnishing at the following conditions: the temperature of treatment was nearly 30°C, the pH initially equal to 3.5 was adjusted to 7 with NaOH (IDF, 1984; BRITZ *et al.*, 2006) and the CODs/NO_3^--N ratio was adjusted to 5 by the addition of KNO_3. The column and support used and their characteristics are listed in table 1. All tests of biological treatment were the means of three repeated determinations on samples and the effectiveness was assessed analytically by following the rate of abatement of nitrates, nitrites and soluble COD.

2.3 Reactor system and bacterial support

The experimental process presented in figure 1 consists of a transparent rectangular bioreactor operating in continuous mode and having about 20 L of total volume; the support occupied an apparent volume of 17 L with a vacuum of 15 L, which is equivalent to 88% of porosity. The characteristics of the continuous bioreactor and the support used are listed in table 1.

Dairy effluent was fed with a peristaltic pump (VELP SP 311) from the reservoir maintained at 4°C using a cooler to prevent any microbial contamination susceptible to contribute to the partial effluent treatment.

To maintain anaerobic conditions in the bioreactor and to achieve mixing, a magnetic stirrer was used. The purpose of the aerobic zone in the higher part of the system was just to remove any organic matter which was not removed in the anoxic zone (Figure 1b). This zone occupies between about 10% and 18% of the total bioreactor area, and the supply of oxygen is naturally by the direct contact with the ambient air. This affords saving in term of cost.

The recirculation of the effluent was carried out so much (to avoid the clogging of the bacterial bed, to dilute the polluting load, to regularize the hydraulic load) that sludge in excess (to increase the biological activity inside the bioreactor and consequently to improve the effectiveness of the treatment).

The biomass was fixed over a plastic ring whose characteristics are listed in table 1. It is a support laid out in bulk, but from time to time, all materials were suspended to avoid the preferential ways of water which can provide unclogging.

To protect the bioreactor sensitive to the problems of clogging, we have used a micro-sieving with an aperture of 150 µm (Figure 1) as a sample pretreatment to remove suspended particles (SM, curd, butter grains, etc.) before the biotreatment itself.

2.4 Microbial selection, adaptation and acclimation

Before treating the effluent, we let the denitrifying biomass develop on the support in the presence of a culture medium rich in organic matter and nitrates which was renewed every three days. Over time, the population of denitrifying micro-organisms grows to the surface of the support. After 21 days, the culture became visible and a biofilm appeared on the surface of the garnishing; this duration represents the necessary time to attain the stationary regime.

Because of the particular composition of the studied effluent (important biodegradable organic load, high contents of nitrates, large quantity of nitrogen and phosphorus, absence of toxic substances), our research focused on the isolation and the selection of mixed populations of heterotrophic denitrifying bacteria. The culture media used for the isolation are of two types: a basic medium (nitrated nutrient broth) and a synthetic mineral medium containing milk powder and supplemented with nitrate, phosphates and trace elements. The basic medium contained per liter of distilled water: 15 g tryptone, 5 g meat extract, 5 g NaCl and 5 g KNO_3. The synthetic culture medium composition in 1 000 mL distilled water was as follows:

Table 1. **Characteristics of bioreactor and support used in the experimental study.**
Tableau 1. ***Caractéristiques du bioréacteur et du support utilisés dans le dispositif expérimental.***

Used material	Nature	Form	Total volume (L)	Cutting[a] (10^{-3} m)	Specific surface ($m^2 \cdot m^{-3}$)	Porosity (%)
Bioreactor	Glass	Rectangular	20	L = 1 000 S = 150	–	–
Support	Plastic	Ring	–	D = 16 x 2 H = 15 ± 1	130	88

[a]L: length, S: side, H: height, D: diameter

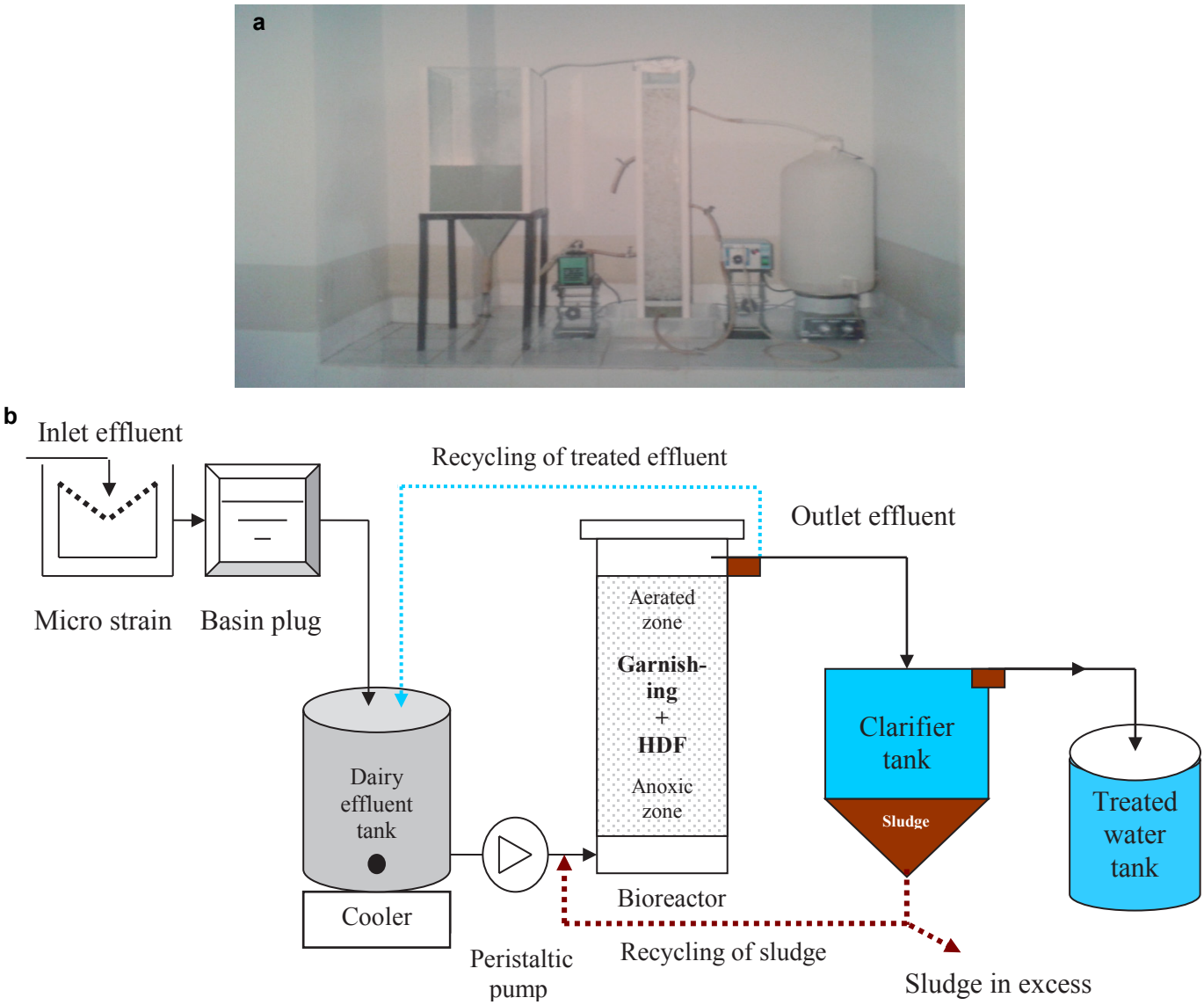

Figure 1. **Experimental system : a) photography taken in the laboratory and b) schematic representation.**
Système expérimental : a) photo prise au laboratoire et b) représentation schématique.

6 g milk powder, 7 g KNO_3, 2 mL of sterilized phosphate solution (20 g·L^{-1} KH_2PO_4 and 50 g·L^{-1} [Na_2HPO_4, $12H_2O$]) and 0.1 mL of oligo-elements solution ($CuSO_4$, $5H_2O$: 50 mg·L^{-1}; $MgSO_4$, $7H_2O$: 50 mg·L^{-1}; $MnSO_4$, H_2O: 100 mg·L^{-1}; $ZnSO_4$, H_2O: 100 mg·L^{-1}; $FeCl_3$, $6H_2O$: 100 mg·L^{-1}; $Mo_7O_{24}(NH4)_6$, $4H_2O$: 500 mg·L^{-1}). The pH was adjusted to 7 ± 0.2 for the basic and the synthetic mediums.

The bacteria were isolated from the sediments and soil from a settling tank and storage basins of dairy effluents, purified and selected in the laboratory. Our choice is justified by the compatibility between the capacities of available pollution degradation to these endogenous bacteria and the nature of the effluent to be treated since they are well adapted to this kind of environment.

The enrichment of the liquid medium consisted of an inoculation of a series of tubes containing 25 mL of culture medium at rate of 10% (v/v) with the already isolated bacteria. These tubes were closed hermetically and incubated in anaerobic jars under atmosphere H_2/N_2 for 48h at 30°C. Once the majority of nitrates were consumed, a new series of tubes was inoculated with 10% of the obtained cultures. This operation of transfer was repeated five times and the last transfer constituted the inoculum rich in denitrifying bacteria. These were adapted to develop in the dairy effluent by successive passage starting from a medium containing more culture medium (22.5 mL: 90%) than effluent (2.5 mL: 10%) to a medium where effluent is predominant. The last passage is carried out in dairy effluent only (100%).

2.5 Determination of growth rates

Microbial growths were calculated by measuring periodically (12h intervals) the absorbance at 620 nm (OD_{620}) of 2 mL samples from one liter cultures. For the HDF, an absorbance of one unit was equivalent to 52 x 10^6 CFU·mL^{-1}.

Some elements contained in the effluent interfere with absorbance at 620 nm. Consequently, we carried out direct enumeration on solid culture medium and we established a correlation between the number of bacteria contained per millilitre of the sample and the OD_{620}. Moreover, the presence of phosphates causes the formation of precipitates which can lead to an over-estimation of the biomass determined by measuring the dry weight. To remove these chemical precipitates, we have referred the methodology used by PACCARD (1995), which consist of a solubilisation of the sample to be analyzed for 60 min at pH 2 under agitation, a centrifugation at 4 000 rpm during 30 min, a resumption of the bottom precipitate in a 9‰ NaCl solution and lastly a second centrifugation. The dry weight is determined by steaming to a constant weight at 105°C; MVS is measured by calcining of the dried sample in the oven at 550°C.

2.6. Calculation of removal percentages

Removal percentage of nitrate, nitrite and COD expressed in percentage was defined by using the following formula:

$$\text{Efficiency}(\%) = \frac{(Ci - Cf)}{Ci} \times 100 \quad (1)$$

where *Ci* and *Cf* are the concentrations before and after treatment respectively.

3. RESULTS AND DISCUSSION

3.1 Quality of dairy effluent before treatment

The mean composition of the influent given in table 2 reveals that the recorded values are characteristic of the wastewater produced by a dairy factory, with the exception of very high nitrate concentration (675 mg·L^{-1}) from the intensive use of nitric acid for cleaning in the milk processing plants, which is also at the origin of an acid pH (3.5). The contents of dissolved COD and BOD_5 were, respectively, 5 865 and 2 810 mg·L^{-1} approaches those obtained in India (RAJESH BANU *et al.*, 2008), in France (CASTILLO DE CAMPINS, 2005) and in Algeria (YAHI and HAMI, 2008). However, these values are higher compared to the results founded by the Institute of Dairy Research in New Zealand (DONKIN, 1997) and lower than those dicted by CRISTIAN (2010).

The CODs/CODt, BOD_5s/BOD_5t, COD/BOD_5 and BOD_5/TKN-N/TP-P ratios are close to 86.5%, 85.9%, 2/1 and to 100/6/1.2 respectively; which indicates that the organic components in the studied effluent are highly degradable by a biological way without any addition of nutritive complements; especially, that we have already demonstrated in another study (HAMDANI *et al.*, 2005) that its physical-chemical treatability is incomplete and largely ineffective when it comes to removing nitrogen and organic matter, whose polluting potential is high.

The nitrates contained in dairy effluent are not only a pollutant load but they also contribute to the eutrophication of the receiving environment wherein it is rejected (Bay of El Jadida City, Morocco). If we add to this the fact that the studied effluent quality largely exceeds the limits imposed by Moroccan standards (MINISTÈRE DE L'ENVIRONNEMENT DU MAROC, 2002), a specific treatment of this effluent becomes an imperative before its discharge into the marine environment.

Table 2. Composition of dairy effluent submitted to biological treatment. Mean values are for 36 measurements (accident data was not taken into account).

Tableau 2. Composition de l'effluent laitier soumis au traitement biologique. Les valeurs moyennes sont pour 36 mesures (les données des accidents n'ont pas été considérées).

Analysed parameters	Mean ± SEM ($mg \cdot L^{-1}$, except pH)	Moroccan standards	Canadian water quality guidelines (seawater)[a]	
			Short-term exposure	Long-term exposure
pH	3.5 ± 0.52	6.5-8.5	–	–
SM	620 ± 159	50	–	–
CODt	6 780 ± 934	500	–	–
CODs	5 865 ± 803	–	–	–
BOD_5t	3 270 ± 813	100	–	–
BOD_5s	2 810 ± 699	–	–	–
TKN-N	170 ± 33	30	–	–
NO_3^- -N	675 ± 128	–	339	45
NO_2^- -N	0.6 ± 0.13	–	–	–
TP-P	35 ± 7.15	10	–	–

[a]These recommendations concern the marine aquatic life protection against the direct toxic effects of nitrate and do not take into account the indirect effects of eutrophication.

3.2 Treatment of the dairy effluent by biodenitrification

3.2.1 Evolution of the temperature and the pH during biodenitrification

Although denitrification is possible between 5 and 75°C because of the abundance and diversity of denitrifying germs, temperature nevertheless constitutes a major variable that must be taken into consideration since it affects the rate of biological reaction (MARTIN, 1979). Figure 2 shows that the temperature evolution over time at the outlet of the bioreactor is relatively stable around a value of 27°C with a fluctuation of ±1°C. This result indicates that the temperature recorded is suitable for a biological activity.

As illustrated in figure 2, at the beginning of the treatment, the pH initially adjusted to 7 underwent a slight acidification in the latency phase, and thereafter, it gradually increased during the phase of denitrification until reaching approximately a value of 9 at the end of biodenitrifying activities. The increase in pH can be explained by a consumption of ions H^+ during the dissimilatrice reduction of nitrates to gaseous nitrogen and to H_2O according to the following reaction, which is in reality a series of 4 oxidation-reduction reactions leading to the appearance of intermediate products: NO_2^-, NO and N_2O (POWERS, 2005).

$$4NO_3^- + 5(CH_2O) + 4H^+ \xrightarrow{\text{Specific enzymes}} 2N_2 + 5CO_2 + 7H_2O$$

The founded values of temperature and pH are close to the optimal temperature and pH for a better biological denitrification: PACCARD (1995) reported that the optimum of biodenitrification ranges between 30 and 35°C, and BOLLAG (1973) indicated that the best elimination of nitrates was obtained for a temperature of 30°C. Some authors mentioned that the optimal pH required for the biodenitrification is generally neutral or slightly alkaline pH value (DAWSON and MURPHY 1972, DODD and BONE 1975). The study of SALEM *et al.* (2007) indicates that the pH range preferred by heterotrophic denitrifiers is between 5.9 and 7.9; although SHEN *et al.* (2009) reported that the optimum pH range in an anoxic/oxic membrane bioreactor with over 99.9% of nitrate removal and without nitrite accumulation was between 7.5 and 8.5.

Nevertheless, these values will have to be considered with reserve because the optimal pH varies for each bacterial species and depends on other parameters: temperature, dissolved oxygen, etc.

3.2.2 Evolution of the concentrations of nitrates and nitrites

Figure 3 shows a total elimination of nitrates present in the dairy effluent after 72h of hydraulic retention time. For an applied load of 0.345 kg NO_3^--$N \cdot m^{-3}$ of unventilated material per day, the eliminated load was 0.344 kg NO_3^--$N \cdot m^{-3} \cdot d^{-1}$, so more than 99.9% of elimination. After 72h, the speed of consumption of nitrates is 16 $mg \cdot L^{-1} \cdot h^{-1}$. These results are attributed to the process using a support that offers a great surface of the adhesion of the bacteria as well as an easy circulation of air (TROIS *et al.*, 2010) and confirm those mentioned by DAHAB and LEE (1988), which indicated that biological denitrification is highly selective for the removal of nitrate ions and the efficiency of this process is very high, reaching nearly 100%, which has not been achieved by any other method available for the reduction of nitrate ions. In the same vein, AKUNNA *et al.* (1994) demonstrated that the

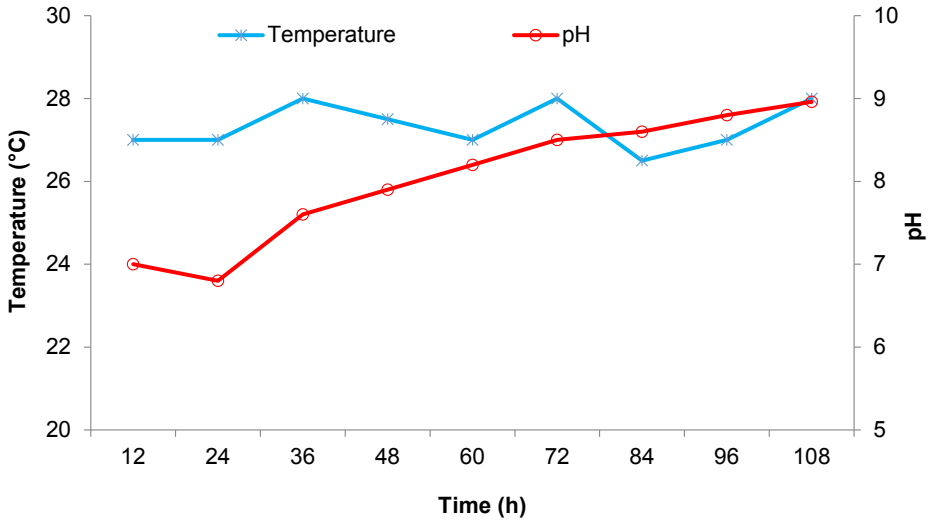

Figure 2. **Evolution over time of the temperature and pH under standard conditions (*T*: 30°C, pH: 7, CODs/NO_3^--N: 5, initial biomass X_0: 1.3 x 10^7 CFU·mL^{-1}).**
Évolution de la température et du pH en fonction du temps en conditions standards (T : 30 °C, pH : 7, DCOs/N-NO_3^-: 5, biomasse initiale X_0 : 1,3 x 10^7 UFC·mL^{-1}).

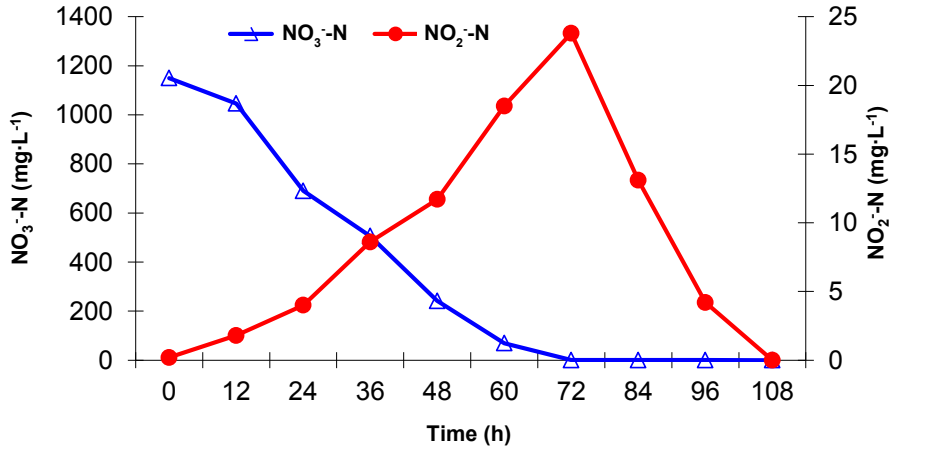

Figure 3. **Changes over time of nitrate and nitrite concentrations under standard conditions (*T*: 30°C, pH: 7, CODs/NO_3^--N: 5, initial biomass X_0: 1.3 x 10^7 CFU·mL^{-1}).**
Variation des teneurs en nitrates et en nitrites en fonction du temps en conditions standards (T : 30 °C, pH : 7, DCOs/N-NO_3^- : 5, biomasse initiale X_0 : 1,3 x 10^7 UFC·mL^{-1}).

ratio of COD/NO_3^--N from 4 to 5 and a high and sufficient concentration of nitrates are in favour of a good denitrifying activity.

During the reaction of denitrification, a transient nitrite accumulation was detected achieving maximum value of 23 mg·L^{-1} after 72h, and tended to disappear afterward until total disappearance (Figure 3). However, at 72h, the pH in the bioreactor was comprised between 8 and 9 (Figure 2), this is in favour of a great activity nitrite reductase: GLASS and SILVERSTEIN (1999) reported that the maintenance of the medium pH to a value beyond 8 is favourable for the denitritation (reduction of nitrites) even to very high concentrations of about 2 g·L^{-1}. According to the same author, the speed of denitritation decreases to pH 7; whereas, the pH 6 involves a total inhibition of the reduction of nitrites with weak concentration (30 mg·L^{-1}). These results agree with other studies which showed that in acid pH, the nitrite reductase would be inhibited (KOSKINEN and KEENEY, 1982; MYCIELSKI, 1983). On the other hand, PACCARD (1995) deferred that a pH near of 7 permits to a mixed culture to completely reduce nitrites present in the culture medium. In addition, when the denitrification is total, the absence of nitrites in the treated effluent is logical, this is in agreement

with the results obtained by TORRIJOS *et al*. (1993), which announced that the complete elimination of nitrates is a guarantee of the absence of the accumulation of nitrites in the treatment by biodenitrification.

3.2.3 Abatement of the soluble COD, growth and characteristics of the biomass

Figure 4 shows that the biodenitrification of the total dairy effluent is accompanied by the oxidation of 81% of the CODs after 72h for an applied load of 1.74 kg CODs·m^{-3} of unventilated material per day. This rate of COD abatement reaches 91.7% within 96h of treatment, especially with installing an aerobic portion in the superior level of the bioreactor, implying that the aerobic bacteria, acting in the presence of oxygen, contribute to the oxidation of residual COD from the anoxic zone.

The important reduction of the soluble COD could be explained by the richness of the effluent of easily biodegradable organic matter (COD/BOD_5 = 2.1 and CODs/CODt = 0.86), which constitutes a source of assimilable carbon for the heterotrophic denitrifying biomass. The results indicated that the organic matter contained in the dairy effluent served as electron donors for total denitrification and that there is no need to add any external carbon contributions, which results in reducing the cost of the carbonaceous substrate in providing.

The stoichiometric ratio of heterotrophic denitrification is 4 mg of CODs, which are necessary to reduce 1 mg of NO_3^--N to N_2 must be corrected to (5:1). This value remains higher than that indicated by MOLETTA and TORRIJOS (1999), who showed that 1 mg of nitrate eliminated request 2.86 mg of COD reduced, and inferior to that mentioned by ZAYED and WINTER (1998): 6 g of COD oxidized per 1 g nitrate nitrogen denitrified.

We believe that the achieved results satisfy the Moroccan standards and thus to better protect humans, fauna and flora living on receiving marine environment from undesirable and serious environmental and health problems caused by the untreated effluent (malodor, eutrophication, infectious diseases, toxicity, etc.). BHARATI and SHINKAR (2013) reported that higher concentration of dairy effluents, including organic matter and nitrogen, is toxic to humans, fish and algae.

The determination of the total count of bacteria (Figure 4) indicates that the decrease of biodegradable carbon content was accompanied with the increase of the absorbance at 620 nm reflecting the increase of the cells numbers (absorbance is proportional to cell density). The number of bacteria seems to be linear from 12h to 60h and the bacteria reached the maximum cell density after 84h (the highest cell numbers coincide with the highest absorbance). The stationary phase was characterized by 0.5 x 10^8 cells·mL^{-1} in the supernatant (Thoma cell counts and enumerations).

We evaluated the amount of adsorbed biomass on support: about 7.5 x 10^9 cells·g^{-1}, the average cell dry weight being 10^{10} g·$cell^{-1}$. Consequently, this result shows that these bacteria adhere to surfaces as plastic support, grow and remove nitrate under experimental working conditions. However, we suppose that cells densities were probably underestimated because there is a part of the microorganism which is detached from support in the bioreactor, particularly in the aerobic zone.

The analysis of biomass composition revealed that this consists of a mixture of total colony counts: total anaerobic heterotrophic flora, denitrifying heterotrophic flora, total aerobic flora and total aerobic heterotrophic flora with proportions of 44%, 26%, 16% and 14% respectively. This heterogeneity can be allotted to the fact that it is very difficult to work under sterile conditions and to maintain a homogeneous culture in the bacterial bed. The interesting thing is the dominance of the anaerobic heterotrophic flora which develops easily depending on the biodegradable organic matter contained in the effluent. The majority of the anaerobic heterotrophic bacteria (in the same sense as aerobic ones), which benefit from their development at the expense of assimilable carbon substrate, are able to reduce nitrate or nitrite to N_2 (DERONZIER *et al*., 2001; CRAFT *et al*., 2002). The denitrifying heterotrophic flora, characterised by its aptitude to use nitrate and other mineral oxygenated compound of nitrogen as a final acceptor of electrons, is clearly abundant: these two types of flora represent 70%; this explains the existence of an important denitrifying activity in the bacterial bed. The composition of the biomass implemented remains stable over time because the experimental device carried out is simple and the operating conditions applied to the bioreactor remain almost unchanged, according to time until reaching balance (applied load, C/N ratio, pH, temperature).

We believe that the aerobic flora grows in the peripheral surface of the support which is supposed to be the soluble organic matter-oxidizers, but the interior considered an anoxic zone was covered by a biofilm developed by the propagation of the anaerobic heterotrophs and denitrifiers flora.

4. CONCLUSION

In this study, the quantitative characterization showed an increase of analysed parameters which exceed the Moroccan standards of wastewaters.

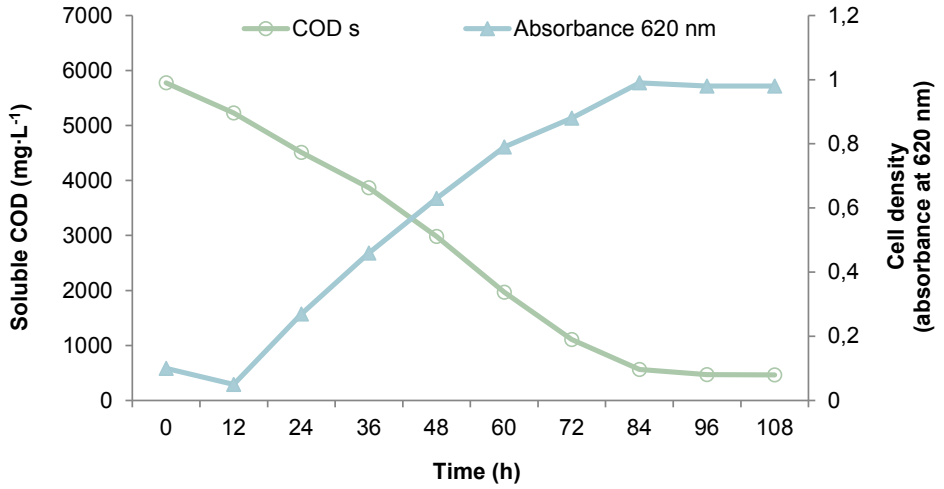

Figure 4. **CODs consumption and bacterial growth over time under standard conditions (*T*: 30°C, pH: 7, CODs/NO_3^--N: 5, initial biomass X_0: 1.3 x 10^7 CFU·mL^{-1}).**
Abattement de la DCOs et croissance bactérienne en conditions standards (T : 30 °C, pH : 7, DCOs/N-NO_3^- : 5, biomasse initiale X_0 : 1,3 x 10^7 UFC·mL^{-1}).

Concerning the treatment, when the experimented system operated at the optimum conditions of temperature (30°C), pH (7) and COD/NO_3^--N ratio (5), it appears capable of reliably achieving successfully simultaneous nitrate and soluble organic matter removal. After 21 days, the start-up of operation, denitrifying reactor was fed with total dairy effluent. The load of 0.344 kg of NO_3^--N per m^3 unventilated material per day was reached after 72h. This induced a total consumption of nitrate coupled with 91.7% reduction of dissolved COD from the dairy effluent and no NO_2^--N was detected.

The process developed in this study is attractive for many reasons: high efficiency for removing the polluting load, less space required, low energy consumption (no aeration needed) and economic operating cost. These advantages make this system a relatively less expensive alternative to treat high nitrate and organic matter containing effluents coming from food-processing and agricultural sectors. This encouraged us to implement it on a pilot scale to better test its feasibility and robustness in real conditions: a STEP named RALBI (anaerobic immersed bacterial bed reactor) was constructed on-site located in the Faculty of Science-El Jadida and co-financed by the Hassan II Academy of Science and Technology and the Chouaib Doukkal University (ASSOBHEI, 2009).

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