

DENITRIFICATION OF WASTEWATER TREATMENT PLANT EFFLUENT USING ANAEROBIC BACTERIAL BED REACTOR IMMERSSED: OPERATING PERFORMANCE

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Abstract:

In this study, a heterotrophic denitrification was designed for domestic wastewater treatment with unexpected water flows at different loading rates. Benefited from excellent removal ability COD, shorten operating time and lower maintenance cost. During the time of operation (six months), injection of nitrate was made in the influent RALBI 1 while the RALBI 2 was fed with sewage without addition of nitrate. The COD concentration in the influent of the two reactors was the same. This two reactors (RALBI1 and RALBI2) were fed with real wastewater for 172 days at 6 h HRT, the average COD removal were 71.55% and 54.82% for RALBI1 and RALBI2 respectively. The results showed that RALBI1 has good removal performance for COD compared with RALBI2. This could be due the injected nitrate in RALBI1 whose effect has further stimulated the activity of denitrifying bacteria.

Keywords: Heterotrophic denitrification; domestic wastewater; treatment; nitrate; organic matter

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INTRODUCTION

Today's world major challenge is the awareness of preserving our living environment from an increasingly growing global warming, causing ecological imbalances with rise in temperatures, flood, drought and especially pollution of vital resources for human survival like air, water and environment. The shortage of water resources has become an exacerbating issue around the world as a result of accelerated industrialization and urban growth as well as changed climatic conditions (Hao *et al.*, 2013; Wu *et al.*, 2012). These effects are more pronounced in poor countries where the availability of water resources, is significantly seen due to severe water supply imbalance caused by the uneven distribution of rainfall, increased in irrigation water demands and high temperatures is the case in Countries in the Middle East and North of Africa (Sánchez *et al.*, 2007). In fact, the pressure on available resources amplifies water degradation (Goh *et al.*, 2015; WWAP, 2012), due to an excessive consumption and high polluting production, generating huge amounts of polluted water that are subsequently released into the environment without any treatment. Among various measures of addressing this challenging problem is the wastewater reclamation (Goh *et al.*, 2015; Yi *et al.*, 2011, Hao *et al.*, 2013). Hence the urgent need of implementing effective treatment of domestic wastewater in small towns, for example Water Framework Directives 2000/60/EC (European Parliament and Council, 2000) in developed countries.

The reclaimed water from municipal wastewater treatment plant (WWTP) can serve for many purposes, such as urban scenic and landscape greening, urban lake and river water replenishment, car washing, construction site water use, urban road sprinkling, industrial circulating cooling water use, irrigation water use (Tahri *et al.*, 2010; Angelakis *et al.*, 1999), and the recharge of groundwater (Llanos *et al.*, 2015; Hao *et al.*, 2013, Hao *et al.*, 2013). In general, the quality of treated and reused water is affected by the available treatment processes whereas many pollutants could not be thoroughly removed (Hao *et al.*, 2013). A treatment process suitable to treat or valorize and recycle this wastewater must be found (Cristóvão *et al.*, 2015). In Morocco wastewater systems are often a huge problem especially in small community who use dirty water without treatment (Tahri *et al.* 2010). The wastewater treatment technologies such as ultrafiltration, reverse osmosis, ion exchange, electro dialysis, and membrane filtration are efficient (Koide & Satta, 2004). However, these technologies have high operational and maintenance costs. However, biological treatment is the most common process used to treat organics-containing wastewaters (Cristóvão *et al.*, 2015; Christensen *et al.*, 2009). Moreover, in some cases the cost per inhabitant

for activated sludge process or even wastewater disinfection to remove germs in a aim reuse this water are relatively expensive and not always within the reach of developing countries like Morocco.

Thus, it was necessary to develop reliable technologies based on an anaerobic system developed lately. This system has been successfully applied for the treatment of wastewater from low resistance, such as domestic wastewater in Morocco, which could be an alternative to conventional aerobic processes. These processes are frequently used since they are more economic and environmentally friendly, using optimized natural pathways to actually destroy pollution, not only transform it into another form (Cristóvão *et al.*, 2015; Gotvajn *et al.*, 2005). As a result, the effective denitrification of wastewater treatment plant effluent is of fundamental importance. Biological denitrification is normally conducted by facultative anaerobes which are in essential need for some food and energy sources which are organic or inorganic (Cast & Flora, 1998; Rijn *et al.*, 2006; Moukhliissi *et al.*, 2014). This process is an efficient, where heterotrophic bacteria convert $\text{NO}_3\text{-N}$ to N_2 . There are studies concentrating on autotrophic denitrification in treatment of wastewater. However, very few real application of heterotrophic denitrification process was found, especially in the developing countries, probably because this process requires sufficient external carbon sources for the nitrate removal (Her & Huang, 1995; Park *et al.*, 2015).

Moreover, almost no study has been carried out on reduction of organic matter by the heterotrophic denitrification process. There is no detailed information on the utilization of this process for the treatment of domestic wastewater at a relatively short retention time and under the natural temperature and pH conditions. Reactor performance is not the unique parameter to be considered in the choice of a heterotrophic denitrification system. Capital, operation and management costs are important factors which determine the actual feasibility of a treatment system. The objectives of this study were : (1) to investigate the feasibility of heterotrophic denitrification in domestic wastewater treatment; (2) to find out the effect of nitrate concentration, organic loading rate (OLR), on organic matter (COD) removal efficiency.

Material and methods

Wastewater characterization

The power of the device studied was wastewater from the sewer system of the Faculty of Sciences of El Jadida. The wastewater is fed by a pump to the basin will be freed which pretreatment of a high proportion of mineral or organic suspended solids and sludge by

settling, before being pumped to the settler. The settler in turn ensures the removal of solid contaminants in suspension, which still persist in the wastewater by settling. At these two stages, the wastewater is free of the major part of the pollution in suspension and ready to be fed to the bioreactors. All these stages are equipped with a by Controlled electromechanical control panel to provide a continuous and automatic system for the treatment of wastewater. **Figure 1** shows the reactor setup and the influent water characteristics are shown in **Table 1**.

The reactor was operated at ambient temperature, no heat exchange was introduced. The reactor was operated in the autumn/winter time where the temperature falls down in winter reaching about 15°C. The average influent wastewater temperature during the experiment is shown in **Table 1**.

Pilot-scale experimental set-up

Figure 1 shows the schematic diagram of wastewater treatment plant. The RALBI consisted of bioreactor in plexiglass, cylindrical with a diameter of 1,6m and a height of 2m. the RALBI was designed to investigate

and evaluate the performance of bioreactor for heterotrophic denitrification of domestic wastewater. As shown in **Fig. 1**, the pilot plant bioreactor wastewater treatment consists of a tank (1), decanter (3) and two cylindrical (4) vessel having an internal volume of 4 m³. Using a peristaltic pump, wastewater was pumped from the influent tank into tank and into two bioreactors through its bottom inlet, and flew through the biofilm on PVC filler, and then flew out from the upper of the reactor. A inside bioreactors (RALBI1 and 2) immersed rings as a medium to support biofilm formation. PVC regular shapes with a size distribution of between 4 and 5 cm, with a double wall, were used as the carrier in the anaerobic reactors to support the growth of microorganisms. The surface area total of particles in reactors are 2.76 cm² including 1.57 cm² swollen and 1.19 cm² integrated surface. Denitrifying bacteria through the metabolic role, degrade the organic matter in water. This bioreactor was found to be effective for reducing the COD and NO₃ serving as the electron donor for heterotrophic denitrification in wastewater treatment.

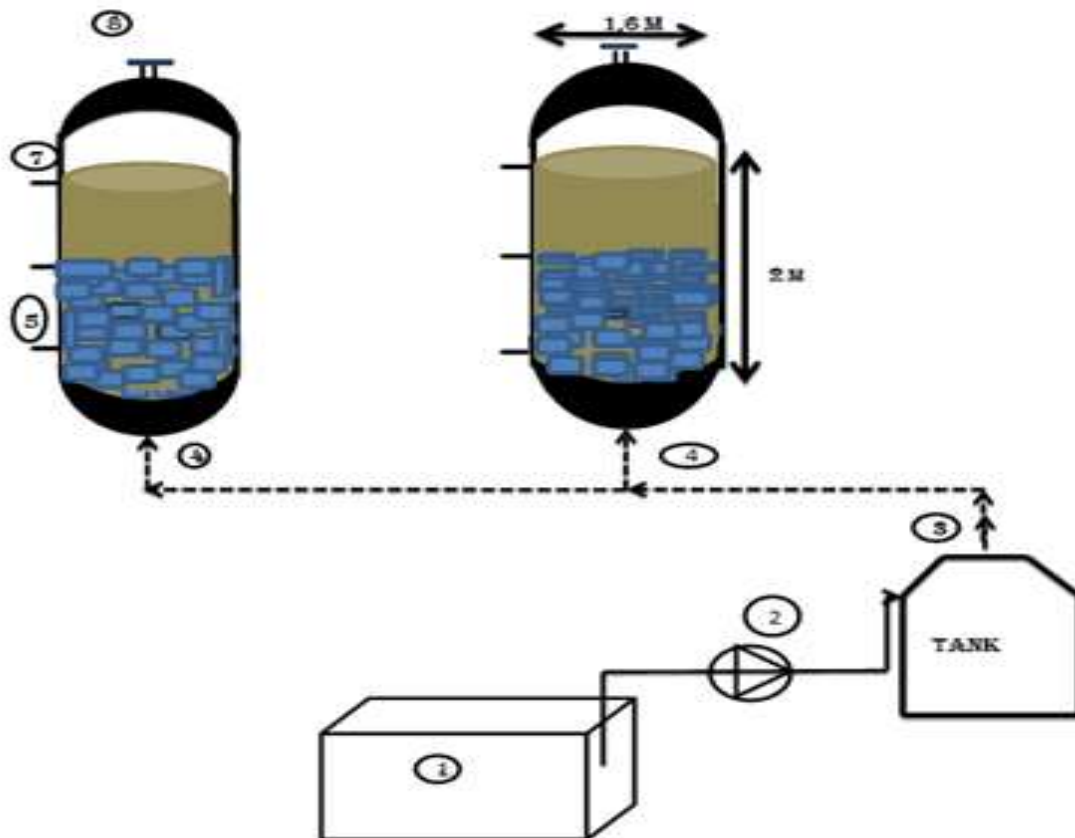


Fig. 1 Schematic diagram of RALBI: tank (1), peristaltic pump (2), decanter (3), up flow(4-5), Sampling point(wastewater, 6), Reactor outlet (effluent, 7) and Gas outlet(8).

Table 1. Wastewater characterization

Parameters	Unit	Min.	Average	Max.
Temperature	°C	15.9	21.8	26.5
pH-value	-	7.50	8.05	8.61
Chemical oxygen demand (COD)	mg L ⁻¹	246.87	464.29	940.00
Biological oxygen demand (BOD)	mg L ⁻¹	111.48	190.66	266.23
Total Suspended Solids (TSS)	mg L ⁻¹	0.3	0.483	0.66
Total Kjeldhal Nitrogen (TKN)	mg L ⁻¹	70.33	110.48	159.6
Ammonia Concentration (NH ₄ -N)	mg L ⁻¹	22.7	69.69	148
Fecal Coliforms	UFC/100ml	1.3 * 10 ⁹	3.6 * 10 ⁹	6.03 * 10 ⁹
Fecal Streptococci	UFC/100ml	5 * 10 ⁶	4.01 * 10 ⁶	13.8 * 10 ⁶

Experimental procedure

The biological denitrification was assessed by studying nitrate reduction and its influence on the reactor performance. The RALBI were operated at room temperatures for 172 days fed with real wastewater. During the time of operation (six months), injection of nitrate was made in the influent RALBI 1 while the RALBI 2 was fed with sewage without addition of nitrate. Samples were collected every day from the influent, Effluent RALBI1 and Effluent RALBI2, and the samples were filtered through a membrane filter before analysis.

Analytical methods

COD was digested using a Digester at 150°C for 120 min and determined by titration using ferrous ammonium sulphate solution according to the APHA (APHA, 2005). TSS was measured by oven drying method with a membrane filter (pore diameter 0.45 µm) and a lab drying oven. pH was measured under magnetic stirring with a Cyberscan 510 pH meter from Eutech Instruments equipped with a combined Ag/AgCl/KCl 4M glass electrode and with a platinum temperature probe. The removal of nitrate and COD was calculated using the following formula:

$$C (\%) = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

where C₀ is the initial concentration of nitrate or COD (mg L⁻¹); C_t (mg L⁻¹) is the remaining concentration of nitrate or COD at time t.

Statistical analysis

The obtained data were submitted to analysis of variance (ANOVA) and compared by Duncan's test (p < 0.05) using the software package SPSS (version 17.0).

RESULTS AND DISCUSSION

Daily fluctuations of influent

The daily averaged concentrations were showed in **Table 2**, and the results of sampling were compared for different days of the week by a paired t-test (critical p < 0.05). The characteristics of the influent are typical for primarily treated domestic wastewater (Korkusuz *et al.*, 2007; Caselles-Osorio *et al.*, 2007b). The COD concentration influent is significantly different during the operating period. It is less than 500 mg L⁻¹ during the months September < August < and October, but it is higher during the months of November < January and December. This fluctuation was caused by frequentation of students at the faculty. These findings indicate that wastewater loading can fluctuate depending on the presence of students at the faculty, with variations lasting at least several days to as long as a week. However, occasional changes in COD concentration of wastewater are studied to test the performance of the reactor according to fluctuations. The COD concentration was higher during December; however, it was not higher during August and September.

COD removal

In this study, we used two anaerobic reactors, with injection of a nitrate solution in a reactor, in order to treat the organic and nitrogenous feed of wastewater. According to the main components of sewage and the various components of the anaerobic degradation pathway, the process was carried out in two anaerobic biodegradation phase. The first reactor is fed of a nitrate solution and nitrate present in the wastewater at the entrance of the reactor (RALBI1) it promotes denitrification wastewater into contact with denitrifying bacteria in the future. This is not the case for the second reactor (RALBI2), the denitrification is done with only the nitrate present in the wastewater of the faculty.

Table 2. Monthly concentration changes of influent

	August	September	October	November	December	January
Temperature (C)	25.2-26.5	25.1-25.3	24.9	21.3-23.2	19.5-20.6	16.3-19.6
pH	7.5-8.21	7.58-8.32	7.77-8.61	8.06-8.5	7.86-8.44	7.75-8.42
COD	388.00-	368.63-	488.96-	735.01- 458.00	940.00 - 456.66	780.00 - 451.00
(mg L ⁻¹)	248.59	246.87	279.67			

Inflow and effluent COD concentrations and the removal rate of COD

The performance of RALBI (1 and 2) for COD removal was given in **Fig. 2**. The two reactors RALBI 1 and 2 were fed with real domestic wastewater of science faculty. During experimental period (from August to January), COD concentration influent was varied between 246 and 940 mg L⁻¹. The average values of COD influent remained relatively stable and low during the first quarter (August, September and October) and fluctuations in the second quarter (November, December and January) which is observed in the COD concentration of influent were caused by continuous frequentation of students at the faculty. From Day 1 to Day 79 (**Fig. 2**), the COD concentration was low than period from Day 84 to Day 172. The concentration average COD increased from 317.84 mg L⁻¹ to 622.28 mg L⁻¹ during the two periods respectively.

On Day 1, the COD concentration decreased from 388 mg L⁻¹ to 88.25 mg L⁻¹ in Effluent RALBI1, and 126 mg L⁻¹ in Effluent RALBI2. The removal efficiencies in

COD were 77.25% and 67.53% on the first day, respectively for RALBI1 and RALBI2. The COD removal efficiencies in RALBI 1 and RALBI2 were maintained stably during the first quarter. The COD concentration in RALBI1 during August and September account 94.25 mg L⁻¹ and 84.45 mg L⁻¹ on average, respectively. However, COD average concentration in RALBI2 is slightly higher than RALBI1 during the same month (121.89 mg L⁻¹ and 125 mg L⁻¹ respectively).

Afterwards, the COD concentration showed a gradually increasing until Day 172, and varied in the range from 107.54 to 174.40 mg L⁻¹ in RALBI1 and between 155.29 and 313.57mg L⁻¹ in RALBI2. However, COD removal efficiency in RALBI1 and RALBI2 exhibited an increase during the second quarter. It was probably due to the fact that The COD concentration influent increased during this period. Throughout the entire experimental period, The COD removal efficiencies in RALBI 1 was higher than RALBI2, suggesting that RALBI1 owned a remarkable removal ability and retaining COD in treating wastewater.

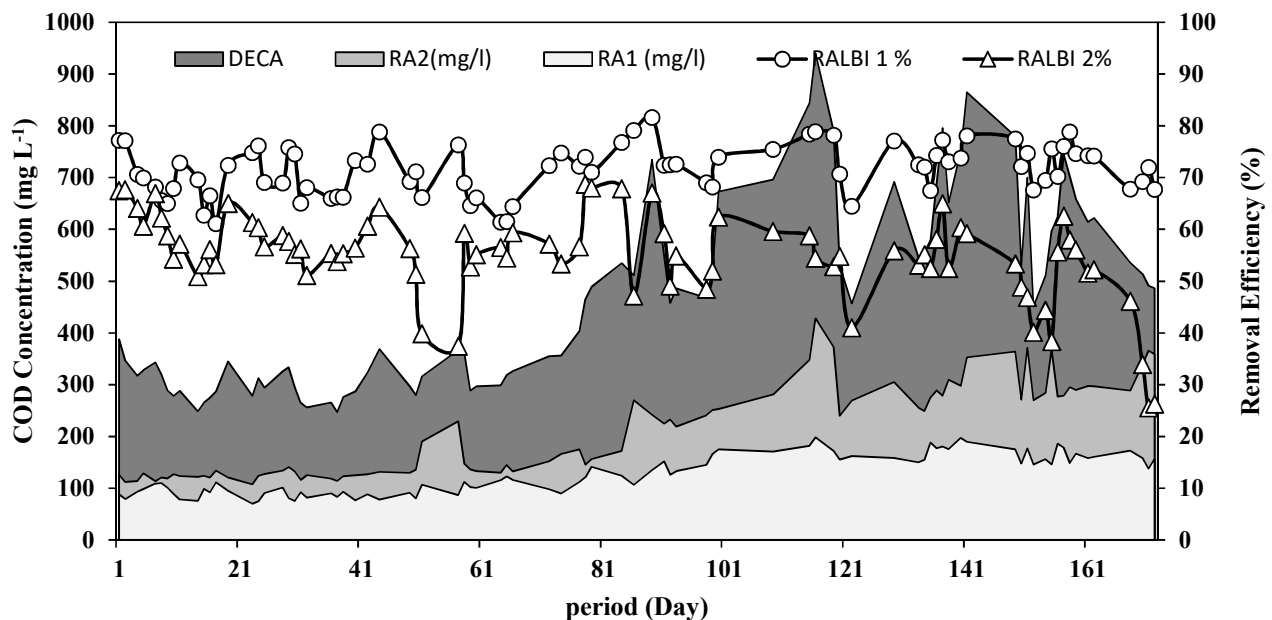


Fig. 2 COD removal performance of RALBI 1 and RALBI 2.

Effect of OLR on The COD and COD reduction efficiency

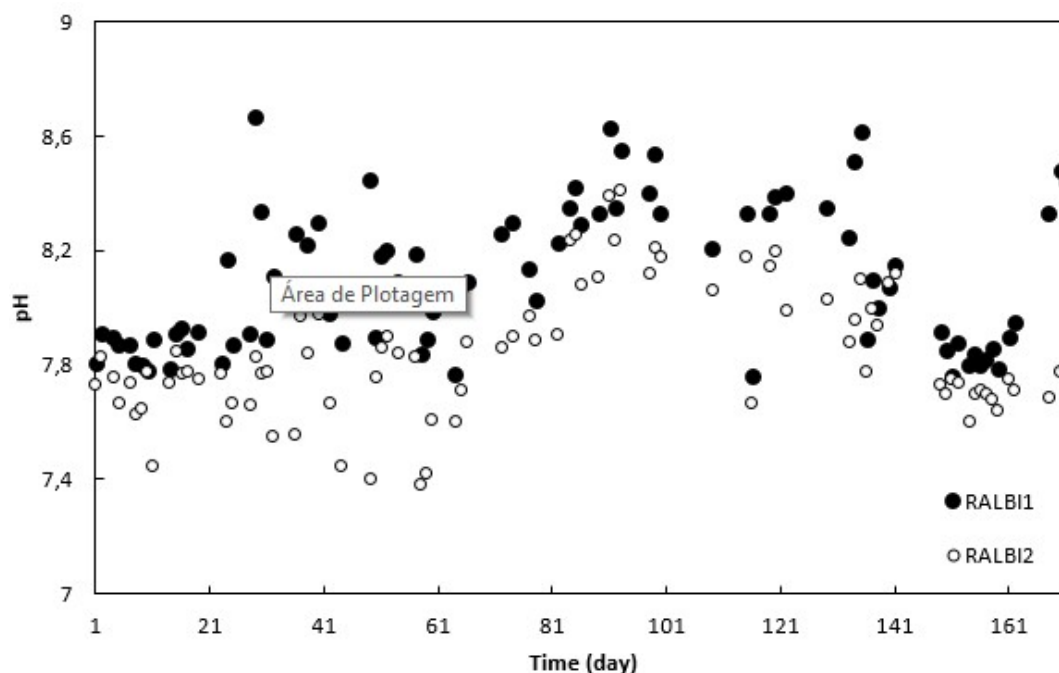
The composition of raw wastewater varied throughout the study (**Table 2**) according to frequentation of students at the faculty. The study covered the comparison of two bioreactors (RALBI 1 and RALBI 2) for treating simulated domestic wastewater performance at different OLRs (HRTs). Hydraulic retention time (HRT) is an important parameter for all bio-reactor to treat wastewater. In this study, The HRT was set at 6h in RALBI 1 and RLBI2 to study the effect of nitrate

concentration increasing on the system performance during 172 days.

The COD removal rate by RALBI2 varied between 25.24% and 68.56% when the influent concentration COD was lower at 500 mg L⁻¹. However, when COD concentration initial was higher at 500 mg L⁻¹, COD removal varied between 33.92% and 67.85%. There were no significant differences in the COD removal by RALBI 2 in contrast to change in the organic loading ratio. As it can be seen from **Table 3**, when the wastewater is treated by RALBI 1, The effluent COD concentration varied between 61.16 % and 78.84 % at

Table 3. Fluctuation of organic loads and degree of bioreactor performance.

	COD < 500 mg L ⁻¹			COD > 500 mg L ⁻¹		
	Min.	Max.	average	Min.	Max.	average
%COD RALBI ₍₁₎	61.16	78.84	69.68	67.51	81.63	74.38
%COD RALBI ₍₂₎	25.24	68.73	55.07	33.92	67.85	54.43

**Fig. 3** Trend of pH in RALBI 1 and 2.

the initial COD lower to 500 mg L⁻¹. The Increase in COD concentration influent (> 500mg L⁻¹) resulted in good COD removal (varied between 67.51% and 81.63%) which is higher than that of RALBI2, which is probably due to NO₃ concentration. As it can be seen in **Fig. 2**, the effluent COD removal efficiencies by RALBI2 were slightly different, but the efficiency by RALBI1 showed significant difference and 81.63 % COD removal was obtained at COD influent > 500mg L⁻¹.

These results indicate the slowest microbial activity with lowest concentration of nitrate, whereas nitrate concentration has a considerable effect on anaerobic degradation of organic substance by denitrifiants microorganisms. In The denitrification process, the nitrate is reduced to nitrogenous oxides by isolates that use nitrate instead of oxygen as electron acceptors and organic matter as carbon and energy source (Moukhliissi *et al.*, 2014). However, The removal of COD was very important in RALBI1 than RALBI2, because the nitrate concentration is superior in RALBI1 than RALBI2. Along with a similar study with a combined system used by Torres and Foresti (2001) for the treatment of domestic wastewater containing 570 mg actual COD L⁻¹ with an average temperature of 21°C was 6 h HRT for time UASB and SBR changed cycle between 24, 12, 6 and 4 h. The total return of the COD was approximately 91% and anaerobic granular sludge UASB inoculated

contribution was 72%. The RALBI used in our study showed a satisfactory processing performance relative to the volume Treaty (4m³) and without combination of the system.

Trend of pH in RALBI 1 and 2

The pH value in RALBI 1 and RALBI2 was illustrated in **Fig. 3**. Since Day 1, the pH of influent varied between 7.50–8.61, but the pH in RALBI 1 effluent (7.76–8.67) was slightly higher than RALBI 2 effluent. The pH of the reactor was maintained at 7 during the beginning of each cycle. As the cycle progressed, in RALBI1, the pH was observed to increase to a range as high as 7.76–8.67. This increase in pH in RALBI1 confirmed the nitrate utilization as suggested in other reports (Tomohide *et al.*, 2001). However, the pH variation in RALBI2 was lower than RALBI1. This difference was due to nitrate concentration.

The NO₃-N removal

Figures 4a–b present the variations of average nitrate concentration and nitrate removal under different conditions during the RALBI treatment process. During the time of operation, injection of nitrate was made in the influent RALBI 1 while RALBI 2 was made with sewage without addition of nitrate, whereas the COD concentration in the influent of the two reactors was the

same. The experiment was conducted for at least 6 month. It was observed that the addition of nitrate in RALBI1 greatly affected the removal of nitrate. It was observed that the concentration of NO₃-N in the effluent of RALBI1 decreased from 29.4 to 3.32 and 38.52 to 4.2 mg L⁻¹ but the removal of nitrate in the reactor 2 (RALBI2) decreased from 10 to 5.14 and from 10.30 to 1.30 mg L⁻¹ during the first two days of operation of the reactor respectively when COD was between 200 and 500 mg L⁻¹ (during the months August, September and October).

Similarly, the concentration of nitrate (NO₃-N) in the effluent decreased from 29.5 to 2.29 and from 17.8 to 11.15 mg L⁻¹ by RALBI1 and 2, respectively, and when The COD of the influent was 940 mg L⁻¹ (6 of December). In general, the denitrification efficiency increased with the addition of nitrate in RALBI1 and when organic load concentration was high for the two reactors. The average NO₃ concentration varies between 24.65 NO₃ mg L⁻¹ and 38.52 NO₃ mg L⁻¹ in the influent during the operation time of RALBI1. Throughout the experimental period, RALBI 1 was able to remove large amounts of nitrate with a maximum removal rate of 95.78%. However, the NO₃ -N removal efficiency remained stable between 77.63% and 95.78%, and the average removal efficiency and effluent concentration during the four months (August, September, November and October) were respectively 88.05% and 3.49 mg L⁻¹. However, the nitrate removal efficiency during the months of December and January varied between 50.91% and 92.24%.

The same trend was found when the influent nitrate concentration which varied between 7.77 and 17.8 mg L⁻¹ at RALBI2, as shown in **Fig. 4b**, yet the removal rate was relatively lower than that at RALBI1. Approximately 57.77 % of NO₃ was removed by RALBI2 with average residual concentration of 4.68 mg NO₃ L⁻¹, suggesting that RALBI1 has a better ability for reducing the nitrate with a relatively higher concentration. The denitrification process in wastewater treatment plant depends on several factors: biological kinetics and physicochemical parameters as pH, temperature, viscosity, substrate concentrations, dissolved oxygen concentration, low COD/N ratio and high nitrite concentration (Beline *et al.*, 1999; Alinsafi *et al.*, 2008; Adouani *et al.*, 2015). The study of the RALBI treating domestic wastewater performance was carried out in different temperatures (**Table 1**) of the six months of operation (hot months: August, September, October and the cold months: November, December and January).

Temperature is a key parameter for the denitrification (Carrera *et al.*, 2003). The experiment started in the hottest days (August, September, November and October), and the high removal efficiency in the beginning proved that the hot weather

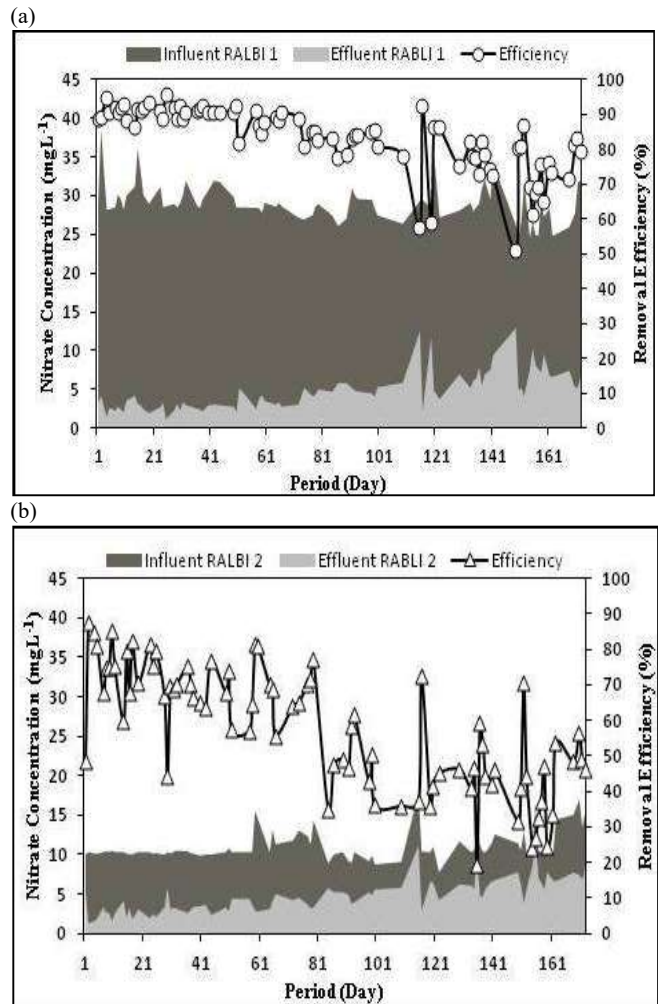


Fig. 4 Nitrate removal performance of RALBI.

(17.9–26.5 °C) was favorable to the denitrification reactor no matter what concentration of nitrates is. Generally, denitrification is performed by mesophilic micro-organisms with an optimal temperature around 30 °C (Bremmer, 1997; Spérandio, 1998). However, when temperature was decreased from 16.2 to 18.3 °C (during days of December and January), and at the removal efficiency dropped greatly. Weili Zhou *et al.* (2011) has shown that temperature is an important factor affecting the denitrification efficiency. According to Adouani *et al.*, 2015, the denitrification activity is very low below 5 °C, and it increases linearly until a maximum around 25–30 °C and thereafter decreases to a minimum at around 65 °C, where growth stops due to enzyme denaturation (Richardson *et al.*, 2009). However, some denitrifying micro-organisms are able to denitrify at temperatures ranging from 5–65 °C (Bremmer, 1997).

To study the nitrate effect on the removal of the COD. **Figure 5** show the correlation between the initial concentration of NO₃-N and the reduced COD. Although the treatment performance under different

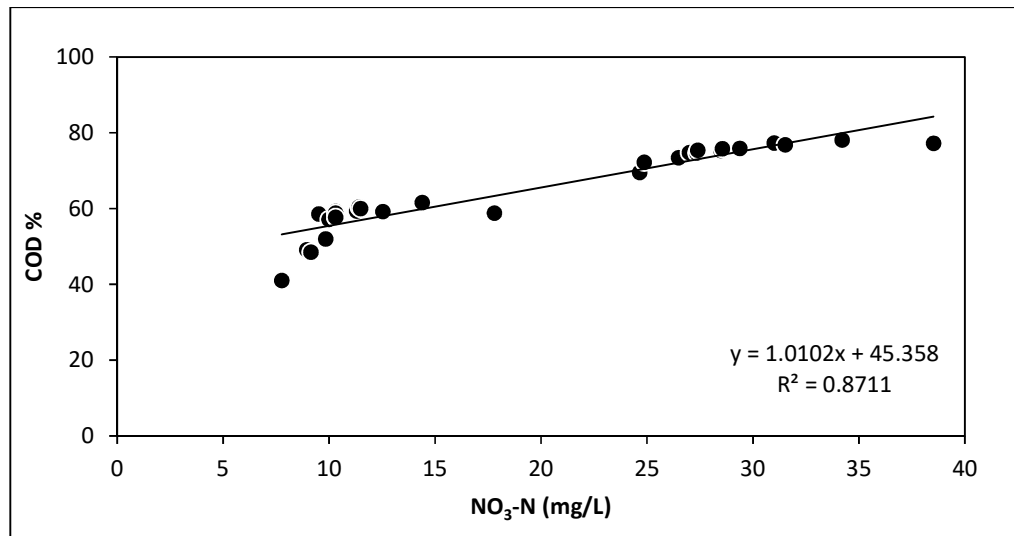


Fig. 5 Correlation between initial concentration of NO₃-N and reduced COD.

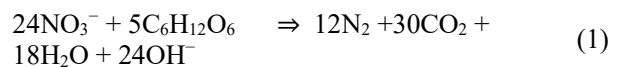
running conditions throughout the entire experiment was quite different, a linear correlation was found for nitrate and DCO removed. In order to reduce 90% of COD, 44.19 mg/L of nitrate had to be consumed. It is indicated that the raw water with a low concentration of nitrate may reduce the COD removal and therefore reduce the reactor performance. Furthermore, when nitrate concentration is lower than 20 mg N/L, the removal of COD is between 41 and 58% whereas it exceeds 70% when the nitrate concentration is greater than 20 mg N/L.

By comparing the two reactors (RALBI1 and RALBI2) studied (with no nitrates) in the second quarter, the effect of the temperature drop since November, is expected resulted in lower reduction rates of COD and reaction times are longer. This observation is not the case of RALBI1, unlike RALBI2 which was low. A study by Kayranli and Ugurlu (2011) found that the yields of the COD were 80% to HRT 11H at 15 ° C, which were much higher than COD removal efficiencies in RALBI1 (72.36%) at HTR 6H. In addition, we found that the denitrification of effluents produced had a level of alkalinity in the range of 8.1 and 7.83 respectively in RALBI1 and RALBI2. This means that the temperature did not have a significant effect on the bacterial activity when processing within RALBI1.

However, the effect of the denitrification may have caused influence on reduction in the COD so that the COD removal rate in RALBI1 increased 2%. In this phase, the denitrification process in the reactor compartment and increasing the elimination of organic matter could be due to the injected nitrate whose effect has further stimulated the activity of the denitrifying bacteria during this period of drop in temperature within RALBI1. Furthermore, in RALBI2 (not nitrate), the denitrification process is performed with only the nitrate present in the influent, so the limit respiration nitrate the

degradation of organic matter especially against a load fluctuation potential wastewater. Even after the change of organic load (COD) showed similar trends to that of the first quarter with a performances improvement of 70.45 to 72.36 % and from 58.68 to 54.38 by RALBI1 and RALBI2 respectively.

Hence the hypothesis of the influence of nitrate on the degradation intensity of loads on the heterotrophic bacteria activity in the two reactors was confirmed. This bacterial activity determines the performance nitrogen removal (NO₃) of 82.24 % and 56.72 %, respectively in RALBI1 and RALBI2 at end of the study. The denitrification had several positive effects on the overall reactor performance, and this was due to the following factors: the use of an oxidisable electron donor in the form of the COD feed and increased system pH the reactors inlet, thus improving environmental conditions (Barber *et al.*, 2000; Ghaniyari-Benis *et al.*, 2010). The denitrification process was carried out by using nitrate by facultative denitrification in the absence of free molecular oxygen to degrade exogenous carbon and obtain energy for cellular activity and synthesis. During anaerobic respiration, nitrate and nitrite are reduced through several pathways. The overall biochemical reaction for denitrification with a carbon source (Roy, 1999; Ghaniyari-Benis *et al.*, 2010) can be expressed by the following theoretical equation:



As a result of this equation and according to Ghaniyari-Benis *et al.* (2010), COD removal efficiency increases in the presence of nitrate. In this study, pH variations during nitrate reduction ranged between 7.38 and 8.41 for RALBI2 and then 7.76 and 8.67 RALBI1. Therefore, these values by comparison two reactors

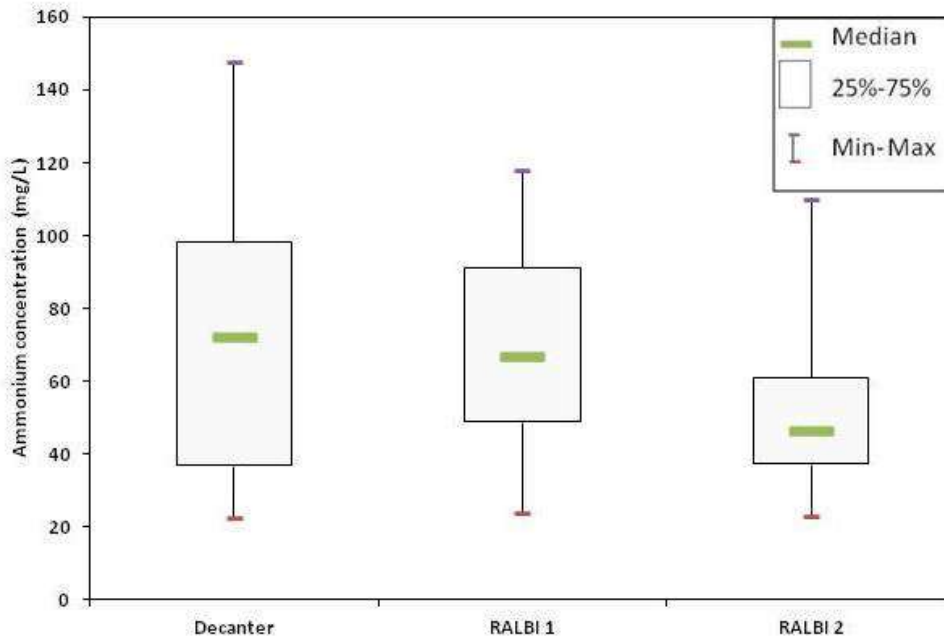
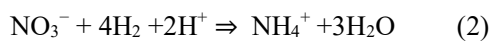


Fig. 6 Box-and-whisker plots for NH_4^+ values at decanter and two reactors (RALBI).

(with where without nitrate addition), the average pH was increased by about 0,22. During the months of fluctuating COD affluent, the addition of nitrate can to further promotes anaerobic respiration denitrifying, leading to increased production of ammonium dissimilative according to Ghaniyari-Benis. But in **Fig. 6** the effluent from RALBI1, facing the degradation of organic load increasingly growing since November, had fairly important content in ammonium. These reactions are usually in anaerobic condition according:



This concentration of ammonium relatively significant for the 6 hours of HRT and the treaty volume (4 m^3), would be due to a mixture of sanitary water (black water) and the nitrate source (NH_4NO_3) used in our study. compared to other studies (Ghaniyari-Benisa *et al.*, 2009) or synthetic wastewater denitrification produced a concentration of 824 mg L^{-1} ammonium from nitrate in the respiration in a multistage biofilter. Other previous reported works have demonstrated that anaerobic digestion can be inhibited by ammonium concentrations in the range of $1500\text{--}3000 \text{ mg/L}$ at pH values above 7.4 and by ammonium concentrations above 3000 mg L^{-1} , regardless of the pH (Calli *et al.*, 2005). At the end of our study, the maximum concentration of ammonium observed was 213.9 mg L^{-1} ; 196.81 mg L^{-1} , respectively RALBI1 and RALBI2, which therefore had no negative effect on the reactor performance.

CONCLUSION

The application of RALBI for the treatment of the Faculty of domestic wastewater to different climatic conditions may be a promising technique and cost effective alternative practice because of its relatively low investment and operating costs. The RALBI technology has been tested in the provision of some pollutants containing wastewater. In addition, the study of two RALBI reactors, with an addition of nitrate and one without it, is to measure the significant potential in removing organic matter and nitrogen domestic wastewater. Likewise, the efficiency of the two reactors biofilm attached in treatment is comparable while the power intensity is greater for RALBI1. The influence of the addition of nitrate concentration 4 g/L (RALBI1) had performance on the COD removal at the end of the study 52.49% and 57% for RALBI2.

However, the reduction of nitrogen (NO_3) was higher in loan RALBI 82.24 % against 56.72% in RALBI2 effluents. The intensity of denitrification was stimulated by the concentration of nitrate in each reactor, which leads to increased production of NH_4 in the reactor. In order to reuse the purified water RALBI, it would be a real problem if the water were to be released into the environment. Any tertiary treatment will be following the RALBI reactors to refine the quality of effluent nitrogen (NH_4) to meet the Moroccan discharge standard (direct), but also avoids the receptor asphyxia environments. In addition, all the studied units (RALBI) showed a considerable potential to eliminate fecal bacteria Faculty of wastewater, providing good

microbiological quality of treated water, which could be a future study in the agricultural irrigation.

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REFERENCES

- Christensen, A., Gurol, M.D., Garoma, T. (2009) Treatment of persistent organic compounds by integrated advanced oxidation processes and sequential batch reactor, *Water Res.*, **43** 3910–3921.
- Gotvajn, A.Z., Zagorc-Koncan, J. (2005) Combination of Fenton and biological oxidation for treatment of heavily polluted fermentation waste broth, *Acta Chim. Slov.*, **52** 131–137.
- Sánchez, A.S., Subiela, V.J. (2007) Analysis of the water, energy, environmental and socioeconomic reality in selected Mediterranean countries (Cyprus, Turkey, Egypt, Jordan and Morocco), *Desalination* **203**, 62–74;
- Angelakis, A.N., Marecos Do Monte, M.H.F., Bontoux, L., Asano, T. (1999) The status of wastewater reuse practice in the Mediterranean basin: need for guidelines. *Water Res.* **33** (10), 2201–2217;
- Calli, B., Mertoglu, B., Inanc, B., Yenigun, O. (2005) Effects of high free ammonia concentrations on the performances of anaerobic bioreactors, *Process. Biochem.*, **40**, 1285–1292.
- Kayranli, B., Ugurlu, A. (2011) Effects of temperature and biomass concentration on the performance of anaerobic sequencing batch reactor treating low strength wastewater, *Desalination* **278**, 77–83;
- Aboussabiq, E.E., Yettefti, I.K., Amine, J., Socias-Viciana M.M., Assobhei, O. (2014) Coagulation - flocculation of domestic wastewater by Moroccan clays: removal of organic matter and suspended solids, *Carp. J. Earth Environm. Sci.*, **9**(1), 33–42.
- Hao, R.X., Li, S.M., Li, J.B., Zhang, Q.K., Liu, F. (2013) Water quality assessment for wastewater reclamation using principal component analysis. *J. Environ. Inf.* **21** (1), 45–54.
- Her, J.J. & Huang, J.S. (1995) Influences of carbon source and C/N ratio on nitrate/nitrite denitrification and carbon breakthrough. *Biores. Technol.*, **54**, 45–51.
- Javier Llanos, Salvador Cotillas, Pablo Cañizares, Manuel A. Rodrigo. (2015) Conductive diamond sono-electrochemical disinfection (CDS) for municipal wastewater reclamation. *Ultras. Sonochem.*, **22**, 493–498
- Park, J.-H., Kim, S.-H., Delaune, R.D., Cho, J.-S., Heo, J.-S., Ok, Y.S., Seo, D.-C. (2015) Enhancement of nitrate removal in constructed wetlands utilizing a combined autotrophic and heterotrophic denitrification technology for treating hydroponic wastewater containing high nitrate and low organic carbon concentrations. *Agric. Water Manag.*, **162** 1–14;
- Jena, J., Kumar, R., Saifuddin, M., Dixit, A., Das, T. (2016) Anoxic-aerobic SBR system for nitrate, phosphate and COD removal from high-strength wastewater and diversity study of microbial communities, *Biochem. Eng. J.*, **105**, 80–89;
- Tahri, L., Elgarrouj, D., Zantar S., Mouhib, M., Azmani, A., Sayah, F. (2010) Wastewater treatment using gamma irradiation: Tétouan pilot station, Morocco. *Radiat. Physics Chem.*, **79**, 424–428;
- Moukhlissi, S., Aboussabiq, F.E., Amine, J., Rihani, M. & Assobhei, O. (2014) Heterotrophic denitrification by Gram-positive bacteria: *Bacillus cereus* and *Bacillus tequilensis*. *Int. J. Scient. Res. Public.*, **4**(4).
- Torres, P. & Foresti, E. (2001) Domestic sewage treatment in a pilot system composed of UASB and SBR reactors. *Water Sci. Technol.* **44** 247–253.
- Wu, P. & Tan, M. (2012) Challenges for sustainable urbanization: a case study of water shortage and water environment changes in Shandong, China. *Procedia Environm. Sci.*, **13**, 919–927;
- Roy, R. & Conrad, R. (1999) Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiol. Ecol.*, **28** 49–61;
- Cristóvão, R.O., Gonçalves, C., Botelho, C.M., Martins, R.J.E., Loureiro, J.M., Boaventura, R.A.R. (2015) Fish canning wastewater treatment by activated sludge: Application of factorial design optimization Biological treatment by activated sludge of fish canning wastewater. *Water Resour. Ind.*, **10** 29–38.
- Hao, R., Li, S., Li, J., Meng, C. (2013) Denitrification of simulated municipal wastewater treatment plant effluent using a three-dimensional biofilm-electrode reactor: Operating performance and bacterial community. *Biores. Techn.*, **143**, 178–186.
- Ghaniyari-Benis, S., Borja, R., Bagheri, M., Ali Monemian, S., Goodarzi, V., Tooyserkani, Z. (2010) Effect of adding nitrate on the performance of a multistage biofilter used for anaerobic treatment of high-strength wastewater. *Chemical Eng. J.*, **156**, 250–256.
- Ghaniyari-Benis, S., Borja, R., Ali Monemian, S., Goodarzi, V. (2009) Anaerobic treatment of synthetic medium-strength wastewater using a multistage biofilm reactor. *Bioresour. Technol.*, **100**, 1740–1745.
- Goh, S., Zhang, J., Liu, Y., Fane, A.G. (2015) Membrane Distillation Bioreactor (MDBR) – A lower Green-House-Gas (GHG) option for industrial wastewater reclamation, *Chemosphere*, **140**, 129–142.
- Watanabe, T., Motoyama, H. & Kuroda, M. (2001) Denitrification And Neutralization Treatment By Direct Feeding of An Acidic Wastewater Containing Copper Ion And High-Strength Nitrate To A Bio-Electrochemical Reactor Process, *Wat. Res.*, **35**(17), 4102–4110.
- Barber, W.P. & Stuckey, D.C. (2000) Nitrogen removal in a modified anaerobic baffled reactor (ABR). 1. Denitrification. *Water Res.*, **34** 2413–2422.
- Zhou, W., Sun, Y., Wu, B., Zhang, Y., Huang, M., Miyayama, T., Zhang, Z. (2011) autotrophic denitrification for nitrate and nitrite removal using sulfur-limestone. *J. Environm. Sc.*, **23**(11) 1761–1769.
- WWAP (2012) *Managing Water under Uncertainty and Risk*. Facts & Figures from United Nations World Water Development Report.
- Yi, L., Jiao, W., Chen, X., Chen, W. (2011) An overview of reclaimed water reuse in China. *J. Environ. Sci.* **23**(10), 1585–1593.
- Beline, F., Martinez, J., Chadwick, D., Guiziou, F., Coste, C.M. (1999) Factors affecting Nitrogen Transformations and Related Nitrous Oxide Emissions from Aerobically Treated Piggery Slurry. *J. Agric. Eng. Res.* **73** , 235- 243.
- Alinsafi, A., Adouani, N., Béline, F., Lendormi, T., Limousy, L., Sire, O. (2008) Nitrite effect on nitrous oxide emission from denitrifying activated sludge. *Process Biochem.*, **43**, 683–689;
- Adouani, N., Limousy, L., Lendormi, T., Sire, O. (2015) N₂O and NO emissions during wastewater denitrification step: Influence of temperature on the biological process. *Comptes. Rendus Chimie.*, **18**, 15–22.
- Carrera, J., Vicent, T., Lafuente, F.J. (2003) Influence of temperature on denitrification of an industrial high-strength nitrogen wastewater in a two-sludge system. *Water SA*, **29**(1), 11–16.

- Bremmer, J.M. (1977) Role of organic matter in volatilization of sulphur and nitrogen from soils, Soil organic matter studies. *In. Atomic Energy Agency*, **2**, 229–240;
- Spérandio, M. (1998) *Développement d'une procédure de compartimentation d'une eau résiduaire urbaine et application à la modélisation dynamique de procédés à boues activées*. Insa, Toulouse, [PhD Thesis].
- Richardson, D., Felgate, H., Watmmough, N., Thomson, A., Baggs, E. (2009) Mitigating release of the potent greenhouse gas N₂O from the nitrogen cycle – could enzymic regulation hold the key *Trends. Biotechn.*, **27**, 388-397.
- Rodier, J., Legube, B., Merlet, N., Brunet, R. (2009) *L'analyse de l'eau*. 9ème édition - Eaux naturelles, eaux résiduaires, eau de mer. Dunod.