Anaerobic Treatment of Black Water and the Effect of Nitrate on Bioreactor Performance (RALBI)

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Abstract
The main challenge that will be faced today is the state of degradation of the environment by discharges or the lack of adequate sanitation for wastewater, which is based on sanitation technologies. Hence the development of anaerobic systems in the treatment of wastewater. In particular, the anaerobic reactor with submerged bacterial bed (RALBI-Upflow) uses biological processes to remove organic matter and nutrients in a single reactor. The experimental system consisted of two units, the first in which one nitrate was injected and the other without nitrate injection with a volume of $4m^3$. This system was fed with raw sewage (black water). The objective was to evaluate the performance of the two reactors for the reduction of the organic load (COD) and the degree of denitrification by different concentrations of nitrates. The removal efficiencies of organic material expressed in COD was 70,25% and 80,11% at nitrate ($RALBI_1$), then 42.28% (COD) and 32.02% (nitrate) for $RALBI_2$ in the treatment of black water.
Keywords: Wastewater, Anaerobic reactor, Upflow, Black water and Treatment

1. Introduction

Traditionally, the conventional septic tank (CST) has been the most commonly used anaerobic system for onsite treatment of domestic wastewater due to its economic affordability, structural and functional simplicity, and electricity-free operation. Shortage of power is a major concern in most of the rural areas of developing countries (Tait *et al*. 2013). The primary treatment of the decanter-digester of domestic wastewater, especially of black water, is limited. Its systems rely on their ability to retain suspended solids by accumulation and sedimentation. Furthermore, most of the dissolved organics and nutrients do not receive significant treatment (Tchobanoglous G *et al*. 2003), but access to drinking water and sanitation is a good indicator of the level of development of a country. Unlike the nitrate concentration, it is very low in blackwater, hence the low risk of nitrate contamination in surface water or groundwater that could be used for human consumption. However, for maximum protection, a maximum level of nitrate contamination has been 10mg/L, CONAVA (2005) in Portugal, which corresponds to the maximum allowed recommended by the World Health Organization (WHO).

Moreover black waters are a natural source of nutrient including nitrogen. Physical–chemical processes such as ion-exchange, electrodialysis, nanofiltration or reversed osmosis to recover nutrients from anaerobic effluents. However, for a reliable operation of these processes the feed water should be free of particles and colloidal material and as low as possible in soluble organic matter (Lorch, 1987; Tanninen *et al*. 2005), but costs are not always within the reach of developing countries. The principal applications of the processes are for removal of the carbonaceous organic matters in wastewater; nitrification; denitrification; phosphorus removal; and waste stabilization. The biological processes are considered the most effective and economic processes in the field of wastewater treatment (Metcalf & Eddy, 2003). But the elimination efficiency of nitrogen for some process such as biological membranes is affected. Because the sludge age does not allow efficient nitrification and the simultaneous nitrification and denitrification (SNdN) process for these systems is affected by the oxygen transfer efficiency (low) (Munch *et al*. 1996; Daigger *et al*. 2007).

Black water is a concentrated version of the domestic sewage, with a COD of around 1000 mg/L and the total kjeldahl nitrogen content (TKN) of around 170–200 mg/L (Murat Hocaoglu *et al*. 2010) the significantly lower COD/N ratio compared to that of domestic wastewater and the high initial TKN level makes it particularly interesting for evaluation and suitable for SNdN. A models has been suggested, the treatment of black waters through denitrification, that is to say Reactor Anaerobic at Bacterial Bed Immersed (RALBI), emphasizing different aspects of its operation, reporting specific results for the investigated problems. The objectives were to study, the denitrification of two reactors, with the absence and addition of nitrate solution. The results of experiments concerning nitrate addition on COD and BOD removals, ammonia and nitrate removal were researched under anaerobic condition. The utilization of this process for the treatment of black water at a relatively short retention time and under the natural temperature and pH conditions. The influence of the addition of nitrogen as an alternative electron acceptor for nitrogen removal in the reactor is explored under a high nitrate concentration, as well as capital, operation and management costs that are important factors determining the actual feasibility of such a process (system) treatment.

2. Material and methods

2.1Wastewater 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**.

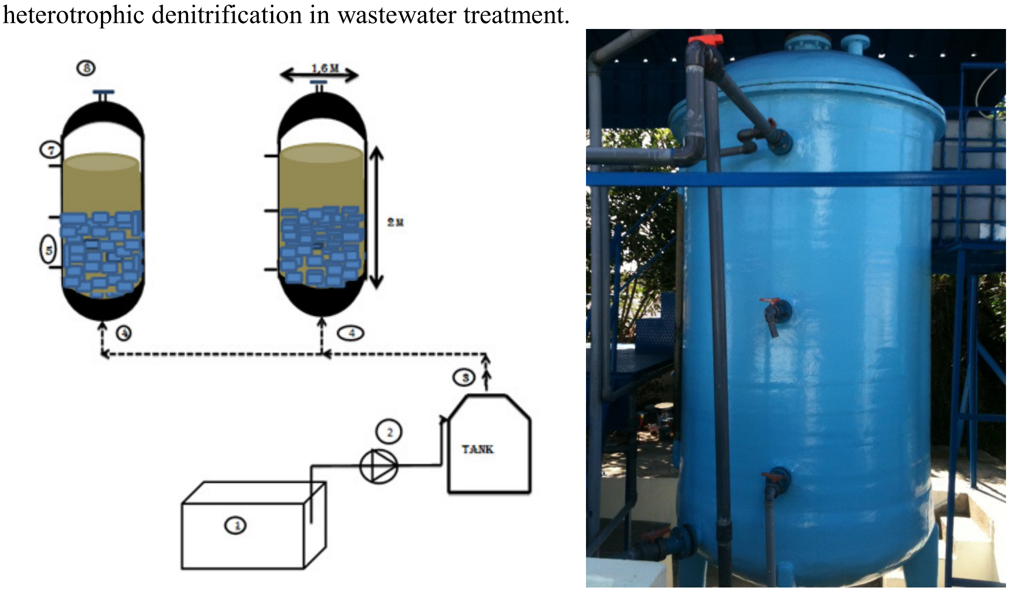
Table 1. Black Water characterization

Parameters	Unit	Min.	Average	Max.
pH-value	-	7,51	8,02	8,54
Temperature	°C	15,9	18,66	23,2
Chemical oxygen demand (COD)	mg L^{-1}	411	691,5	1150
Biological oxygen demand (BOD)	mg L^{-1}	372,41	476,96	388,38
Total Suspended Solids (TSS)	mg L^{-1}	0,457	0,543	0,662
Ammonia Concentration (NH_4–N)	mg L^{-1}	131	220,25	376,8
Nitrite Concentration (NO_2)	mg L^{-1}	0,07	0,126	0,29
Nitrate Concentration (NO_3)	mg L^{-1}	1,03	3,66	8,07
Fecal Coliforms	UFC/100ml	$6,8. 10^9$	$7,2. 10^9$	$8. 10^9$

The reactor was operated at ambient temperature, no heat exchange was introduced. The reactor was operated in the winter/spring time where the temperature falls down in winter reaching about 15°C. The average influent black water temperature during the experiment is shown in **Table1**.

2.2 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, 6 m 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., the pilot plant bioreactor wastewater treatment consists of a tank (1), decanter (3) and two cylindrical reactor (4&5) vessels having an internal volume of 4 m^3. Using a pump, wastewater was pumped from the influent tank into decanter-digester and by gravity into two biorectors through its bottom inlet, and flew through the biofilm on PVC filler, and then flew out from the upper of the reactor. Inside bioreactors (RALBI 1 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 Medias in reactors are 2,76 cm^2 including 1.57 cm^2 swollen and 1.19 cm^2 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 NO_3 serving as the electron donor for heterotrophic denitrification in wastewater treatment.



(A) **(B)**

Fig 1: (A) 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) and (B): Picture of Reactor Anaerobic at Bacterial Bed Immersed (RALBI)

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2.3 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 140 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 $RALBI_1$ and Effluent $RALBI_2$, and the samples were conduit and analyzed after sampling.

2.4 Analytical methods
COD was digested using a 5B-1F Digester at 150°C for 120 min and determined by titration using ferrous ammonium sulphate solution according to the APHA (APHA. 2005) and a lab drying oven. pH was measured using a pH meter (model E-201-9 provided by Shanghai Yoke Instrument Co., Ltd., China). TSS, BOD_5, Ammonium nitrogen (NH_4^+), were analyzed in the laboratory according to the methods prescribed in AFNOR(French national organization for standardization) handbook (AFNOR, 1999, 2001a); Nitrate (NO_3), Nitrite (NO_2) were analyzed by (Jean, R., *Ed. Dunod, Paris*. 2009).

3. Results and discussion
3.1 Black water characteristics
Daily grab sampling was selected to represent the actual characteristics of black water. The detailed characteristics of black water during the study are summarized in **Table 1**. The organic matter present in the black water can be estimated by calculating BOD to COD ratio. The average value of this ratio was observed more than 0,63 which indicated the suitability of the black water for biological treatment (Metcalf and Eddy, 2003).

3.2 Load fluctuations of black water and monthly distribution.
It was noted that the daily loading of black water was slightly lower than the values reported by Murat Hocaoglu *et al*. (2010) and Kujawa-Roeleveld and Zeeman (2006). The variation in pollutant loading mainly depends upon the facultor activity and primary settling.

3.3 Black water treatment and elimination efficiency of bioreactors ($RALBI_1$ & $RALBI_2$)
The system was started up in May, 2011 and operated for a period of 24 weeks for study, after rehabilitation period in June 2015. For the first 8 weeks of operation, the system was considered to be acclimate stage. **Fig. 2** shows the results related to the variation of COD concentrations throughout the two reactors for the different periods studied and an influent COD and BOD concentration max of 1150 mg O_2/L, 488mgDBO/L. As can be seen, the COD removal efficiencies varied according of season and bioreactors (RALBI 1& 2) at retention time of 6h. At HRT of 6 h, the total COD and removal efficiencies reached 70, 25 and 42, 28% respectively (RALBI 1&2) and the role played by denitrifyants on an effective during months on the organic matter reduction process. Bioreactors, acclimated or matured after almost 100 days of operation when significant removal efficiency (up to 82,15%) was observed for COD within $RALBI_1$, while $RALBI_2$ were at 61,38%.

The effluent COD concentration from the first four month reached 184,22; 230,62; 181,38 and 183,95 mg/L $RALBI_1$ and for $RALBI_2$ reached 328,14; 357,48; 384,45 and 321,14 mg/L, respectively. The average effluent pH was observed as 8, 16 ± 0.13 and 7,88 ±0,11 (RALBI 1&2), above within the observed range of 6.5–7.5 for anaerobic digestion (Metcalf and Eddy, 2003) caused by suggesting no excessive accumulation of organic acids within the treatment unit.

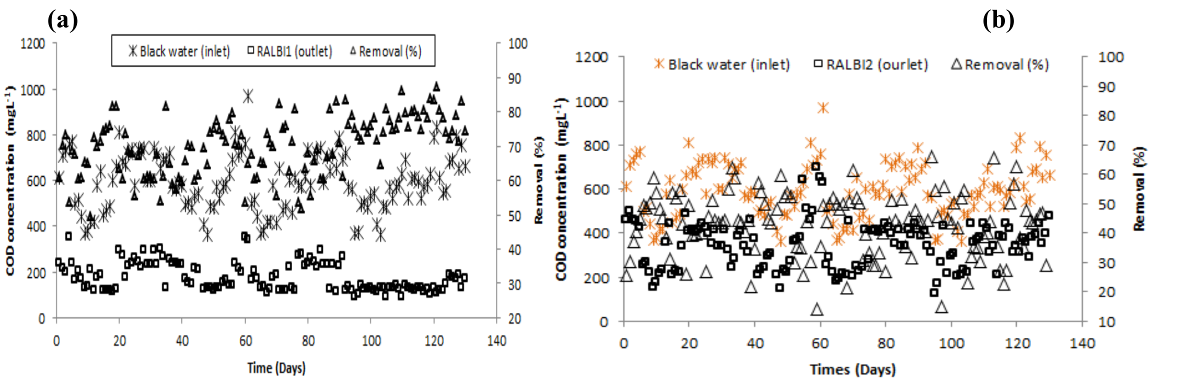

Fig 2: Time course of the concentration in influent and effluent with removal efficiency of bioreactors RALBI 1 (a) and 2 (b) systems for COD.

Effluent primary at a T° 16-20°C, the outlet COD and BOD concentrations from the first reactor reached 175,5 and 98,75 mg/L, respectively. Therefore, almost half of the existing organic contaminants in the black waters were removed in the RALBI1, that is why COD and BOD removal efficiencies achieved at this HRT were steady and increasing 66,96% and 70,22% respectively, January at April. The average effluent quality was increasingly important for a treatment volume of 4m3. The removal efficiency (COD) was highest for $RALBI_1$ of 74,5-78,09%, while it was quite low for RALBI2 (42,51) respectively at 21-23°C, May and June 2016. Each removal efficiency was observed for nitrate injection and no nitrate, which might be due to the degree of bacterial stimulation of the activity for the degradation of the organic load during anaerobic digestion in RALBI 1 &2.

The concentration of nitrate could accelerate the elimination of organic matter, but especially avoid the accumulation of organic intermediare (organic acids) probably the case $RALBI_2$. As indicated the results ($RALBI_2$) revealed that 58,34% COD proportion were present in the effluent. Similar results were reported in the literature by (Al-Jamal and Mahmoud, (2009) for anaerobic treatment of strong sewage. Hence, the dissolved fraction would be consisted of soluble microbial products, which are generally resistant to biodegradation. The increased level of COD in the effluent might be due to increase in the effluent VFA concentration (Sharma *et al.* 2014). Inside regions of this reactor (RALBI 1 et 2) were filled with hollow-sphere carriers made of PVC (approximately 2,5cm in diameter) in settled form. The average BOD and TSS removal efficiency after acclimatization were 72, 03% and 84, 15% respectively for $RALBI_1$. The increase in removal efficiency over time was due to sufficient development of biomass at the bottom of the primary chamber and enhanced bacterial immobilization on the surface of the filter media. The upflow mode of liquid-flow also contributed significantly in achieving a higher removal efficiency.

An increase in pH values was observed at the first reactor ($RALBI_1$) (**Fig**. 1B). This rise in pH in reactor like this system is caused different by phase (denitrification). pH values in each period had an increasing trend, which could also be caused by the production of compounds that increase the alkalinity by substrate degradation.

3.4 Phase II: influence of nitrate addition on the reactor performance.

Figures below presents the behavior of the concentration influent and effluent the oxidated nitrogen forms (nitrate + nitrite) the efficiency of removal presented for RALBI 1&2. It is perceived that the efficiency of denitrification presented for the bioreactors was well steady, a sufficient level for the adhesion of bacteria to the media (PVC) and the degree of denitrification.

With the addition of nitrate at a concentration of 5000 mg/L and a variable organic loading of 360 at 970 mg COD/L/d. During the experiment, the permanent injection of nitrate showed an influence on the increase and the time required (6h) for the elimination by the denitrifiers. Fig. 4 shows the variation trend of the nitrate and nitrite produced. As can be seen in Fig. 4, the main part of the existing nitrate at feed was removed in the compartment, so that nitrate concentration in efluent $RALBI_1$ reached 1,85; 2,87; 3,94; 2,39; 6,8 and 4,08 mg/L, respectively. Therefore, virtually all of nitrate present at feed was removed, the denitrification efficiencies in the first, second and third months being 82,64%, 81,3% and 82,43%, respectively. However, $RALBI_2$ had on average 2,29mg/L of nitrate in the effflueut either 32,02% at the end of the experience **Fig5**. The produced nitrite concentration measurement inside the reactor showed that only traces of nitrite were found in the effluent treated, with nitrite concentrations on average of 0,22 and 0,098 mg/L in the $RALBI_1$ et $RALBI_2$, respectively. However, in the study (S. Ghaniyari-Benisa *et al.* 2010) dealing with synthetic wastewater, by baffled reactors, had significant nitrite concentrations of 138 and 24 mg / L in the first and second chambers, respectively

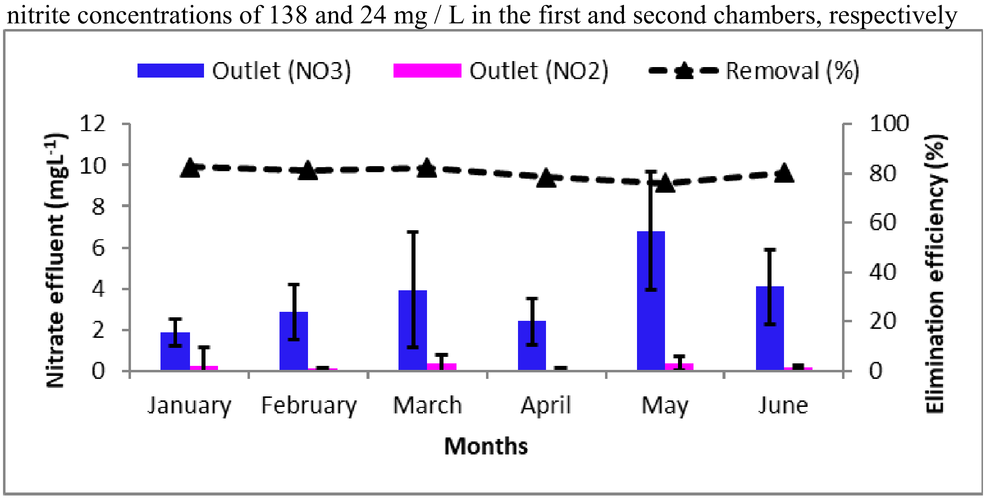

Fig 4: Profile of the nitrate and nitrite concentration variations according months ($RALBI_1$)

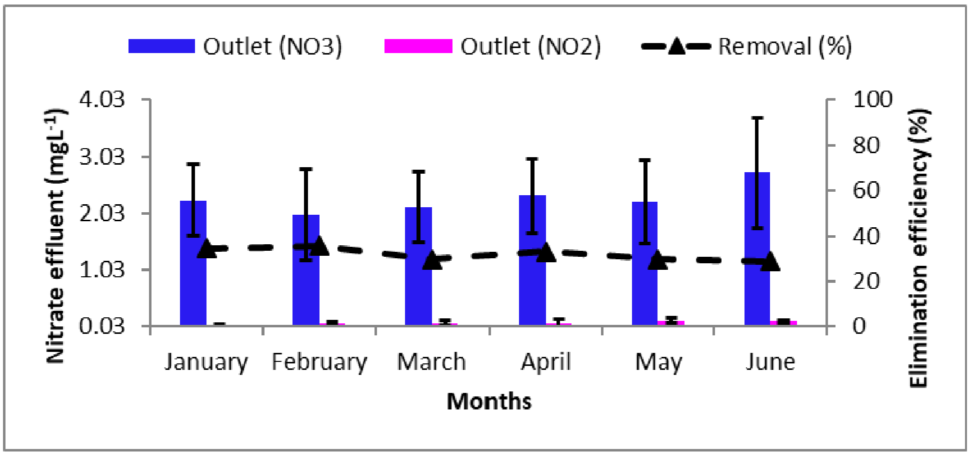

Fig 5: Profile of the nitrate and nitrite concentration variations according months (RALBI$_2$)

(**Fig. 2a-4**) illustrates the denitrification effect on the organic matter removal concentrations. The effluent COD and BOD concentrations from the reactor reached 175,25 and 98,76 mg/L, which is equivalent to COD and BOD removal efficiencies of 70,25% and 73,03%, respectively. This denitrification within RALBI$_1$ studied (with nitrate injection), denitrification resulted in an increase in COD, BOD removals, so that the COD elimination efficiencies increased by more than 20 %, relative to RALBI$_2$. In this phase, the process of denitrification in the compartment (lower chamber of the reactor) begins the degradation of the organic matter and thus improves this elimination of the COD at the outlet of RALBI anaerobic bioreactor with flow (ascending) and 2m high. This increase in the removal efficiency is due to the oxidation of some COD feed for the required energy and carbon source supply for nitrate reduction.

Denitrification had several positive effects on 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 at the reactor inlet, thus improving environmental conditions (W.P. Barber & D.C. Stuckey, 2000). The denitrification process was carried out by using nitrate by facultative denitrificants 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 (R. Roy & R. Conrad,1999) can be expressed by the following theoretical equation: **Eq**.

$$24NO_3^- + 5C_6H_{12}O_6 \Rightarrow 12N_2 + 30CO_2 + 18H_2O + 24OH^- \quad (1)$$

As a result of this equation, COD removal efficiency increases in the presence of nitrate. pH variations during nitrate reduction ranged between 7,82 and 8,39 in the present study. Therefore, these values by comparison with those reported in the RALBI$_2$ experiment (without nitrate addition), the average pH was increased by about 0, 24. Therefore, the addition of nitrate to the process has a some momentous and contrary effects on biofilter performance (M.H. Gerardi 2002):

(a) Quick reduction of NO_3^- by facultative anaerobes through anaerobic respiration.

(b) Foaming formation as a consequence of the rising gaseous nitrogen. These two observations were observed during our study of the RALBI bioreactor (Fig 6). Finally the (c) increasing redox potential of the sludge system. An increase in redox potential hampers the methanogeneous activity of the microorganisms that transform VFAs to methane. Adding nitrate would can raised the redox potential of the system (K.C. Chen & Y.F. Lin, 1993). This would make it possible to judge the effect of nitrate, and bacterial activity in biological processes, in a concern for competition for the substrate whose interaction between denitrification and methanogenes that can take place within the bioreactor.

Fig. 6: Foaming formation as a consequence of the rising gaseous nitrogen (outlet $RALBI_1$)

The continuous addition of nitrate ($5gL^{-1}$) by injection into $RALBI_1$ could produce and accumulate nitrite, but throughout the experimental period, no accumulation of nitrite was observed throughout the process and all of the nitrite produced was transformed, it can therefore be considered that the inhibition effect of the other intermediate product (N_2O, NO) of accumulation denitrification would not have occurred (production of N_2).

Table 2 shows the concentrations of ammonium observed in the bioreactors for conditions of nitrate absence and presence (denitrification). Production of ammonium from nitrate is an ordinary reaction in anaerobic conditions as was demonstrated by some authors as **Eq. (2)** (J.C. Akunna *and al.* 1994):

$$NO_3^- + 4H_2 + 2H^+ \Rightarrow NH_4^+ + 3H_2O \quad (2)$$

As can be seen in Table 2, without nitrate (252,06 mgL^{-1} primary effluent) the concentration of ammonium in the $RALBI_2$ reactor effluent were 203,94 mg/L, while the $RALBI_1$ reactor effluent reached a maximum content of 240,56 mg/L. In the present study, the relatively large concentration of ammonium observed would be caused by nitrate source used in experiance (NH_4NO_3) in bioreactor ($RALBI_1$). It is perceived that it did not have significant removal of these nitrogen forms, presenting concentration sufficiently raised in the effluent. This is due to the origin of raw affluent (black water) in the entrance of the reactor (José Tavares de Sousa1 *et al*. 2008) and the treatment method used, however therefore had no negative effect on the reactor performance.

Table2. Ammonium concentration present in bioreactors effluent.

Months	Primary Effluent mgL^{-1}	RALBI 1 (adding nitrate)	RALBI 2 (without nitrate)
January	**233,8**	**226,07**	**171,62**
February	**238,93**	**243,8**	**205,75**
March	**224,62**	**223,77**	**175,95**
April	**260,05**	**241,55**	**219,05**
May	**278,41**	**268,09**	**217,27**
June	**272,77**	**234,49**	**233,53**

In short, the treatment of wastewater (black water) is characterized by factors that must be monitored in conventional systems such as the retention of soluble components for hydraulic retention time (activated sludge), mass transfer limitation for dissolved oxygen (DO) diffusion and utilization And soluble microbial products, which accumulation is known to be inhibitory for nitrification activity (Chudoba, 1985, Ichihashi et al., 2006, Jiang et al., 2009). However, this was not the case for the RALBI process during our study, the RALBI system after optimization on the semi and pilot scale, presented the data of a large sampling campaign over a period of 6 months, Allowing to give an indication of the treatment undergone by primary effluents feeding the bioreactor by gravity (upflow). The addition of nitrate resulted in an increase in the effective removal of organic carbon from the black water via the supply of carbon and energy for cellular activity and the synthesis of new denitrifying bacteria in a system anaerobic, which eliminates the constraint of exogenous carbon and energy and thus an energy, economic autonomy for the rural and periurban zones of the developing countries.

Conclusion

Several regions of Africa are already suffering the consequences of climate change, and will be hit especially hard by the ongoing rise in global temperatures. This will aggravate the water context, reduced rainfall and longer droughts will take their toll, and the regions will struggle to meet the basic demand for water. Poorer communities have fewer resources to cope with the impacts of climate change and will be hit hardest. Declines in agriculture will increase rural unemployment, driving large numbers of people to crowded cities. However, the potential of wastewater should be considered as a non-negligible resource to be developed in a controlled reuse framework. Hence the need to develop environmentally safer and economically viable domestic wastewater treatment technologies (Kumar et al. 2014).

The process of the submerged bacterial anaerobic reactor (RALBI), based on the results of the analyzes, carried out during our study proved to be a very promising alternative for rural areas and small communities without a sewer system. High reduction efficiencies for COD, BOD, TSS and NO3 in our experiments prove the reliability of the process regardless of the type of polluted water treated and the environmental conditions. In addition, the bioreactor (RALBI) in terms of on-site primary blackwater effluent treatment and other favorable economic and operational characteristics could be a possible alternative to the conventional septic tank for rural and peri-urban areas in developing countries. This is why its secondary effluents may be needed and adapted in reuse applications of non-potable water, such as infiltration, irrigation, recycled for water flushing. Nevertheless, additional treatment of its effluents could be envisaged, taking into account the rather large load of nitrogen especially for ammonium.

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