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Research Article
Effect of Adding Nitrate on the Performance of a Reactor in an Immersed Bacterial Bed Used for Anaerobic Treatment of Domestic Wastewater

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Abstract

Background and Objective: The difficulty of sanitizing wastewater as it arises acutely in small communities, rural areas etc., requires that appropriate solutions be taken. The discharge of raw wastewater into the wild in Morocco accounts for nearly 54% on the coastline, reflecting the impact of a lack of hygiene and sanitation for the population and the environment. To improve the treatment of wastewater in rural areas that lack sanitation systems, the aim of this study was to introduce 2 anaerobic bioreactors in a pilot wastewater denitrification process to determine if those bioreactors could help decrease the chemical oxygen demand and nitrate levels in the wastewater. **Materials and Methods:** During operation, the decanted primary effluent separately supplied the 2 anaerobic bioreactors (hereinafter "bioreactor 1" and "bioreactor 2": The bioreactor 1 was fed with external nitrate plus the nitrate present naturally in the primary effluent, whereas, the bioreactor 2 was just fed with nitrate from the primary effluent) with an ascending flow. This allowed the annual average organic loading rate (chemical oxygen demand) of 0.652 g/day to be applied to the bioreactors. The primary effluent nitrate load applied to the bioreactors ranged from 1.94×10^{-3}-14×1.10^{-3} g/day. In addition, 600 mg L^{-1} of nitrate was added in the bioreactor 1 treatment throughout the experiment at a 6 h hydraulic retention time. Data was statistically analyzed by one-way ANOVA using SPSS. **Results:** The overall organic loading rate in effluents treated with bioreactor 1 was 0.036 g m^{-3} day, a 72.16% reduction and 0.064 g m^{-3} day, a 48.9% reduction, for ARIBB 2. An average nitrate reduction performance of 80.31% was observed for bioreactor 1 and 42.81% for bioreactor 2 at the end of the experiment in June. **Conclusion:** The bioreactor 1 with nitrate addition showed better performance than the nitrate-free bioreactor 2, compared with the different chemical oxygen demand (COD) loads and environmental conditions. The relatively low cost of external nitrate facilitates access to the process.

Key words: Heterotrophic denitrification, domestic wastewater treatment, chemical oxygen demand reduction, nitrate reduction performance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to the growing, global water shortage problem, stemming from accelerated industrialization, urban growth and climate change[1,2]. Cost-effective and feasible wastewater treatment options are necessary to counter the harmful impacts that pollutants have on water resources. Like increased chemical oxygen demand (COD), nitrate levels and organic matter have harmful effects on the environment. These types of wastewater treatment options are vitally important in poor countries. This is especially the case in the Middle East and North Africa[3], where water resources are scarce, due largely to a water supply imbalance caused by uneven precipitation distribution and increased demand for irrigation water. The resulting pressure on available resources amplifies water degradation[4,5] because of excessive consumption and high pollutant production, which generates huge quantities of liquid waste that are released into the environment without any treatment.

Among the various measures taken to address this growing problem are wastewater recovery efforts[1,4,6], which are urgently needed in small cities and rural areas. Typically, the quality of treated and reused water depends upon the available treatment processes and in spite many pollutants cannot be completely eliminated[1]. Accordingly, a solution suitable for the treatment or recovery and recycling of such wastewater must be found[7]. In Morocco, sewage systems often present significant problems, especially in small communities that use untreated dirty water[8]. Biological treatment mechanisms, such as activated sludge and treatment membranes are the most widely used processes for treating wastewater that contains organic matter[7,9]. But these technologies are relatively expensive and are not always available to small communities and rural areas of developing countries such as Morocco. Therefore, it has been necessary over the last few decades to develop reliable technologies based on an anaerobic (denitrification) system.

This type of wastewater treatment system could be an alternative to conventional aerobic processes. Anaerobic systems are more economical and environmentally friendly, using natural methods optimized to biodegrade pollutants into reusable matter[7,10]. Biological denitrification is normally carried out by facultative anaerobic processes that use organic and inorganic wastewater sources as sources of carbon and energy. However, in developing countries, in particular, the heterotrophic denitrification process is rarely observed-probably because this process requires sufficient external carbon sources for nitrate removal[11]. Moreover, because some countries have lax discharge standards for certain compounds, nitrogen in particular, there is a minimal demand to implement systems for wastewater treatment.

The aim of this study was to carry out a low cost (6 euro/month), anaerobic treatment of domestic wastewater by heterotrophic denitrification of 2 anaerobic reactors system with an immersed bacterial bed (ARIBB) separated, one using an external supply of nitrate (hereinafter "ARIBB 1") and the other without an external addition of nitrate (hereinafter "ARIBB 2"). This permitted an evaluation of the stability of wastewater treatment applied with different organic loads as well as the level of denitrification efficiency in the removal of the organic matter from the ARIBB 1 and ARIBB 2 bioreactors of the treated wastewater. Other experiments could be carried out, like the introduction of an external carbon source (in the event of a low carbon in the wastewater) that could provide suitable amounts of electron donors (given that such an external carbon source would be essential to the successful application of the ARIBB process in different environments). However, this study focused on demonstrating which anaerobic bioreactor was best suited for reducing COD and nitrate levels in wastewater.

MATERIALS AND METHODS

Wastewater characterization: This experiment applied the ARIBB wastewater treatment process to treat wastewater from the sewer system of the Faculty of Science of El-Jadida. The wastewater was pumped to the pilot plant basin. After pretreatment, the proportions of mineral or organic suspended solids were decanted before being pumped to the settling tank. The settler in turn removed the suspended solids from the sewage. After these 2 steps, the wastewater was free of most of the suspended pollution and was ready for the bioreactors. Each step was equipped with an electromechanical control panel, which controlled the station's batch and automatic wastewater supply. The characteristics of the various sources of wastewater used in this experiment are provided in Table 1.

Experimental setup: To begin the experiment, a semi-pilot bioreactor was used to treat wastewater from the city of El-Jadida, which was transported in 25 L drums to the Biomare laboratory. Subsequently, the ARIBB bioreactors pilot scale was used to treat domestic wastewater from the Faculty of Science and the nearby village.

Semi-pilot reactor: Figure 1 is a diagram of the bioreactor used before the installation of the ARIBB station in this study.

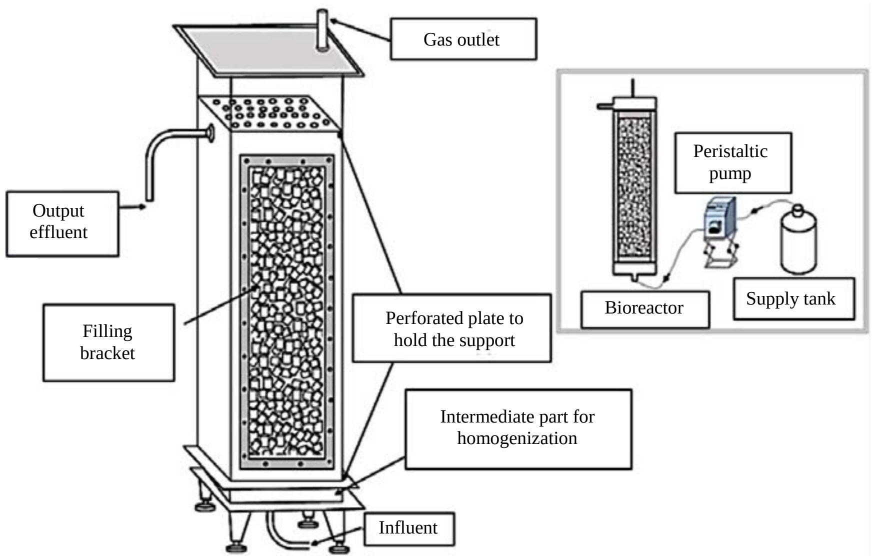

Fig. 1: Configuration semi-pilot bioreactor

The semi-scale bioreactor was filled with plastic support (PVC) for biofilm adhesion. A peristaltic pump was used to feed the bioreactor continuously. A tank was used for the storage of wastewater before treatment. A plastic connection pipe supplied the semi-pilot bioreactor with an upflow. After the treatment, the effluent exits through the upper part of the bioreactor and where there is also an outlet for the gases. The bioreactor was rectangular (1 m long and 0.25 m sided) made of stainless steel, with a volume of 64.5 L. The bioreactor was equipped with a recirculation loop, through which a flow rate of 4.96 L h^{-1} was maintained.

Inoculum: The seeding medium consisted of a mixture of diluted wastewater, taken from the sewer network in the city of El-Jadida and the denitrifying flora, obtained from inoculum subcultures in media of cultures composed of nutrient broth nitrate. This inoculum was enriched by the addition of KNO_3 at a rate of 1 g L^{-1}. The process allowed for the anaerobic cultivation of important biomass in cans with a capacity of 27 L. The incubation of this biomass was carried out at ambient temperature of 20-5°C for 24 h (in order to obtain cells in their exponential phase of growth). Next, the seeding medium of the semi-pilot bioreactor was used to inoculate the ARIBB bioreactors (pilot scale).

ARIBB bioreactors (pilot scale): Figure 2 shows the schematic of the wastewater treatment plant. The ARIBB, which was designed to study and evaluate heterotrophic denitrification performance by the external addition of NO_3 from domestic wastewater, was filled with rings that promoted the formation of biofilm. This biofilm formed a kind of bed that remained immersed in the influent of the bioreactor (cylindrical)-giving the bioreactor its name, an anaerobic reactor with an immersed bacterial bed or ARIBB. Regular forms of double-walled rings, made with PVC, were distributed every 4-5 cm to support the growth of microorganisms. The total area of a single support in the reactors was 2.76 cm^2, of which 1.57 cm^2 were interior surfaces and 1.19 cm^2 were external surfaces. The bioreactor had a diameter of 1.6 m and a height of more than 2 m. Once constructed, the denitrifying bacteria were introduced into the bioreactors to colonize the other supports present within each ARIBB. The metabolic role of this bacterial flora degraded the organic matter in water according to the nitrate concentration.

As indicated, the bioreactor wastewater treatment pilot plant consisted of a reservoir (1), a decanter (3) and an ascending stream of decanted wastewater for cylindrical reactors (4) with an internal volume of 4,000 L for each bioreactor. The wastewater was pulled by gravity into the 2 bioreactors through their lower inlets. The up flow passed through the reactors filled with support (PVC) to which the active biomass adhered. The treated water then left the reactor through the upper outlets (6).

The sewage treatment plants are designed based on the nature of the tributaries that are to be treated. The ARIBB station was sized according to the flow of pollution that a small rural community or tourist complex can generate, which is 1000 Eq/H of domestic wastewater of the connected population. With biochemical oxygen demand (BOD_5) per

Table 1: Characterization of experimental wastewater sources

Wastewater source	Oxygen demand: Chemical (COD, mg L^{-1})	Oxygen demand: Biological (BOD, mg L^{-1})	Total suspended Solid s (TSS, mg L^{-1})	Total Kjeldahl Nitrogen (TKN, mg L^{-1})	Ammonia Concentrate (NNH_4^+ mg L^{-1})	Nitrate (NO_3^-)	Fecal coliform (CF, ufc/100 mL)	Fecal streptococcus (SF, ufc/100 mL)	T°C	pH
[a]City of El-Jadida	776.41-1481.66	534.65-850.65	533.46-1002.31	nd	nd	3.93-8.09 mg L^{-1}	7.39-7.98	7733	24.5-30.6	66.2-7.5
[b]Semi-scale	nd	810.5	300	104.4	74	1.9 mg L^{-1}	30×10^{10}	nd	23	nd
[c]Pilot scale (ARIBB)	246.87-970	294.23-488	140-560	70.21-147.4	26.89-123.8	1.94×10^{-3}-14.09×10^{-3} g/day	7.48-8.92	5.08-6.83	15.9-26	77.5-8.6

a: Wastewater of the city of El-Jadida, b: Wastewater of the city of El-Jadida experienced in the laboratory (semi-pilot), c: Wastewater from the faculty and the small village treated by ARIBB (pilot scale), nd: Not determined

EqH = 60 g/day, including a daily load of BOD_5 = 60 kg and MES EqH = 80 g/day, the daily load of effluent MES was 80 kg. The hydraulic load was based on a daily input of 100 L/EqH. In this ARIBB station, the volume of wastewater that could be treated was 100 m^3/day. Thus, the average flow was distributed over 24 h, with a treatment of 4.2 m^3 each hour. Based on these specifications, the ARIBB proved to be a solution that can adapt well within the framework of zones that do not have collective sanitation systems. A good indicator of anaerobic reactor's functionality is its ability to manage sludge, which is an important factor in achieving higher COD reduction rates. However, sludge management was not addressed in this manuscript because it was being examined in another scientific study.

Experimental procedure: Biological denitrification was evaluated by studying the resulting COD reduction with the addition of nitrate in ARIBB 1 and without such an addition in ARIBB 2 as well as by studying the influence on the performance of the reactors. ARIBB was used at ambient temperatures during an annual cycle and fed with domestic wastewater from the Faculty of Science during the study. During operating time, the external nitrate was injected continuously by a metering pump into the ARIBB 1 inflow, while the ARIBB 2 was fed with the same influent without the addition of nitrate. Samples were taken daily from raw sewage and effluent from bioreactors (ARIBB 1 and ARIBB 2) and were subsequently analyzed in the laboratory. A batch wastewater treatment of the ARIBB bioreactors was provided by the main pump at the level of the screening basin, which was regulated by the floats of the basin and that of the settling tank, in order to avoid any external overflow of the wastewater.

Analytical methods: Organic matter (COD) was digested using a digester at 150°C for 120 min and determined by titration using a ferrous ammonium sulfate solution according to the French National Organization for Standardization[12]. Total suspended solids (TSS) was measured through an oven drying method with a membrane filter (pore diameter 0.45 μm) and a lab drying oven. The water's 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 ammonium (NH_4) was analyzed in the laboratory according to the methods prescribed by AFNOR[12]. Nitrate (NO_3) were analyzed according to Rodier *et al.*[13].

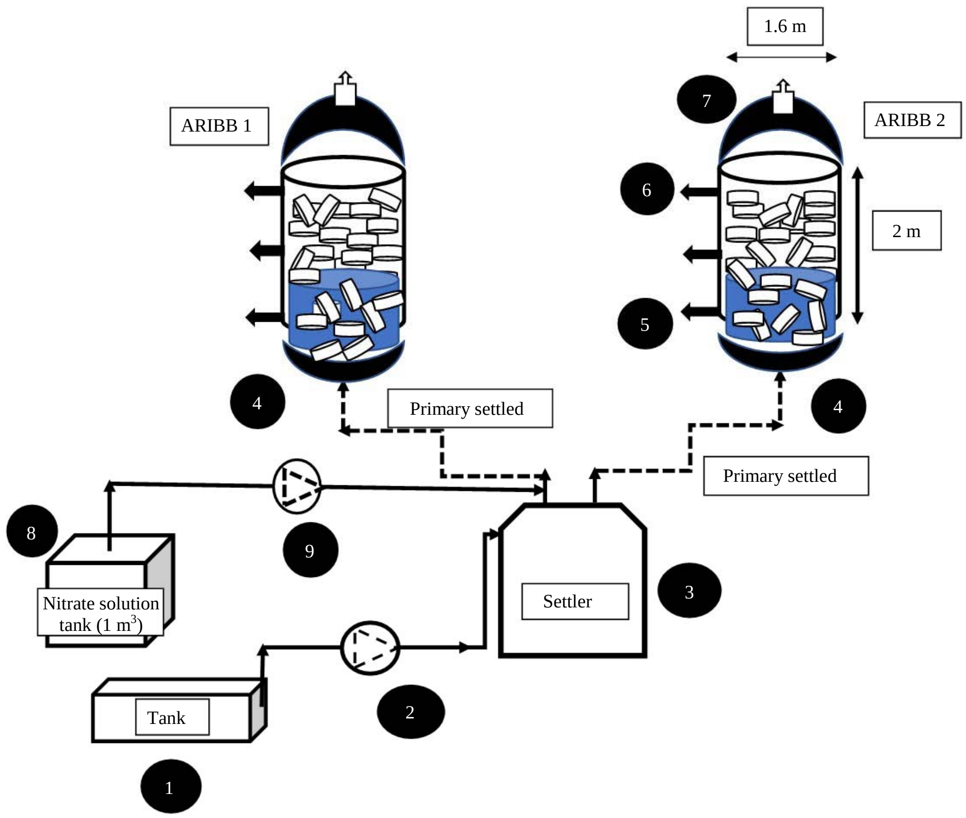

Fig. 2: Schematic diagram of ARIBB: (1) Tank, (2) Pump, (3) Settler or decanter, (4) Up flow, (wastewater, 5) Sampling point, (effluent, 6) Reactor outlet, (7) Gas outlet, (8) Nitrate solution tank and (9) Metering pump

Statistical analysis: The one-way analysis of the variance was used as a statistical test. This test applies when one or more categorical explanatory variables are measured that have an influence on the distribution of a continuous variable to explain. This is called a one-factor analysis when the analysis is based on a single-variance, two-factor analysis or multifactor analysis model. And compared by Duncan's test ($p<0.05$) using the software package SPSS (version 17.0).

RESULTS

Daily fluctuations of influent: Variations in daily concentrations and sampling results were compared for different days of the week. Characteristics of the influent were almost similar to the city's raw sewage. The concentration of COD (influent) differed significantly during periods of operation. It was less than 0.5 g/day during summer and early autumn but highest during autumn to spring. This fluctuation was based on the seasons as well as the activities of the campus population.

Accordingly, these results indicate that the loading of wastewater can fluctuate, with variations of several days to several weeks. However, any occasional modification of the COD concentration of the waste water was taken into account in order to evaluate its impact on the performance of the bioreactors (ARIBB 1 and 2). The COD concentration was higher in autumn-winter.

At the pilot scale, the concentration of nitrate injected by the metering pump was set at 600 mg N/L (optimal concentration at the semi-pilot scale) at the inlet of the ARIBB 1 bioreactor in order to obtain an optimal treatment such as experienced on the semi-scale reactor in the laboratory. The average rate of loading of NO_3 from the decanted effluent applied to the reactors varied from a minimum of 1.94×10^{-3} to a maximum of 14.1×10^{-3} g/day. Depending on the main components of the wastewater and the different components of the anaerobic degradation pathway, the process of external nitrate feeding (ARIBB 1, Fig. 2) to improve anaerobic biodegradation favored the denitrification of wastewater at contact with the denitrifying bacteria. In contrast, the RALBI 2

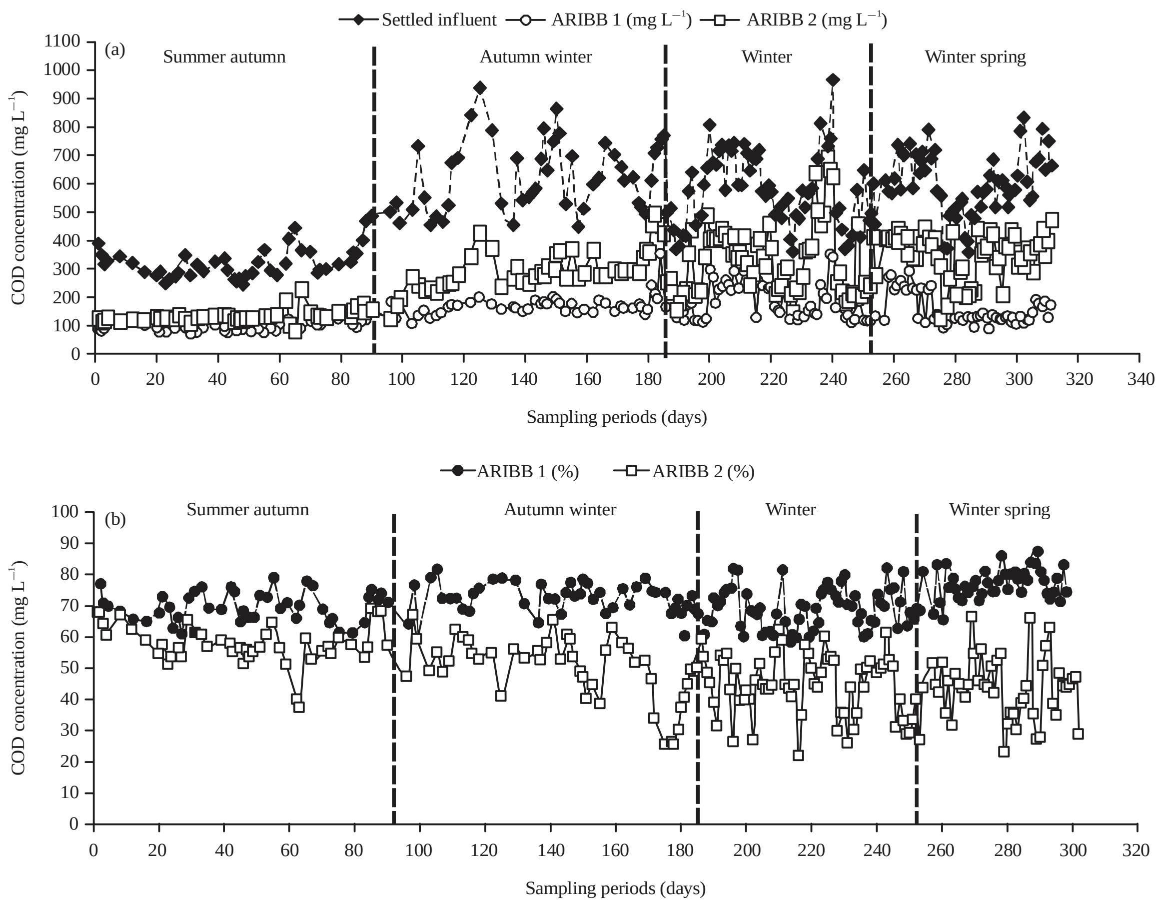

Fig. 3(a-b): (a) Influent and effluent COD concentrations, (b) The %COD removal of the ARIBB 1, ARIBB 2

bioreactor was fed only with nitrate present in the same decanted effluent (primary effluent) at a low concentration. Based on a sampling from the 2 reactors, the nitrate concentration ranged from 17.51-41.14 mg L^{-1} and 1.52-17.8 mg L^{-1}, respectively ARIBB 1 and ARIBB 2.

Influent and effluent COD concentrations and the removal rate of COD: The ARIBB (1 and 2) COD reduction performance is provided in Fig. 3b. During the experimental period (August-January), the concentration of decanted COD (primary effluent) varied from a minimum of 246 mg L^{-1} to a maximum of 940 mg L^{-1}. From summer to fall, the applied COD load averaged 0.453 g/day, well below the average COD concentrations in the spring. This was probably related to the increase in water consumption due to warmer weather in summer and moreover, related to the fact that the station was fed during this period by the small village nearby. In the fall and early winter, COD concentrations of sewage increased to 0.746 g/day, due to an increased concentration of organic matter. In winter, the average amount of COD (0.712 g/day) was greater than the soluble COD in autumn. Accordingly, the characteristics of fluctuations in organic loads (COD) were based on factors like seasons and university activities as such factors impact water consumption and organic loading.

From day 1-79 days (Fig. 3a), the COD concentration was low compared to the period from day 84-172 days. Initially, the concentration of COD (primary effluent) increased from 388-88.25 mg L^{-1} in effluent ARIBB 1 and 126 mg L^{-1} in effluent ARIBB 2. The COD elimination efficiency was 77.25 and 67.53% on the 1st day, respectively for ARIBB 1 and ARIBB 2. The average COD concentration in the primary effluent increased by 310.77 mg L^{-1} (August-October) and 601.82 mg L^{-1} (November-January). This COD performance in ARIBB 1 and ARIBB 2 was stable in the first quarter of the annual cycle.

The effluent concentration (COD) in ARIBB 1 on the 1st day of sampling from August to 60 days (September) averaged 94.25$\pm$11 and 84.45$\pm$9 mg L^{-1}, respectively. However, the average concentration of COD in ARIBB 2 was slightly higher than that of ARIBB 1 over those 2 months (121.89$\pm$6 and 125$\pm$8 mg L^{-1}, respectively). Subsequently, the organic loading of COD increased progressively, peaking

Table 2: Soluble COD abatement rate and bioreactor performance (February-June)

	Average COD >0.5 g/day					
	Winter			Winter-Spring		
	Minimum	Maximum	Average	Minimum	Maximum	Average
COD (ARIBB 1) (%)	60.00	82.15	68.85	65.51	87.32	76.57
COD (ARIBB 2) (%)	22.03	62.46	44.67	23.17	66.22	43.35

at 940 mg L^{-1} on 125 days (December) in the decanted effluent. The application of its fillers during this 1st stage to January gave an average of 149.86-174.40 mg L^{-1} COD in the effluents treated with ARIBB 1 and 225.9-313.57 mg L^{-1} COD in ARIBB 2. In addition, the COD abatement rate in ARIBB 1 increased by more than 80% on the 105th day (November) and stabilized around 70% on the 179th day in January. However, the abatement rate in ARIBB 2 on the same day (105th day) was 54.93% in November and decreased to 30.14% on the 179th day before the end of January. The volume of wastewater treated and the external supply of nitrate made it possible to maintain stable systems, particularly for ARIBB 1, in terms of the elimination of COD whose higher organic matter loads explained this observation during this phase. Throughout the experimental period (summer-spring), the efficiency of COD reduction in ARIBB 1 was greater than ARIBB 2, which showed that ARIBB 1 possessed a high COD reduction capacity in wastewater.

Effect of the organic load applied and the elimination efficiency

First phase (August-January): The composition of the loading rate of the primary effluent varied throughout the study according to the seasons. The necessary contact time of wastewater and active biomass or hydraulic retention time (HRT) is an important parameter for all bioreactors. In this study, HRT was fixed at 6 h in ARIBB 1 and ARIBB 2 to study the effect of nitrate concentration on the performance of the system during the experimental period. The rate of reduction of COD by ARIBB 2 varied from 37.47-68.73% at 25°C or 0.033 g m^{-3} day, when the average load of CODs applied was less than 0.5 g COD/day. However, when the initial average COD concentration was greater than 0.5 g/day, the COD elimination ranged from 25.56-67.85% at 18.3°C or 0.072 g m^{-3} day. Unlike ARIBB 2, when the wastewater was treated with ARIBB 1, the rate of reduction of COD (effluent) varied between 61.16 and 78.84%, i.e., 0.022 g m^{-3} day, when the same average COD loads were applied (<0.5 g COD/day). Subsequently, the application of a high average COD load (>0.5 g/day) led to a good COD removal with 0.041 g COD m^{-3} day, ranging from 64.12-81.63%, which was higher than the ARIBB 2.

Second phase (February-June): Results for changes in organic loads (COD) in the decanted primary effluent continued to be significant in the second stage from late January-June, with a second peak at 970 mg L^{-1} (240 days, Fig. 3a) in March, while the COD concentration of raw sewage reached a maximum of 1.150 g COD/day.

As demonstrated by the experience of the pilot station, which treated water from November through June, COD loads varied with the season. In this second phase (from February until June), the overall efficiency of COD elimination was conditioned by the role played by the denitrifying flora in the reduction of organic matter. In winter, the average load (0.712 g COD/day) of primary effluent was even higher than COD in autumn (0.453 g COD/day). Therefore, soluble COD (COD> 0.5 g L^{-1}) had a reduction rate of 0.0485 g COD m^{-3} day, ranging from 60% (291 days) to a maximum of 82.15% (243 days) after several days of adaptation (16-19°C) of the COD in the effluent treated with ARIBB 1, whereas, the effluent of ARIBB 2 was 22.03-62.46% (Table 2).

In the spring, effluent quality began to improve, especially for a treatment volume of 4 m^{-3}. The elimination of the pollutant load (COD) in the ARIBB 1 effluent varied from 65.51-87.32%, whereas for ARIBB 2, it varied from 23.17- 66.22% (21-23°C), respectively, in the treated effluent of 249 and 289 days (April-June). Based on the average organic loading rate of 0.697 g/day (Fig. 3b, Table 2), ARIBB 1's average load of primary effluent was 0.044 g COD m^{-3} day for this period (February-June) and, ARIBB 2's, 0.086 g COD m^{-3}. This observation, similar to past seasons, is favored by the external nitrate, which makes it possible to maintain the efficiencies observed continuously in the COD abatement rates.

NO_3 removal

Nitrate concentration: Fig. 4a, b show the concentration and removal of nitrates under different environmental conditions during the ARIBB treatment process when the COD loads in the decanted sewage (primary effluent) increased gradually. Experience has shown that the external addition of nitrate in

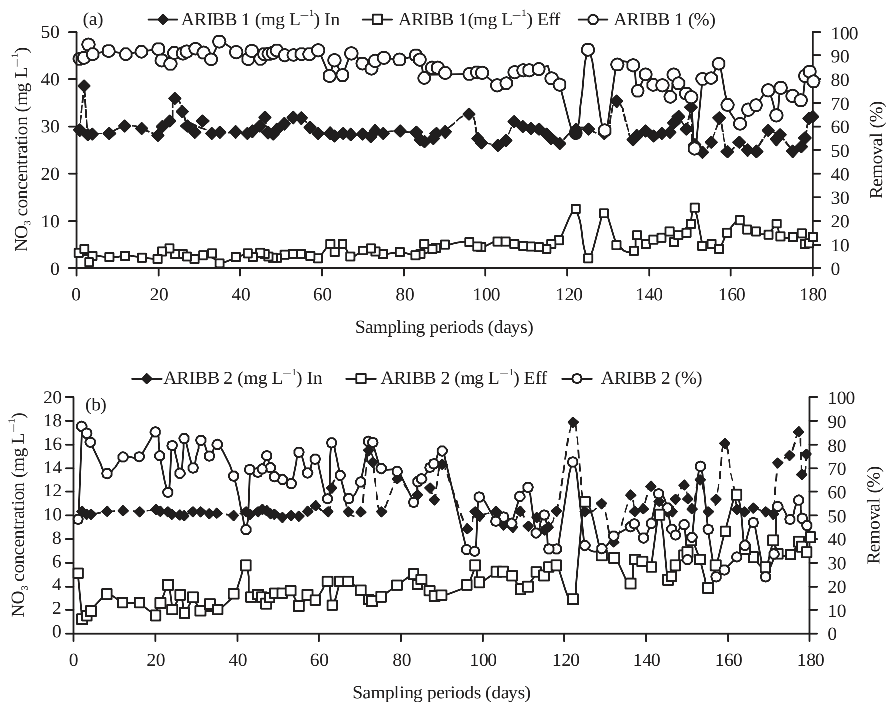

Fig. 4(a-b): Trend of nitrate treatment (a) ARIBB 1 and (b) ARIBB 2

ARIBB 1 strongly affects the concentration of COD in the treated water. The r ate of nitrate applied to the reactor and NO_3 injected (600 mg L^{-1}) in ARIBB 1 provided a concentration of 29.4 and 38.52 mg L^{-1} for the 1st and 2nd day within ARIBB 1. After treatment, the concentration of nitrate decreased from 29.4-3.32 mg L^{-1} on the 1st day and from 38.52-4.2 mg L^{-1} on the 2nd day in treated effluents (August). Then 10 mg L^{-1} (internal measurement of the ARIBB 2 reactor without nitrate injection) to 5.14 and 10.30-1.30 mg L^{-1} for the 1st and 2nd day of August in the effluent ARIBB 2, when COD was between 300 and 400 mg L^{-1} (summer-autumn).

Similarly, the concentration of nitrate (NO_3) in the reactors increased from 29.5 mg L^{-1} for ARIBB 1 and 17.8 mg L^{-1} for ARIBB 2-2.29 and 11.15 mg L^{-1} of the treated water (125 days) of ARIBB 1 and ARIBB 2, respectively, when the organic COD load of the decanted effluent reached 940 mg L^{-1} at the beginning of December. In general, the efficiency of denitrification, i.e., improved COD removal, increased with the external addition of nitrate in ARIBB 1 as well as the concentration of high organic load in the decanted wastewater. The mean concentration of nitrate varied between 24.65 mg NO_3 L^{-1} (153 days) and 38.52 mg NO_3 L^{-1} (2 days) in the ARIBB 1 influent during this phase (August-January). Throughout the wastewater treatment monitoring, ARIBB 1 was able to remove large quantities of nitrate with a maximum removal rate of 95.78% on 35 days (September).

However, the NO_3 abatement rate remained stable between 77.63 and 95.78% from the 1st day of August to 103 days in November. In addition, the average effluent discharge during the summer to early fall was 88.05%, with an average of 3.49 mg NO_3 L^{-1} in the effluent. In addition, the nitrate abatement rate between December and January ranged from 50.91-92.24% for ARIBB 1 (Fig. 4a).

The same trend was observed when the nitrate concentration (ARIBB 2 internal measure) varied between 7.77 mg L^{-1} (136 days) and 17.8 mg L^{-1} (125 days) in Fig. 4b and had a lower average abatement rate from August-January than ARIBB 1. Approximately 4.68 mg NO_3 L^{-1} (treated effluent), i.e., 57.77% NO_3 removed by ARIBB 2 at the end of this phase in January, with a minimum of 24.27% on 171 days and a maximum of 87.38% on 2 days.

Study of the effect of nitrate on the improvement of COD elimination: There is a link between the initial concentration of NO_3 and the reduced COD, although the performance of the treatment under different operating conditions throughout the experiment is quite different. For a reduction of at least 50% organic loading of COD, an average concentration of 10 mg L^{-1} nitrate should be available for COD

<0.5 g/day on average in the primary effluent (Fig. 4a, b). This would produce 70.74% nitrate reduction in ARIBB 2. On the other hand as soon as the COD> 0.5 g/day, the mean concentration of nitrate in the ARIBB 2 influent was 11.27 mg L^{-1} from November to the end of January, which degraded its nitrate performance to only 44.71% nitrate reduction (Fig. 4b). While ARIBB 1, with an average of 28.5 mg L^{-1} of nitrate (ARIBB 1 internal measure), improved its performance to a total of 76.83% nitrate removal in treated water (Fig. 4a). The interesting point to note is that the external addition of nitrate in our study, in contrast to the long and complex biological nitrification process and, given the different organic loads of COD applied to ARIBBs, made it possible to judge the level of the denitrification process compared to other processes in the literature is discussed below.

DISCUSSION

Anaerobic digestion processes occur in many places where organic matter is available and the redox potential is low (zero oxygen). The availability of electrons in organic carbon compounds is one of the most important factors controlling the activity of denitrifying heterotrophic flora[14]. The anaerobic conditions of the bioreactors explained the average effluent COD removal rates, particularly that of ARIBB 1 (0.041 g COD m^{-3} day), because the average organic load (0.746 g/day) of the wastewater was high since that time. ARIBB 1 showed a maximum rate of 81.63% COD reduction, which was obtained when COD> 0.5 g/day. This observation can also be explained by the concentration of the electron acceptor (600 mg L^{-1}), the external nitrate (NO_3), which was pumped at the inlet of the ARIBB 1 bioreactor.

In the denitrification process, nitrate is reduced to nitrogen oxides by isolates that use nitrate, instead of oxygen as electron acceptors and organic matter as a carbon and energy source[15]. The maximum removal of nitrogen by denitrification is achieved when the organic material is used an electron donor, this was the case with the different COD loads applied to the ARIBB bioreactors. This is the concentration of the nitrate in the influent, which allowed ARIBB1 a better treatment of the organic load. Although it was lower than the COD yields in the combined system (UASB and SBR), i.e., 91% used by Torres and Foresti[16] but was comparable to that of the up flow anaerobic sludge blanket and sequencing batch reactors (UASB), with 72%. Similarly, Kayranli and Ugurlu[17] showed that the yields of COD were 80% at HRT 11 h and 15°C. In addition, the same observation was made by Moharram *et al.*[18] with HRT of 15 h and internal recirculation (IR) = 200% of the effluents. But compared to our system, ARIBB 1 had the best-operating conditions the most economical. Due to the external contribution of nitrate to the elimination of COD in ARIBB 1 (73.4%) but also the maintenance of efficacy without internal recirculation at HRT of 6 h. This could be a significant contribution to other existing systems.

So, whatever the organic load, the addition of nitrate promotes a high and continuous percentage of denitrification potential in the anaerobic reactor (ARIBB 1)[19]. In the same way that the demand for oxygen is directly related to the organic charge, so too is the demand for nitrates (denitrification potential) because oxygen and nitrate act as electron acceptors for the same degradation reactions. Thus, at least 71% (COD), representing the organic load fraction applicable of the wastewater, have been removed by denitrification (ARIBB 1). Unlike ARIBB 1, the same load applied to the ARIBB 2 bioreactor is considerably higher than the available nitrate (7.09 mg L^{-1}). Therefore, the stability of the ARIBB 2 system cannot be maintained. Consequently, the organic fraction (COD) remains concentrated in the effluent (since there is no acceptor sufficient to transfer electrons), which gives a removal efficacy of 44% for ARIBB 2 over the entire duration, from February to June of the study.

The process of denitrification in a wastewater treatment plant depends on several factors: Biological kinetics and physicochemical parameters such as pH, temperature, viscosity, substrate concentrations, dissolved oxygen concentration, low COD/N ratio and the high concentration of nitrite[20-21]. However, the domestic wastewater used in our study typical to domestic influent from Korkusuz *et al.*[22], Caselles-Osorio *et al.*[23], had significantly lower parameters, particularly nitrites because the nitrate concentration was still low. But also, the ARIBB functioned in an anaerobic environment and therefore no nitrification. It could also be seen that the temperature did not have a significant effect on bacterial activity during the winter period within ARIBB 1, with a nitrate variation between 27.49 mg L^{-1} (day 198) and 58.6 mg L^{-1} 299 days in May (Fig. 5a, 2nd phase). Zhou *et al.*[24] showed that temperature is an important factor that affects the efficiency of denitrification. According to Adouani *et al.*[21], denitrification activities are minimal below 5°C and increases linearly to a maximum of about 25-30°C and then decreases to a minimum at about 65°C where growth is stopped due to enzymatic denaturation activity[25].

This was not observed during the nitrate-associated ARIBB 1 study. Instead, the COD removal was improved with the addition of nitrate because the bacteria and denitrifiers consuming COD are heterotrophic and would favor a

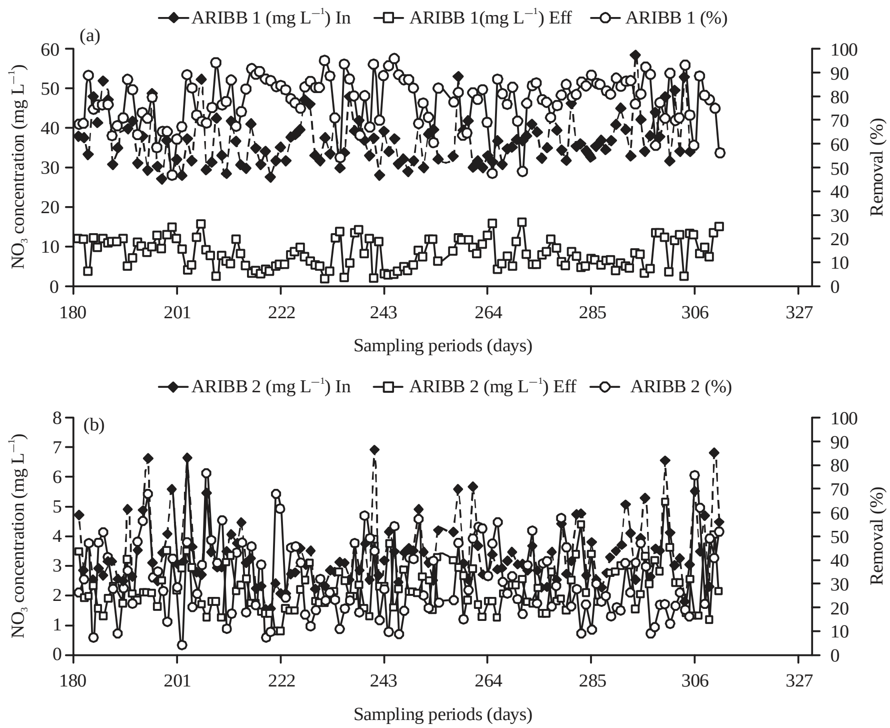

Fig. 5(a-b): Evolution of reactor nitrate loading and performance (a) ARIBB 1 and (b) ARIBB 2

symbiotic ecosystem since the denitrifiers consume COD (electron donor) and require nitrogen (NO_3). Under these circumstances, a sufficient concentration of terminal electron acceptors became a growth factor. But also could be a solution to the doubling of nitrifiers, which require 10-20 times longer than for other heterotrophic bacteria[26].

The low rate of nitrate loading in the applied primary effluent became a limiting factor in the denitrification process for ARIBB 2 compared to the high COD electrons (> 0.5 g/day) to be transferred, constraining the activity and above all, the synthesis of new cells.

Thus, the higher the nitrate loading, the higher the denitrification potential in the anaerobic reactor, reflecting a 73% reduction (COD) in ARIBB 1 and 45.62% reduction in ARIBB 2 (COD) by the end of the experiment, which ran November through June (Fig. 2).

Thus, the hypothesis that nitrate influences the intensity or the improvement of the degradation of the organic loads of COD by the activity of the optional heterotrophic bacteria in the two reactors was verified. This bacterial activity determined nitrogen (NO_3) suppression of 77.57 and 38.59%, respectively, in ARIBB 1 and ARIBB 2 from November-June (Fig. 4 and 5).

Furthermore, the concentration of nitrate that an anaerobic reactor can denitrify biologically, i.e., the denitrification potential of this reactor, depends on the presence or absence of the nitrate feed in the anaerobic digester. This affects denitrification because the denitrification potential is a function of the nitrate concentration[19]. This observation could explain the overall performance of ARIBB 1, i.e., 72.16% owing to the nitrate injected permanently for a treatment of 6 h. Because, for each 1 mg NO_3 denitrified with N_2 gas in the anoxic zone, 8.6 mg COD is used (theoretically). The oxygen equivalent of nitrate is 2.86 mgO_2/mg NO_3, which means 1 mg NO_3 denitrified with N_2 gas at the same electron acceptability as 2.86 mg oxygen[19].

CONCLUSION

Based on the experimental results, the treatment of domestic wastewater by ARIBBs bioreactors, allowed us to study various effects of operational parameters such as the organic loads of COD and nitrate applied on the quality of the treated water of the faculty. The ARIBB 1 bioreactor carried out a qualitative treatment of the primary effluents, in spite of the great variations of the organic load, owing to the external addition of nitrate. It was able to maintain stability and efficiencies in accordance with the seasons. The influence of nitrate concentration on performance was able to achieve a different denitrification level for each bioreactor (ARIBB 1 and ARIBB2). The heterotrophic denitrification in the ARIBB 1 reactor reached a sufficient potential, owing to the permanent injection of a nitrate concentration, which improved the average removal of organic material and with that of nitrate, respecting the requirements of the discharge standards in Morocco.

SIGNIFICANCE STATEMENTS

This study demonstrates how wastewater can be effectively and inexpensively treated by passing through an anaerobic reactor system with an immersed bacterial bed and added nitrates. Accordingly, this study can help small communities, that are suffering water shortages, reclaim their wastewater for home or agricultural use through this denitrification process.

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