# Water reuse: dairy effluent treated by a hybrid anaerobic biofilm baffled reactor and its application in lettuce irrigation

K. A. Santos, T. M. Gomes, F. Rossi, M. M. Kushida, V. L. Del Bianchi, R. Ribeiro, M. S. M. Alves and G. Tommaso

## ABSTRACT

There is a synergy between the large quantities of organics-rich effluents generated by the dairy industry and the continually increasing water needs for crop irrigation. In this sense, this study aimed at evaluating the effect of decreasing the hydraulic retention time (HRT) on the stability and efficiency of a hybrid anaerobic biofilm baffled reactor (HABBR) treating simulated fat- and salt-rich dairy wastewater, followed by its agricultural reuse. The reactor was monitored over 328 days, during which 72, 24, and 12 h were the hydraulic detention times. After achieving steady-state, the reactor presented organic matter removal greater than 90% and produced biogas with 41 ± 23%, 53 ± 3%, and 64 ± 12% of methane for HRTs of 72, 24, and 12 h, respectively. The best process performance was observed for an HRT of 24 h, and thus, a lettuce culture was irrigated with the treated effluent. The irrigation was performed in five different treatments, for which the amount of treated effluent added to tap water varied from 0 to 100%. Both the effluent and the harvested vegetables were evaluated for microbial contamination. Apart from the 75% effluent supply condition, there were no losses in leaf mass or area observed; instead, there was an increase of these parameters for the 25% and 50% effluent supply treatment. The use of dairy effluent treated by the HABBR allowed for microbiologically safe food production. Therefore, the process offered both potential cost reduction for fertilizers, preservation of water resources, and a renewable energy source.

**Key words** | agro-industrial effluent, anaerobic digestion, hybrid anaerobic biofilm baffled reactor, *Lactuca sativa*

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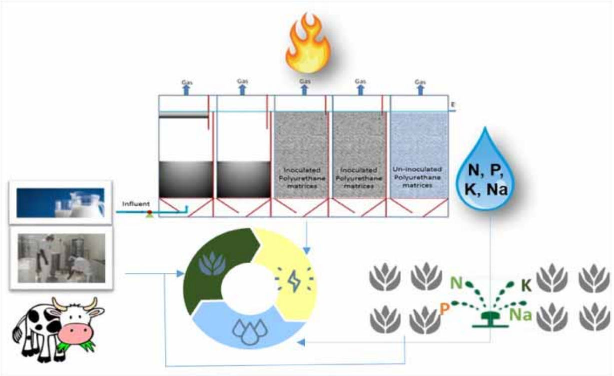
## HIGHLIGHTS

- The anaerobic baffled reactor was stable during treatment of fat-rich salt dairy wastewater.
- The methane content was enhanced with hydraulic retention time reduction.
- The use of dairy effluent reduced the needs related to N fertilizer by up to 50%.
- The use of drinking water for lettuce irrigation has been reduced by 50%.
- The effluent did not present a risk of contamination in lettuce production.

doi: 10.2166/ws.2020.276

## GRAPHICAL ABSTRACT



## INTRODUCTION

The milk processing industry is one of the most important sectors in the food industry. However, as a consequence, there is a large volume of fat-rich and salty effluents generated. Anaerobic digestion has many advantages, in addition to treating wastewater, because it also produces a methane-rich biogas that can be recovered and used as a fuel, providing 20% of energy requirements from the dairy factory and reducing the total carbon footprint emissions by 13%, according to Stanchev *et al.* (2020). Although several studies have highlighted advantages of using anaerobic digestion for the treatment of dairy effluents (Demirel *et al.* 2005; Karadag *et al.* 2015), the effective treatment of such effluents remains a challenge due to its high lipid content and the inhibitory effect that long-chain fatty acids may present in certain concentrations (Alves *et al.* 2009).

The anaerobic baffled reactor (ABR) is a simple and efficient configuration for many wastewater treatments. The main advantage is the natural capability of phase separation, allowing acidogenesis and methanogenesis optimization (Cohen *et al.* 1982; Weiland & Rossi 1991). Consequently, different microbial groups can develop under favorable conditions. This configuration also has longer biomass retention time as an advantage, in addition to the resistance to organic and hydraulic shocks (Barker & Stuckey 1999).

Proposed by Bachmann *et al.* (1985) and extensively studied since then (Zhu *et al.* 2015; Soh *et al.* 2020), ABRs have been widely used for treating several types of effluent, either in their original configuration or presenting some optimizations. ABR are used in the treatment of sanitary effluent (Nasr *et al.* 2009), fish meal wastewater (Putra *et al.* 2020), vinasse from corn ethanol production (Sayedin *et al.* 2018), slaughterhouse wastewater (Al Smadi *et al.* 2019), vegetable waste (Gulhane *et al.* 2017), as well as effluents from oil refineries (Arvin *et al.* 2019) or heavy oil that produce water with high salt concentrations and poor nutrients (Ji *et al.* 2009).

Although very versatile, this reactor presents some limitations. According to Sayedin *et al.* (2018), the main conventional ABRs limitations are related to low biomass growth rate and biomass washout. Problems related to sludge washout are commonly reported in literature when granular reactors are used to treat dairy effluents (Passegi *et al.* 2012). One option to overcome such problems is the granular or flocculent biomass combination with fixed films. These reactors are called hybrid biofilm reactors and they were defined by Büyükkamaci & Filibeli (2002) as a combination of the upflow anaerobic sludge blanket (UASB) in the lower part, with a filter in the upper part. According to the authors, hybrid reactors may provide the advantages of the UASB and anaerobic film reactors, reducing their limitations. According to Karadag *et al.* (2015), hybrid biofilm reactors have been widely applied for dairy wastewaters treatment. Gomes *et al.* (2011) have observed organic matter removal efficiencies greater than 90% using an hybrid UASB reactor equipped with a superior polyurethane bed for dairy effluents treatment, even with high volumetric organic loads, such as 16 $kg \cdot m^{-3} \cdot d^{-1}$. The concept of hybrid biofilms reactors can easily be applied to ABRs, due to its natural compartmentalization. In this way, Fujihira *et al.* (2018) introduced a polyurethane bed inside an ABR with four chambers treating a solid/lipid-rich wastewater, while Nguyen *et al.* (2020) included a fixed-bed stage outside an ABR used for synthetic dyeing effluents treatment.

Even with an elevated performance, anaerobic reactors have not been able to produce effluents that meet such strict standards required for treated effluent discharge in water bodies, both in relation to residual organic matter and nutrient concentrations. The complete treatment is expensive because it aims to remove nutrients and salts, thus including tertiary and advanced level (thus including tertiary and advanced level), and small- to medium-sized dairy plants have therefore frequently encountered difficulties in controlling their emissions. Based on this scenario, the anaerobically treated effluents reuse would be advantageous for resources recycling, eliminating the need for a further post-treatment. In fact, the reuse of anaerobically treated wastewaters for crop irrigation can be an alternative for small wastewater treatment plants, which saves resources related to moisture and nutrient provision (Bame *et al.* 2014; Gomes *et al.* 2015).

Although dairy wastewater is a moisture and nutrient source, its salinity may restrict the agricultural reuse (Matsumoto *et al.* 2012). On the other hand, it is important to consider that different cultures may show different behavior related to salinity tolerance and nutrient uptake rates (Oliveira *et al.* 2011; Bourazanis *et al.* 2016). Lettuce (*Lactuca sativa L*) is considered 'moderately sensitive' to the presence of salts (Ayers & Westcot 1999) and is a vegetable known for its high vitamin A content. It adapts well to greenhouse cultivation and has a low cost of production with a short production cycle (Filgueira 2008). However, the needs for lettuce irrigation are high. These characteristics make this cultivar a candidate for irrigation with agro-industrial effluents.

Besides crop yields, another paramount aspect of agricultural reuse is the microbiological security of produced vegetables. The major concern is focused on the farmer health care, as well as agricultural products consumers, especially in the case of vegetables grown on the ground and consumed raw. Most studies that consider agro-industrial reuse have been carried out to evaluate the quality of effluents and post-harvested vegetables in relation to physic-chemical parameters, and further studies are required on their microbiological quality (WHO 2006).

Considering a circular economy scenario implementation, through water reuse in agriculture, this research has aimed to present data on a modified ABR, a hybrid anaerobic baffled biofilm reactor (HABBR), treating simulated fat-rich and salt dairy wastewater, posteriorly reused for irrigation of lettuce crops. Regarding the reactor operation, the objective was to verify the hydraulic retention time (HRT) influence, and hydraulic and organic shocks application on the overall organic matter removal efficiency. Our hypothesis was that, if properly operated, the HABBR could combine advantages from suspended biomass use to the treatment of fatty rich wastewaters, as preconized by Alves *et al.* (2009), while also preventing biomass losses, as observed by Gomes *et al.* (2011), providing a solid-free effluent as the necessary, considering irrigation purposes. The treated effluent from the best operational conditions was used to irrigate a lettuce crop to verify the influence on the amount of effluents used on the crop yield as well as on the biological safety of those produced vegetables. The results presented here have proved that the anaerobic digestion may be a key technology to an integrated approach to the Food–Energy–Water Nexus, thus providing security for the following generations.

## METHODS

### HABBR: configuration, operation, and monitoring

#### Reactor operation

A bench-scale HABBR was monitored in three operational conditions, namely OC1, OC2, and OC3, which were differentiated according to the applied HRT, which were 72, 24, and 12 h, respectively. The HABBR reactor (Figure 1) was constructed using acrylic glass, with five chambers with a total volume of 25 L. The

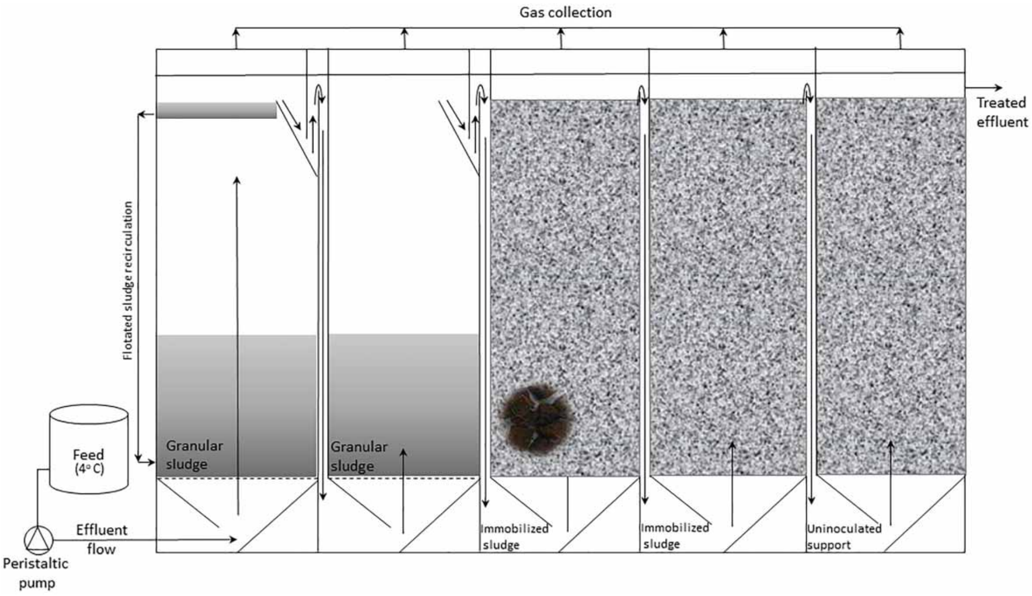


**Figure 1** | Scheme of the HRT – HABBR.

first and second chambers contained granular biomass, and cubic polyurethane foam matrices (1 cm side length) were used as a support for biomass immobilization in chambers 3 and 4. Chamber 5 was provided only with polyurethane matrices. The granular inoculum was obtained from an efficient UASB reactor treating effluent from starch production. The reactor was kept inside a temperature-controlled chamber (37 ± 2 °C) to achieve temperature stability. During OC2, a hydraulic and organic load shock experiment was carried out to verify the possibility of decreasing the HRT from 24 to 12 h. The procedure was performed three times and consisted of reducing the HRT to 12 h for 36 h, subsequently subjecting the system to 24 h of HRT for 72 h. In all conditions, the HRT was maintained by a peristaltic pump (Gilson Mini plus) with four channels connected to ports distributed equidistantly across the reactor's width.

## Substrate

Formulated dairy effluent with organic matter concentration expressed as chemical oxygen demand (COD) of 3 $g{\cdot}L^{-1}$, oil and grease of 310 $mg{\cdot}L^{-1}$, and a conductivity of 4.5 $mS{\cdot}cm^{-1}$ was used to feed the HABBR. The effluent was supplemented with 1 $g.L^{-1}$ sodium bicarbonate ($NaHCO_3$), and its pH was corrected with a 10% hydrochloric acid (HCl) solution to a range within pH 7.0–7.5. The formulated wastewater composition was based on the characterization previously performed by Cichello *et al.* (2013), which is presented in Table 1. The composition of macronutrient and micronutrient solutions were prepared according to Zhender *et al.* (1980). The substrate reservoir was maintained at a temperature below 4 °C to minimize biochemical reactions. Before entering the reactor, the substrate was heated to 37 ± 2 °C.

## Reactor monitoring

The reactor was monitored twice a week. Organic matter concentration expressed as COD, Kjeldahl nitrogen (total and

**Table 1** | Formulated dairy wastewater composition

| **Component** | **Concentration** |
|---|---|
| Skim powdered milk | 2 g $COD{\cdot}L^{-1}$ |
| Milk cream (35% fat) | 1 g $COD{\cdot}L^{-1}$ |
| Peracetic acid | 0.0181 $ml{\cdot}\mathbf{L}^{-1}$ |
| Hydrogen peroxide (33%) | 0.028 $ml{\cdot}\mathbf{L}^{-1}$ |
| Nitric acid | 0.39 $ml{\cdot}\mathbf{L}^{-1}$ |
| Macronutrients solution | 1.8 $ml{\cdot}\mathbf{L}^{-1}$ |
| Micronutrients solution | 1 $ml{\cdot}\mathbf{L}^{-1}$ |

ammoniacal) concentration solids (gravimetric method), phosphorus (spectrophotometric method), sodium (using flame photometer), and chloride (using inductively coupled plasma–atomic emission spectrometry – ICP–AES), were performed according to *Standard Methods for the Examination of Water and Wastewater* (APHA 2012). The pH value was measured with a calibrated potentiometer. Bicarbonate alkalinity and total volatile acid content were determined by titration, according to the methods proposed by Ripley *et al.* (1986) and Dillalo & Albertson (1961), respectively. The biogas composition was determined by gas chromatography, using a thermal conductivity detector (Shimadzu) equipped with a Carboxen 1010 PLOT column, 30 m × 0.53 mm, according to recommendations contained at Rosero-Henao *et al.* (2019).

## Agricultural reuse

Agricultural reuse was performed using simulated dairy effluent treated in the HABBR after achieving a steady state during the second operational condition. The experiment was conducted in a greenhouse with 100 $m^2$, in polycarbonate, and a gable roof. It consisted of a randomized block using factorial design 5 × 4 with four replicates. The treatments (T) consisted of different proportions of tap water (TW) and the anaerobically treated dairy wastewater (ANE): T1, 0% ANE +100% TW; T2, 25% ANE +75% TW; T3, 50% ANE +50% TW; T4, 75% ANE +25% TW; and T5, 100% ANE +0% TW. The lettuce seedlings were placed in 2.5-L pots on 24 April 2015. The predominant soil in the region, classified as Rhodic Hapludox, was used to fill the pots (EMBRAPA 1999). A composite sample was taken and sent to the Laboratory of Agricultural Sciences/ ZAZ/FZEA (Table 2).

The characteristics presented in Table 2 were defined for lettuce crop fertilization, as suggested by Raij *et al.* (1996). The pots were filled with 3 kg of soil, which were previously adjusted adding 2.00 $t{\cdot}ha^{-1}$ of limestone (100%), 100 $kg{\cdot}ha^{-1}$ of N, 400 $kg{\cdot}ha^{-1}$ of phosphorus pentoxide ($P_2O_5$), and 100 $kg{\cdot}ha^{-1}$ of potassium oxide ($K_2O$), supplied by ammonium nitrate ($NH_4NO_3$) (34% of N), simple superphosphate (18% of $P_2O_5$ and 16% of Ca and 8% S), and potassium chloride (KCl) (60% of $K_2O$). The N fertilization was 100, 75, 50, 25, and 0% for T1, T2, T3, T4, and T5, respectively, and it was complementary to the addition of the treated effluents.

Irrigation management was based on the replacement of estimated crop evapotranspiration (ETc). This was calculated using a reduced Class A evaporation pan. The evaporation measure was multiplied by the culture coefficient (Kc) proposed by Marouelli *et al.* (2008) for different crop development stages. The correction coefficient (Kp) used for the reduced pan inside the greenhouse was 1, as recommended by Farias *et al.* (1994). The frequency of irrigation adopted was 2 days, in which the water volume was applied manually with the help of a graduated bottle.

The seedlings were harvested 41 days after transplanting. The analyzed parameters were the wet and dry weight of roots and leaves and the leaf area measured by LI-Cor model LI 3100. The data were subjected to a wide analysis range. For situations in which there were significant differences, the means were subjected to regression analysis.

**Table 2** | Result of soil analysis used to fill the experimental portions

| **pH ($CaCl_2$)** | **P (res) ($mg{\cdot}dm^{-3}$)** | **S ($mg{\cdot}dm^{-3}$)** | **K (res) ($mmolc{\cdot}dm^{-3}$)** | **Ca ($mmolc{\cdot}dm^{-3}$)** | **Mg ($mmolc{\cdot}dm^{-3}$)** | **H + Al ($mmolc{\cdot}dm^{-3}$)** | **OM ($g{\cdot}kg^{-1}$)** |
|---|---|---|---|---|---|---|---|
| 5.5 | 11 | 20 | 2.1 | 21 | 9 | 44 | 11 |
| SB | CTC | V | B | Cu | Fe | Mn | Zn |
| ($mmolc{\cdot}dm^{-3}$) | ($mmolc{\cdot}dm^{-3}$) | (%) | ($mg{\cdot}dm^{-3}$) | ($mg{\cdot}dm^{-3}$) | ($mg{\cdot}dm^{-3}$) | ($mg{\cdot}dm^{-3}$) | ($mg{\cdot}dm^{-3}$) |
| 32 | 76 | 42 | 0.58 | 0.5 | 10 | 1.5 | 0.5 |
| Total sand | Clay | Loam | | | | | |
| ($g{\cdot}dm^{-3}$) | ($g{\cdot}dm^{-3}$) | ($g{\cdot}dm^{-3}$) | | | | | |
| 640 | 341 | 19 | | | | | |

OM, organic matter; SB, sum of bases; CTC, cation exchange capacity; V, base saturation.

Sample of leaves were washed with HCl solution (0.1%) and dried in a forced circulation oven (65 °C) until reaching constant weight. Subsequently, they were processed in a mill and sent of nutritional diagnosis analyses for the macronutrients N, K, Ca, S, P, Mg, and Na in accordance with Malavolta *et al.* (1997).

## Microbial analyses

### Effluents

Samples of 20 mL of the treated effluent were collected aseptically in a sterile container and analyzed weekly for 4 weeks. Decimal dilutions in saline peptone water were prepared according to the estimated contamination and analyzed for the presence of total coliforms and *Escherichia coli presence*, which were determined by inoculation, in duplicate, in Compact Dry EC plates (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan). Heterotrophic bacteria were analyzed by inoculation on to the surface of Plate Count Agar (PCA), and incubated at 35 ±2 °C for 48 h (Vanderzant & Splittstoesser 2001; Silva *et al.* 2010).

### Plants

Cellulose sponges soaked in buffered peptone water were used to aseptically recover microorganisms from the surface of lettuce leaves before harvesting them to sample the many conditions of soil contact, composition, and hydrophobicity. Decimal dilutions in saline peptone water were prepared according to the estimated sample contamination, which were determined by inoculation performed in duplicate. The total quantification of total mesophilic counts (PCA plates at 35 ±2 °C for 48 h), psychrotrophic (PCA plates at 7 ±2 °C for 10 days), molds, and yeasts (Dichloran Rose Bengal Chloramphenicol Plate (DRBC) at 25 ±2 °C for 10 days) were performed according to Vanderzant & Splittstoesser (2001) and Silva *et al.* (2010). Total coliforms and *E. coli* were analyzed by inoculation on Compact Dry EC plates (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) at 35 ±2 for C/24 hours. The quantification of *Staphylococcus aureus* was performed by inoculation on Compact Dry X-SA plates (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) at 35 ±2 °C for 24 h. The presence of *Salmonella* spp. was verified according to the Association of Official Analytical Chemists International (AOAC) Official Method 2003.09 (AOAC International 2007), using the BAX System PCR Assay for *Salmonella* (Du Pont Qualicon Co.) as certified by the AOAC Research Institute No 100.201.

## Statistical analysis

Data regarding agricultural reuse were subjected to multiple analysis. For situations in which there were significant differences indicated by the Tukey test (5% significance level), the means were subjected to regression analysis. The software used was SISVAR 5.3 (Ferreira 2011). The results from the microbial analyses were analyzed by the Kruskal–Wallis statistical test, often used to test null hypothesis where all treatments have equal distribution functions against alternative hypothesis that at least two treatments present different distribution functions. The null hypothesis have argued that the effluent use and concentration in the irrigation medium did not statistically interfere in the microbiological contamination. The alternative hypothesis have argued that treatments generate different results.

# RESULTS AND DISCUSSION

## Reactor startup and monitoring

Figure 2(a) shows the efficiency of removing organic matter in each HRT over the entire operating time. The average concentration of organic matter expressed in COD was 3,078 $mg{\cdot}L^{-1}$ at the reactor inlet and 273 $mg{\cdot}L^{-1}$ at the system outlet, which represented an efficiency of 91 ± 2.4%. An average filtered COD of 2.4 $g{\cdot}L^{-1}$ was observed at the entrance and 0.23 $g{\cdot}L^{-1}$ at the output, which represented COD removal efficiency of 91 ± 3% in relation to the filtered sample. The average methane fraction in the analyzed samples throughout the operation was 46 ± 23%. The observed results are in the same range of values observed by Langenhoff *et al.* (2000) who evaluated the performance of an ABR with eight compartments and total volume of 10 L. The reactor was fed with diluted semi-skimmed milk with COD of

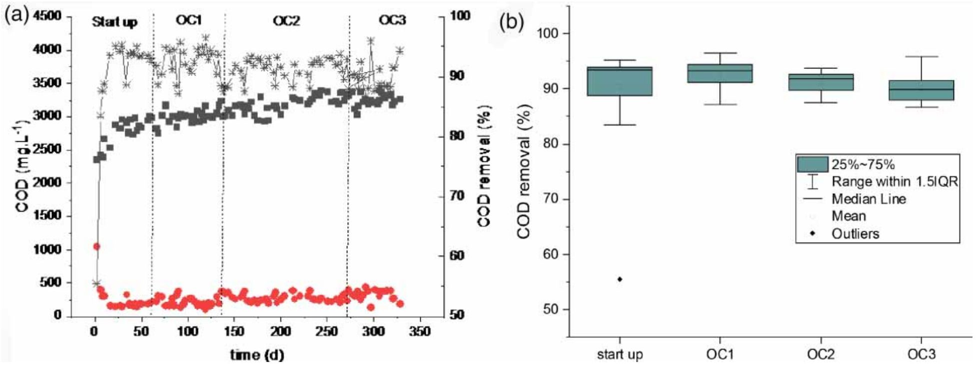


**Figure 2** | Values of organic matter concentration expressed in COD and removal efficiency along the startup, operational condition 1 (OC1), operational condition 2 (OC2) and operational condition 3 (OC3): affluent COD (■), effluent COD (●) and COD removal efficiency (Ú) (a); boxplot graphic for COD removal efficiency obtained for the startup, OC1, OC2 and OC3 (b).

0.5 g·L$^{-1}$ and it was operated with HRT ranging between 80 and 10 h. For all applied HRT, COD removals with values above 80% were achieved.

The reactor showed stable operation throughout all conditions studied, which could be observed through consistent bicarbonate alkalinity, as well as rising methane content in the biogas. The reactor robustness can also be stated, taking into account the low variability in the values obtained for COD removal, as shown in Figure 2(b).

## Start up and first operational condition

The reactor was operated over 149 days with a hydraulic detention time (TDH) of 72 h and an organic loading rate (OLR) of 1 g.L$^{-1}$·d$^{-1}$, comprising the reactor startup and OC1. During the first 28 days, the organic matter removal efficiency went from 51% to 90%. The production of bicarbonate alkalinity was verified since the beginning of the operation; however, the startup period was considered concluded within 59 days of operation due to the consistency of such production and because the organic matter removal had a variation below 2% (Seborg *et al.* 1989). The results are similar to those observed by Jürgensen *et al.* (2018), who studied an ABR (HRT of 1.6 d) preceded by an continuous stirred tank reactor, which served as a buffer tank. According to the authors, the system startup treating dairy wastewater (organic loading rate (OLR) ranging from 1.25 and 4.50 g.L$^{-1}$·d$^{-1}$, total HRT of 9.2 days) lasted 90 days, after which 82% of organic matter removal was achieved. After 60 days, Gomes *et al.* (2011) achieved, on average, 90% of organic matter removal in a hybrid UASB reactor treating dairy wastewater fed with an OLR of 1.1 g·L$^{-1}$·d$^{-1}$.

At the end of OC1, the HABBR showed removal averages of organic matter of 92 ± 3% and 92 ± 2% (referring to the results of the last 90 days of operation) for unfiltered and filtered samples, respectively. The methane fraction in the biogas was, on average, 41 ± 23%. The results were comparable to those verified by Wang *et al.* (2004) who applied a TDH of 96 h in an ABR treating glucose-based wastewater with COD of 2.5 g·L$^{-1}$. The authors observed that COD removal efficiency was 94% at the end of the experiment. The HABBR showed stability throughout OC1, with an average production of alkalinity to bicarbonate of 266 ± 126 mg $CaCO_3$·L$^{-1}$. At the end of this condition, a ratio between intermediate alkalinty and partial alkalinity (IA/PA) of 0.1 was verified at the reactor output, which reinforces the reactor stability. Riplay *et al.* (1986) proposed to determine the relationship between intermediate alkalinity (related to the volatile acids) and partial alkalinity (related to bicarbonate) to address the anaerobic processes stability. Considering this, values higher than 0.3 indicated the occurrence of disturbances in the process. Biomass flotation occurred due to the applied oil and grease load, which was 0.105 g COD·L$^{-1}$·d$^{-1}$. However, each chamber was equipped with deflectors to biomass containment, which prevented sludge displacement to other compartments.

## Second operational condition

The HABBR was monitored during 105 days with a TDH of 24 h and OLR of 2.96 g.$L^{-1}$·$d^{-1}$ during OC2. The average COD removal efficiency was 91 ± 1.8% for unfiltered and filtered samples of 91 ± 2.0%. Biogas presented a methane fraction of 53 ± 27%. The reactor was operated with stability, with an average alkalinity production of 380 ± 32 mg $CaCO_3$·$L^{-1}$. After 12 days at this operational condition, a biomass flotation in the first chamber due to an oil and grease load of 0.31 g COD·$L^{-1}$·$d^{-1}$ was observed. The floated biomass was grayish in color. This phenomenon was previously verified by Gomes *et al.* (2011) while operating a hybrid UASB treating dairy effluents (OLR ranging from 0.5 to 15.8 g·$L^{-1}$·$d^{-1}$). The authors verified a biomass flotation when the OLR of 4.6 g COD·$L^{-1}$·$d^{-1}$ was applied, which is higher than in OC2 when considering the entire reactor volume. Nonetheless, it is important to consider that ABR resemble a sequence of complete mix reactors in series (Barker & Stuckey, 1999). In the first chamber of the HABBR, the OLR was 9.5 g·$L^{-1}$·$d^{-1}$. Thus, clogging problems have occurred due to suspended biomass displacement to the third compartment, which contained adhered biomass. Bottom discharges and floated biomass removal were sufficient to solve such problems that did not occur during the entire operation. According to Alves *et al.* (2000), anaerobic biomass is susceptible to long-chain acids acclimation, which reverses the inhibitory effects of such molecules. Thus, when adapted to a higher concentration of oil and grease, the HABBR biomass was able to be degraded in such fraction, avoiding this substrate accumulation or its degradation intermediates (i.e. long-chain fatty acids), consequently impairing biomass flotation.

After 90 days of operation, a hydraulic and organic load shock experiment was performed. The experiment consisted of reducing detention time to 12 h for 36 h, and subsequently submitting the reactor to detention time of 24 h for 72 h. The procedure was repeated three times, and according to the monitored performance during this process, the alkalinity values were comparable to those verified in the OC1, with average production values of 346 ± 26 mg $CaCO_3$·$L^{-1}$. The values of the IA/PA ratio were lower than 0.3 for the three sequential shocks applied, which indicated that the HABBR quickly absorbed shocks without suffering disturbances due to accumulations of volatile acids. The average removals of organic matter were 85 ± 4.5%, 85 ± 4%, and 83 ± 4.8% for the three sequential shocks applied. The biogas composition showed an increase in the methane fraction, which was, on average, 64 ± 1.3%. During application periods that lasted 24 h, between the shock applications, the removal of organic matter was, on average, 90.5 ± 0.5%. Manariotis & Grigoropoulus (2002) studied the performance of an ABR containing three compartments and treating synthetic effluent with an OLR ranging from 0.303 and 0.662 kg COD·$L^{-1}$·$d^{-1}$. For HRT of 24 h and 12 h, the authors obtained an efficiency COD removal of 83% and 80%, respectively.

## Third operational condition

During the third experimental condition, the system was operated for approximately 71 days, with a hydraulic detention time of 12 h and an OLR of 5.92 g.$L^{-1}$·$d^{-1}$. During this period, the average methane fraction in biogas was 62 ± 12%. The reactor showed consistent production of alkalinity, which was, on average, 355 ± 55 mg $CaCO_3$·$L^{-1}$, with IA/PA of 0.2.

Unfortunately, during this condition the reactor constantly presented problems, such as liquid leakage and a partial biogas outlet clogging due to the obstruction caused by the biomass. There was a biomass migration to the fourth compartment, and the treatment using minimum operational maintenance was not viable. For the HABBR operation, with part of its immobilized biomass, the system's floated sludge management must be constant. In this way, Passeggi *et al.* (2012) presented an interesting configuration of UASB reactor equipped with a scum extraction device and a lamella settler. According to the authors, the problems associated with the lipid hydrolysis low-rate lipid and foam accumulation have been resolved by extracting the scum, and 90% removal of organic matter has been verified in their system, which was on an industrial scale. This solution could easily be applied to the; however, if the intention is to keep the operation as simple as possible, conditions similar to those applied at OC2 should be maintained, if the effluent has comparable oil and grease concentrations.

## Agricultural reuse

The research results on water reuse in agricultural crops are different in relation to production maintenance and quality of crops irrigated with treated effluents (Ayoub *et al.* 2016; Shahrivar *et al.* 2019), mainly due to cultivated species variability, the characteristics of these waters, and the specificities of each cultivation site, such as climate and soil. Thus, the application of the HABBR effluent to lettuce irrigation is presented and discussed in the following paragraphs.

The fresh and dry mass of lettuce roots, as well as the concentration of K, Ca, and P in leaf tissue, showed no significant changes among the tested treatments. On the other hand, the dry and fresh leaves mass values, leaf areas, and the values of N, Na, Mg, and S in the leaf tissue were influenced by effluent dosage, with a correlation coefficient greater than 0.92 (Figures 3 and 4).

Thus, an increase in the values of such biometric parameters was observed when T2 and T3 were applied. T2 showed a 37% and 6% increase for fresh and dry leaf mass, respectively, and a 37% increase in leaf areas compared to T1. The observed results are different from those observed by Oliveira *et al.* (2011), who investigated different lettuce cultivars exposed to five levels of water salinity. They observed a linear reduction in the leaf number, leaf area, and mass with an increasing salt concentration. Cáceres *et al.* (2015) applied the nitrified liquid phase from an anaerobic digester fed with cow manure to a lettuce crop without any nutrient supplementation. Comparing the present crop yields to a crop that received standard fertilization revealed no differences.

When the effluent supply was 75%, leaf weights and areas decreased compared to to all other treatments. On the other hand, when the effluent supply was 100% (T5), an increase in weight and leaf area was observed (Figure 4), suggesting that the input of organic matter provided by T5 offset the salt stress caused by it, which was not observed when the effluent supply was only 75%. The organic matter soil supply, especially in tropical soils, can bring physical, chemical, and biological benefits to crop development and, in the case of vegetables, such increase is even more beneficial (Zandonadi *et al.* 2014).

Better yields are attributed to plants submitted to irrigation with organic leachates, not only because of the high concentration of nutrients but also because of the presence of humic substances (Tejada *et al.* 2008; Singh *et al.* 2010). The interaction between humic substances application with different concentrations of sodium chloride in wheat cultivation were observed by Asik *et al.* (2009). Under saline stress, the application of humus (1 g soil·kg$^{-1}$) have resulted in dry mass increase due to the nutrients input increment.

The uptake of N, Na, and S in lettuce leaf tissue with increasing effluent doses between T1 and T5 were 42%, 238%, and 242%, respectively (Figure 4). The increase of N caused by the augment of the effluent dose was reflected in the absorption of these nutrients by the plant. Nevertheless, it did not cause yield enhancement, as expected. This result must be related to the high salinity levels provided by the effluent and also to the N form. The N concentration in the effluent was divided between the organic form (49.46%) and NH4$^+$ (50.54%). In a vast bibliographic review Andrews *et al.* (2013) concluded that the N form available to plants can affect seed germination time and rate, leaf expansion and function, dry matter partition between shoot and root, and root architecture. The magnitude of these effects depends not only on N supply, but also on environmental factors.

Britto & Kronzucker (2002) stated that a high $NH_4^+$ concentration can be phytotoxic to fertirrigated crops, and competes with the absorption of other cations due to the need to maintain the electroneutrality in the plant. Results of this competition can be verified by the reduction of Mg in the lettuce leaf when increasing effluent doses are applied (Figure 4(c)). In this way, the Mg concentration (Figure 4(c)) presents the same trend depicted in Figure 3 for the leaf mass and area. In plant metabolism, magnesium is a component of the chlorophyll molecule (vital for photosynthesis) and is involved in enzymatic activation, protein synthesis, and translocation of synthesized compounds (Guo *et al.* 2016).

As for the Na and S concentrations, in addition to the N concentration, the increase in dairy effluent dose increases availability of these elements to lettuce plants (Figure 4). Pereira *et al.* (2011) evaluated the impact on the soil–plant system that used treated sewage effluents in tropical conditions for cultivatating citrus plants and they verified the benefits in soil productivity and acidity. However, in

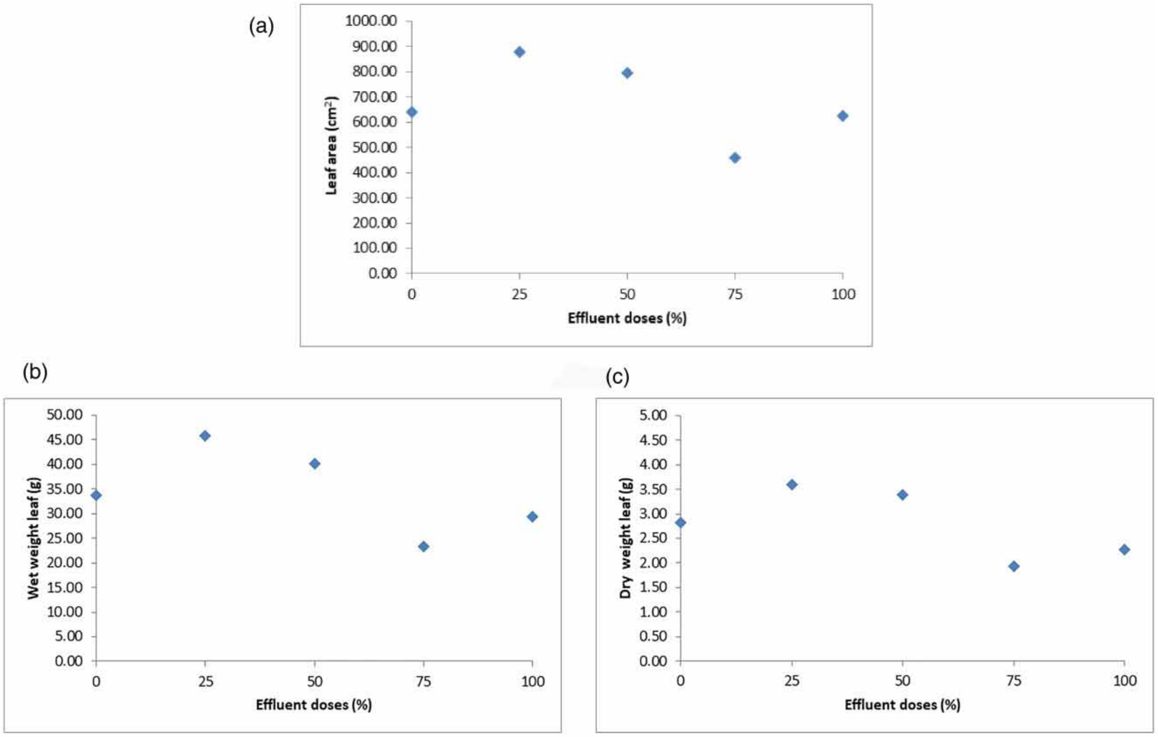


**Figure 3** | Effect of different proportions of dairy effluent, 0%, 25%, 50%, 75% and 100% (T1, T2, T3, T4, and T5), on the leaf area (a) and wet (b) and dry (c) weight leaves of the lettuce. Significant at $P < 0.05$.

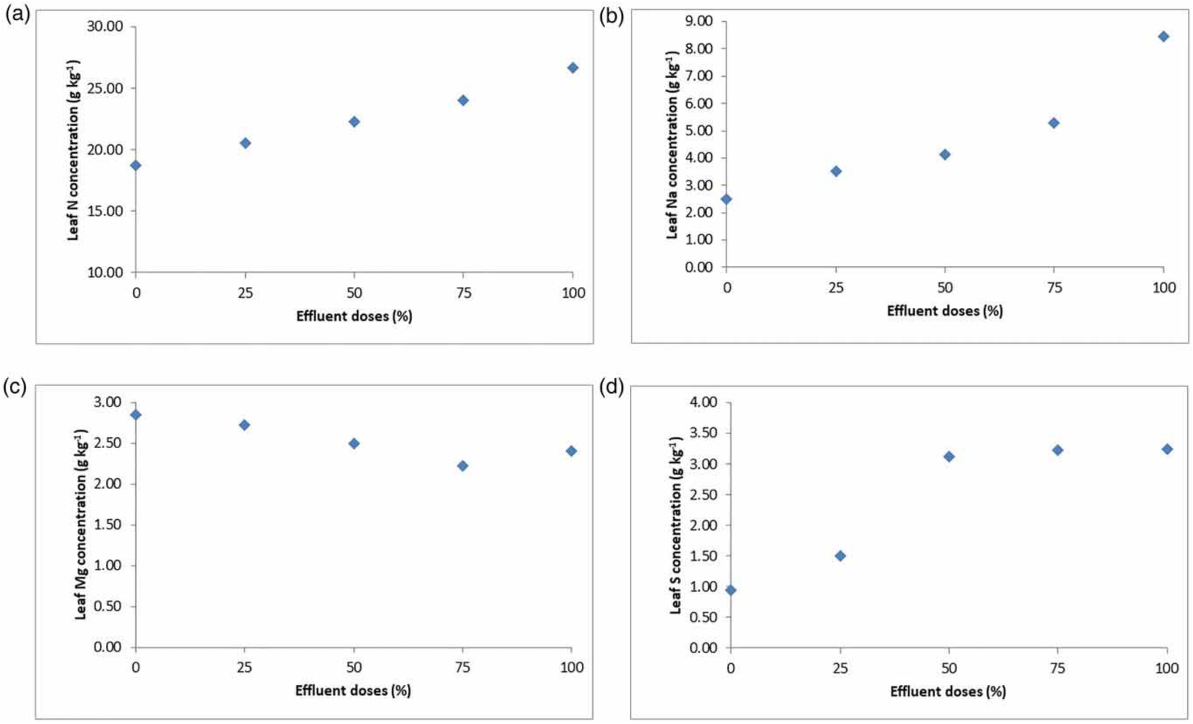


**Figure 4** | Effect of different proportions of dairy effluent, 0%, 25%, 50%, 75%, and 100% (T1, T2, T3, T4, and T5), on the concentration of N (a), Na (b), Mg (c), and S (d) in leaf tissue. Significant at $P < 0.05$.

treatments with irrigation sheeting superior to plant demand, there were nutritional imbalances due to the high Na and S concentration.

The results of applying the effluent from HABBR to lettuce cultivation were promising, allowing savings of up to 50% in N fertilizer. The agricultural reuse technique can also complement the wastewater treatment with high organic and nutrient concentrations, avoiding the necessity of reaching the high quality parameters required when discharges in water sources surface are considered. The research should continue and focus the assessment on nutrient dynamics in plant and soil in relation to the N source provided by these treatments, as well as the high salt content.

## Microbial analyses

Microbial analyses were performed to verify the contamination potential of the reactors for food security of the produced vegetables, because the inoculum from the anaerobic reactors can often come from domestic wastewater treatment plants, which also present potential contamination by pathogenic microorganisms. Although the HABBR was fed with simulated dairy effluent, thus theoretically free of microorganisms, it may not be the case for industrial full–scale situations, where the level of asepsis in the dairy products can be very high and where such production units do not mix their domestic effluents with their industrial effluents.

### Effluents

The results for counting heterotrophs and total coliforms in the effluent over a 4-week period are shown in Table 3. *E. coli* contamination was not detected in any of the effluent samples, which were, therefore, within the limits recommended by the World Health Organization (WHO). The presence of fecal coliforms is indicative of poor effluent quality and, according to the WHO, the legislation restricts the presence of *E. coli* in irrigation water to a maximum of 3.0 log colony forming units (CFU)/100 mL$\cdot^{-1}$ (WHO 2006).

During the 4 weeks of lettuce cultivation, the effluent produced by the HABBR showed similar concentrations, without statistical differences, for total coliforms and for heterotrophic bacteria, as shown in Table 3. Heterotrophic bacteria, as well as total coliforms, are microorganisms naturally present in water samples and, by analogy, in effluents. Their presence does not create immediate health risks; however, values above 6.0 log CFU·100 mL$^{-1}$ (WHO 2006) may represent contamination risks to the culture that has been irrigated with such source. For the present analysis, the results of counting these microorganisms remained within acceptable limits.

**Table 3** | Microbiological analysis results for the anaerobically treated effluent

| | **Week** | | | |
|---|---|---|---|---|
| **Microbiological analyses (log CFU·g$^{-1}$)** | **1** | **2** | **3** | **4** |
| Heterotrophic bacteria | $4.9^A$ | $5.0^A$ | $5.9^A$ | $5.3^A$ |
| Total coliforms | $4.7^A$ | $4.9^A$ | $5.1^A$ | $3.8^A$ |

CFU – colony forming unity.
Matching upper case letters in the lines do not differ statistically by the Kruskal–Wallis test ($P < 0.05$).

### Plants

Table 4 shows the mean values among the four replicates of lettuce microbiological characteristics produced under different doses of treated dairy effluent.

The presence of *E. coli* and *Samonella sp.* has not been detected in any of the samples, meaning that they adhere to the requirements of Brazilian legislation that regulates microbiological standards for fresh vegetables. The law requires the absence of *Salmonella sp.* in 25 g of food, and the limit for thermotolerant coliforms is 2 log CFU at

**Table 4** | Results of microbiological analyses on lettuces irrigated with different effluent concentrations

| | **Effluent dosage (%)** | | | | |
|---|---|---|---|---|---|
| **Microbiological analyses (log CFU/g)** | **0** | **25** | **50** | **75** | **100** |
| Mesophilic bacteria | $4.9^A$ | $5.3^A$ | $4.3^A$ | $5.9^A$ | $5.4^A$ |
| Total coliforms | $<1^A$ | $<1^A$ | $1.6^A$ | $3.5^A$ | $3.0^A$ |
| Molds and yeasts | $3.6^A$ | $3.6^A$ | $3.9^A$ | $3.3^A$ | $3.6^A$ |
| Psychrotrophic bacteria | $2.9^A$ | $3.3^A$ | $3.7^A$ | $3.9^A$ | $4.1^A$ |
| *Staphylococcus aureus* | $2.1^A$ | $2.6^A$ | $3.0^A$ | $3.0^A$ | $3.0^A$ |

Matching upper case letters in the lines do not differ statistically by the Kruskal-Wallis test ($P< 0.05$).

45 °C·g$^{-1}$ (BRASIL 2001). From the statistical point of view, the null hypothesis that the five treatments did not have significant difference in relation to the total counts of mesophilic, psychrotrophic, *S. aureus* aerobes, total coliforms, molds, and yeasts was accepted at a 5% level of significance, inferring that irrigating lettuce crops with 25%, 50%, 75%, or 100% effluent yields leads to a microbiological contamination statistically equal to irrigation with only water. It is important to note that, in addition, the current results remained within the accepted limit considered safe for human consumption in Brazil (BRASIL 2001). Thus, it is possible to state that even irrigation with a 100% effluent did not represent significant increase in microbiological load compared to irrigation with drinking water alone. This is in accordance with the data obtained in the effluent analyzes previously presented (Table 3), demonstrating that it is safe to use the effluent from the dairy treated in ABBR.

## CONCLUSIONS

The HABBR has been proven to be a reliable option for fatty-rich salty wastewater treatment, as demonstrated by the consistent alkalinity production, high organic matter removal (above 90%), and rich methane biogas production. HRT of 24 h provided the optimal operational conditions among those tested, although hydraulic shocks using 12 h of HRT were well tolerated.

When carefully conducted, the agricultural reuse of the anaerobic-treated effluent could guarantee yields of the lettuce crops. The 50% dose of dairy anaerobically treated effluent resulted in the best conditions for residue utilization in agriculture, presenting an equal lettuce production without nutritional disorders and a reduction in 50% of necessary nitrogenous mineral fertilizer.

Throughout the irrigation time, the treated effluent did not show potential for microbiological contamination, proven by the results of the analysis carried out on the plants, where the count of ended mesophilic aerobes, psychrotrophic, *S. aureus*, molds and yeasts, and total coliforms were below levels allowed by legislation, thus guaranteeing food safety.

## ACKNOWLEDGEMENTS

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for the grants to L. K. A. Santos (Finace Code 001) and to M. M. S. Alves (PVE 123/2012, process no. 23038.009805/2012-39), as well for the financial support.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 30 June 2020; accepted in revised form 2 October 2020. Available online 20 October 2020