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# Efficiency of a denitrifying sludge in a UASB reactor fed with whey

# Eficiencia de un lodo desnitrificante en un reactor UASB alimentado con lactosuero

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## Abstract

Whey is a by-product of the cheese industry that, when discharged into the environment, causes the eutrophication of natural water bodies. Although various alternatives for its utilization have been studied and proposed, a significant portion is still discarded into the environment. Therefore, the objective of this study was to evaluate the digestion of whey using denitrifying sludge in an UASB reactor. The UASB reactor was set up and operated for 100 days in two stages. In the first stage (0-50 days), it was fed with acetate ($CH_3COO^-$) until reaching a denitrifying steady state. In the second stage (50-100 days), the reactor was fed with whey as the carbon source. The reactor was fed with 117 mg $NO_3^-$-N $L^{-1}$ $d^{-1}$, maintaining a C/N ratio of 1.8 in both stages. In the first stage, $E_{CH_3COO^-C}$ and $E_{NO_3^-N}$ of 93.35 ± 4.5% and 97.72 ± 1.24%, respectively, were achieved. In the second stage, $E_{whey\text{-}C}$ and $E_{N\text{-}NO_3^-}$ of 95.69 ± 0.61% and 95.68 ± 1.78%, respectively, were obtained. In both stages, $Y_{C\text{-}HCO_3^-}$ and $YN_2$ were close to one. No accumulation of secondary metabolites was quantified, indicating that the sludge had the capacity to biodegrade the organic load of the whey.

*Keywords*: whey, UASB, dairy industry, digestion, denitrifying sludge.

## Resumen

El lactosuero es un subproducto de la industria quesera que, al descargarse al medio ambiente, provoca la eutrofización de cuerpos de agua natural. A pesar de las diversas alternativas estudiadas para su aprovechamiento, gran parte sigue siendo desechada al medio ambiente. Por ello el objetivo del presente estudio consistió en evaluar la digestión de lactosuero a través de un lodo desnitrificante en un reactor UASB. Se operó un reactor UASB por 100 días en dos etapas. En la primera (0-50 días), se alimentó con $CH_3COO^-$ hasta alcanzar el estado estacionario desnitrificante. En la segunda etapa (50-100 días), el reactor se alimentó con lactosuero como fuente de carbono. En ambas etapas, se alimentó con 117 mg $NO_3^-$-N $L^{-1}$ $d^{-1}$, manteniendo una C/N de 1.8. En la primera etapa, se obtuvieron $E_{C\text{-}CH3COO\text{-}}$ y $E_{N\text{-}NO3\text{-}}$ de 93.35 ± 4.5 y 97.72 ± 1.24 %, respectivamente. En la segunda etapa se obtuvieron $E_{C\text{-}lactosuero}$ y $E_{N\text{-}NO3\text{-}}$ de 95.69 ± 0.61 y 95.68 ± 1.78 %, respectivamente. En ambas etapas, los $Y_{C\text{-}HCO3\text{-}}$ y $Y_{N2}$ fueron cercanos a uno. No se cuantificó la acumulación de metabolitos secundarios, por lo que el lodo tuvo la capacidad de biodegradar la carga orgánica del lactosuero en compuestos inocuos.

*Palabras clave*: Suero, UASB, industria láctea, digestión, lodo desnitrificante.

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# 1 Introduction

Whey is a by-product derived from the dairy industry, characterized by a yellow-greenish color and a sweet-sour taste. It is composed of proteins, carbohydrates, vitamins, lipids, enzymes, and minerals (Mahmoudi *et al*., 2024; López-Torres, 2024). Annually, up to 190 million tons are produced worldwide, and in most cases, it is discharged directly into the environment without any form of treatment (Charalambous *et al*., 2023).

The components of whey can vary significantly according to factors such as the composition of the milk, the type of cheese, the cheese-making process, the treatment of the cheese milk, and the post-fermentation processing of the milk (curd) (Zhubanova *et al*., 2023). This by-product contains milk in amounts ranging from 85% to 95% and nutrients in about 55% (Mahmoudia *et al*., 2024). Additionally, it is characterized by containing (kg $m^{-3}$) lactose (0.18–60), fats (0.08–11), proteins (1.4–100), and minerals (10–189) within pH ranges of 4.6 to 6.4 (Caltzontzin-Rabell *et al*., 2024; Ramos-Suárez *et al*., 2024; Gómez & Sánchez, 2019). Furthermore, it has been reported to contain B-group vitamins such as thiamine, pantothenic acid, riboflavin, pyridoxine, nicotinic acid, cobalamin, and ascorbic acid concentrations of 2.2 mg $mL^{-1}$ (Besediuk *et al*., 2024).

Despite the aforementioned benefits, the presence of whey in the environment causes severe problems, making the cheese industry one of the most polluting sectors within the agri-food industry, as this by-product still lacks commercial value for its utilization (Ashton & Syed, 2020; Caballero *et al*., 2021). The presence of whey in natural water bodies has caused various contamination issues, as it increases the biological oxygen demand (BOD) to between 35 to 60 g $L^{-1}$, the chemical oxygen demand (COD) to 60 to 70 kg $m^{-3}$, and the dissolved organic carbon (DOC) to ranges of 50 to 102 g $L^{-1}$ (Chourasia *et al*., 2022). Additionally, whey has been reported to have low alkalinity (<0.22 g $L^{-1}$ $CaCO_3$), high phosphate content (2.0 to 4.5 g $L^{-1}$), and inorganic nitrogen (1 g $L^{-1}$) (Casallas-Ojeda *et al*., 2024). These factors contribute to the eutrophication of natural water bodies, resulting from changes in soil composition due to alterations in nitrogen and phosphorus cycles (Casallas-Ojeda *et al.,* 2024). To mitigate these issues, whey has been used as a dietary supplement and for ethanol production, among other proposed alternatives that are still under study. For example, Trejo *et al*. (2015) proposed the reuse of whey for the commercialization of products such as fermented beverages, ricotta cheese, and petit-suisse-type cheese, as it contains large amounts of nutrients, proteins, and solids. Nevertheless, whey discharges have been estimated at up to 10 billion tons per year, with 50% of this amount not being repurposed for the production of new products, resulting in the remainder being released into the environment (Besediuk *et al*., 2024; Cárdenas-Medina *et al.*, 2020; Lech & Holownia *et al*., 2015). Consequently, the study of processes capable of degrading or fully eliminating these contaminants from water is imperative.

While conventional wastewater treatment processes have helped mitigate water pollution, they do not completely eliminate the high organic loads caused by the presence of whey, resulting in high levels of COD. However, various technologies for treating water contaminated with cheese industry by-products have been studied. Bazrafshan *et al*. (2021) investigated the treatment of dairy industry wastewater in a coagulation system combined with a membrane bioreactor of metal-organic frameworks. They reported a removal of 26% and 75% of the organic matter present in whey wastewater in the coagulation and activated sludge processes, respectively, with an initial COD of 10,000 mg $L^{-1}$. The total COD reduction in the system was around 98.8%. Additionally, whey was diluted with deionized water in this system due to the high organic loads present in the water. Moreover, whey is characterized by high nitrogen concentrations. Elia *et al*. (2023) reported total nitrogen (TN) concentrations ranging from 340 to 1040 mg $L^{-1}$, $NO_3^-$ levels between 4 to 8 mg $L^{-1}$, and dissolved organic carbon ranging from 75,300 to 80,300 mg $L^{-1}$.

In addition to whey pollution, large quantities of nitrogen are also discarded daily from various domestic and industrial anthropogenic activities. Nitrogen is an important component of all living organisms. However, the presence of species such as $NO_3^-$ and nitrite ($NO_2^-$) in the environment can be due to the ecosystem's geology and, in the worst and most concerning case, anthropogenic activities. The main sources of $NO_3^-$ discharge are domestic wastewater, agricultural runoff, and the mineralization of organic nitrogen in soil and water (Xu *et al*., 2024). Exposure and ingestion of $NO_3^-$ in humans can lead to an increase in methemoglobin in the blood (methemoglobinemia), reducing oxygen transport through the blood. According to the World Health Organization and the European Drinking Water Directive (98/83/EC, 1998), the threshold value for $NO_3^-$ intake and consumption in drinking water is 0.8 mM (Margalef-Marti *et al.,* 2019). In the environment, high nitrogen concentrations increase the eutrophication of natural water bodies, which decreases the photosynthetic activity of aquatic plants and thus reduces the concentration of dissolved oxygen (Luo *et al.,* 2022).

Table 1. Composition of the UASB feed sources in the two operational stages.

| **Composition of the feed source** | | **First stage (mg L$^{-1}$)** | **Second stage (mg L$^{-1}$)** |
|---|---|---|---|
| **Carbon source** | $CH_3COO^-$-C | 1000.00 | - |
| | $KH_2PO_4$ | 1000.00 | 500.00 |
| | $Na_2MoO_4 \bullet 2H_2O$ | 120.00 | 60.00 |
| | $CaCl_2 \bullet 2H_2O$ | 60.00 | 30.00 |
| | $MgSO_4 \bullet 7H_2O$ | 400.00 | 200.00 |
| | Whey-C | - | 1000 |
| **Nitrogen source** | $NO_3^-$N | 555.55 | 510.42 |
| | $CuSO_4 \bullet 5H_2O$ | 20.00 | 20.00 |
| | $FeCl_3 \bullet 6H_2O$ | 400.00 | 400.00 |

There are various chemical, physical, and biological treatment technologies for the removal of nitrogen from wastewater, but biological processes are more commonly used due to their low cost and energy consumption. These are classified into anaerobic-anoxic-oxic (AAO) processes, anaerobic/oxic (AO) processes, which are carried out in sequencing batch reactors (SBR), moving bed biofilm reactors (MBBR), upflow anaerobic sludge blanket reactors (UASB), among others (Fan *et al.*, 2024). Denitrification is a biological process that consists of the reduction of nitrate ions ($NO_3^-$) to gaseous nitrogen ($N_2$), while an organic source as $CH_3COO^-$ is oxidized to $CO_2$ and $HCO_3^-$ (Equation 1), depending on working conditions (Camacho *et al.*, 2023; Martínez *et al.*, 2021).

$$CH_3COO^- + 1.6NO_3^- \rightarrow 2HCO_3^- + 0.8N_2 + 0.2H_2O + 0.6OH^- \quad (1)$$

This process has achieved complete biodegradation of various recalcitrant compounds from the petrochemical and pharmaceutical industries in batch systems to environmentally harmless compounds (Islas-García *et al.*, 2017). The study of these types of processes in continuous systems could represent a sustainable and efficient alternative for the removal of nitrogen and carbon components present in whey. Therefore, the objective of this work was to evaluate the digestion of whey by denitrifying sludge in an Upflow Anaerobic Sludge Blanket (UASB) reactor.

## 2 Methodology

### 2.1 *Degradation of Whey in the UASB*

A 1.340 L upflow anaerobic sludge blanket (UASB) reactor was set up and operated, inoculated with 3.58 g L$^{-1}$ of sludge from the Atotonilco de Tula Water Treatment Plant, Hidalgo, Mexico. The reactor was monitored for 100 days in two stages and was fed with a separate carbon and nitrogen source. In the first stage (50 days), the reactor was fed with $CH_3COO^-$-C and $NO_3^-$-N at a C/N ratio of 1.8, following the composition shown in Table 1, until achieving a steady denitrifying state. In stage 2 (from day 51), the UASB was fed with whey as the carbon source and $NO_3^-$, maintaining the C/N ratio of 1.95 (Table 1). The whey used to feed the UASB was sourced from an artisanal cheese industry in Tulancingo de Bravo, Hidalgo.

The UASB operated at a constant temperature of 20°C, with a hydraulic retention time of 2.2 days and a pH of 9.47 ± 0.25. Liquid and gas samples were taken at the reactor outlet over the 100-day operation period for subsequent analysis. Liquid samples at the UASB outlet were centrifuged for 10 minutes at 5000 rpm, and the supernatant was filtered through a 0.22 $\mu$m nylon membrane pore size. Biogas production at the UASB outlet was measured by displacement using an inverted column fully submerged in a pH 3 saturated NaCl solution (360 g L$^{-1}$). Substrate and product concentrations were evaluated until the UASB reached steady-state with a coefficient of variation (CV) of less than 10%.

### 2.2 *Evaluation of UASB*

The biodegradation capacity of the sludge in the UASB reactor was evaluated by calculating substrate consumption efficiencies (E), as shown in Equation 2.

$$E = \left(\frac{C_o - C_i}{C_o}\right) * 100 \quad (2)$$

Where Co is the initial substrate concentration and Ci is the final substrate concentration. Additionally, product yields (Y) were calculated using Equation 3.

$$Y = \frac{C_P}{C_o - C_i} \quad (3)$$

Where $C_P$ is the concentration of the generated product. Furthermore, a mass balance of carbonaceous and nitrogenous compounds at the inlet and outlet of the UASB was conducted to rule out the accumulation of intermediate compounds and the production of metabolites from the denitrification process.

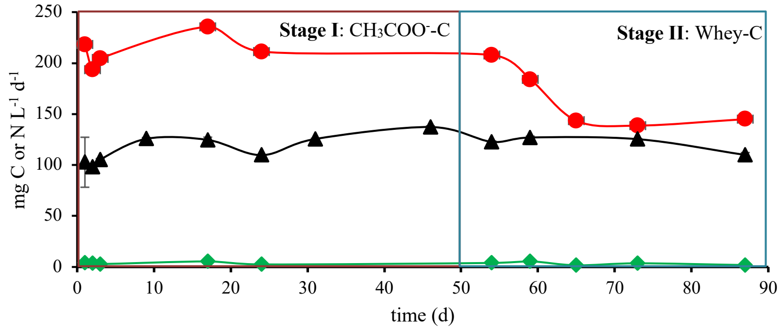


Figure 1. Feeding profile of the carbon and nitrogen source to the UASB reactor. (●) TOC, (▲) TN, (♦) $HCO_3^-$-C.

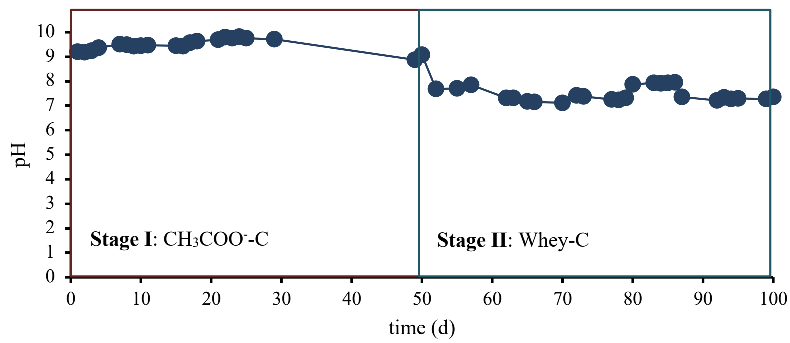


Figure 2. pH evaluation in the UASB reactor fed with acetate and whey. (●) pH

### *2.3 Analytical methods*

Concentrations of total carbon (TC), total organic carbon (TOC), inorganic carbon (IC), and total nitrogen (TN) were evaluated using a Shimadzu total organic carbon analyzer. Concentrations of $N_2$ and $CO_2$ were evaluated using a Perkin Elmer Autosystem XL gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and an HP-PLOT/Q capillary column from Agilent Technologies (USA) with dimensions of 30 m length, 0.530 mm internal diameter, and 40 $\mu$m particle size. Ultra-high purity argon was used as the mobile phase at a flow rate of 10 mL min$^{-1}$. The oven, injector, and detector temperatures were set at 60°C, 100°C, and 200°C, respectively. Total suspended solids (TSS), fixed suspended solids (FSS), and volatile suspended solids (VSS) were evaluated following the APHA standard (1998).

## 3 Discussion and results

### *3.1 UASB Reactor Operation*

The UASB reactor was operated and monitored for 100 days in two stages of 50 days each. In the first stage (0-50 days), the reactor was fed with 1000 mg $CH_3COO^-$-C L$^{-1}$, and in the second stage (51-100 days), whey was added as the sole carbon source, with the total organic carbon (TOC) concentration adjusted to 1000 mg L$^{-1}$. This was because whey contains high carbon loads, which could modify sludge metabolism, and the C/N ratio was maintained at 1.8. For the nitrogen source, the reactor was continuously fed with 117 mg $NO_3^-$-N L$^{-1}$ d$^{-1}$, maintaining a C/N ratio of 1.8 in both stages. The profile of organic loads from the carbon and nitrogen sources in the UASB is shown in Figure 1. This figure illustrates that carbon and nitrogen loads remained constant throughout the operation.

The UASB reactor was maintained at a temperature of 20 °C during the 100 days of operation. The pH in the first stage was 9.47 ± 0.25, and when whey was fed, it decreased to 7.46 ± 0.28 as shown in Figure 2. This decrease is attributed to whey being an acidic byproduct (pH 4.2 – 5.2), causing a two-unit drop in the medium pH (Besediuk *et al.*, 2024). However, this change did not have negative effects on the denitrifying respiratory process, as efficient performance has been reported within pH values ranging from 7 to 9 (Camacho *et al.*, 2023; Islas *et al.*, 2017). Moreover, under these conditions, a predominant production of $HCO_3^-$ is ensured, minimizing $CO_2$ release (Guerrero-Martínez *et al.*, 2024).

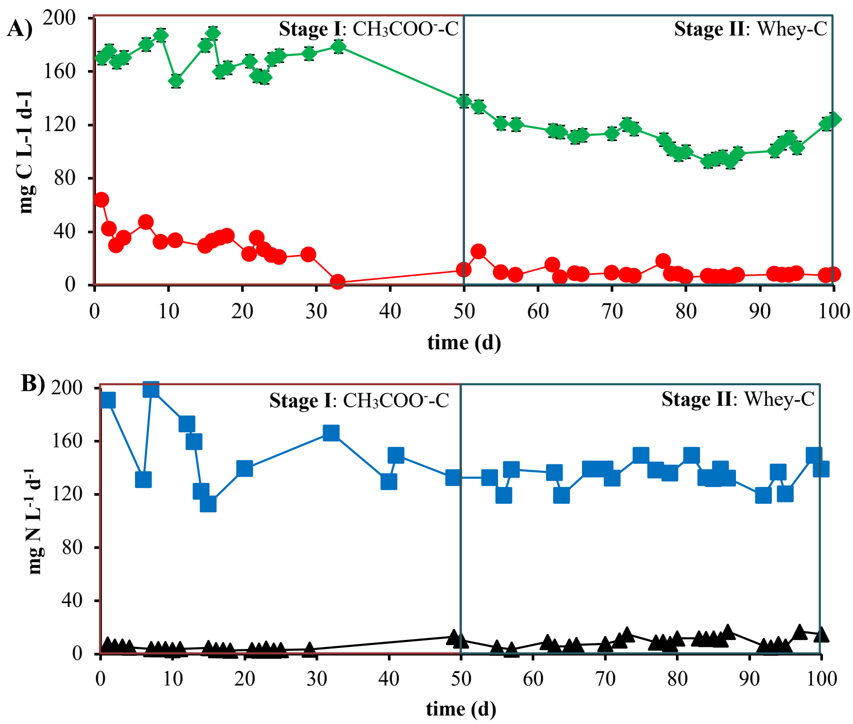


Figure 3. Effluent profile of the UASB reactor fed with acetate and whey. A) Carbonaceous compounds: (●) TOC, (♦) $HCO_3^-$-C, B) Nitrogenous compounds: (▲) TN, (■) $N_2$.

Table 2. Substrate consumption efficiencies and product yields in the UASB reactor fed with acetate and whey.

| Stage | Efficiencies (%) | | | Yields (Y) | |
|---|---|---|---|---|---|
| | $CH_3COO^-$-C | Whey-C | $NO_3^-$-N | $HCO_3^-$-C | $N_2$ |
| 1 | 93.35 ± 4.50 | - | 97.72 ± 1.24 | 0.98 ± 0.08 | 1.30 ± 0.24 |
| 2 | - | 95.69 ± 0.61 | 95.68 ± 1.78 | 0.70 ± 0.08 | 1.20 ± 0.08 |

### *3.2 Evaluation of UASB reactor with acetate and whey*

The results of the profile of effluents from the UASB reactor fed with acetate and whey are shown in Figure 3. During the first 50 days of operation, a gradual increase in $CH_3COO^-$-C consumption was observed, reaching an $E_{CH_3COO\text{-}C}$ of 93.35 ± 4.5% (Table 2). Simultaneously, $HCO_3^-$-C was generated as the sole product from acetate biodegradation, achieving a $Y_{HCO_3\text{-}C}$ of 0.94 ± 0.08. Regarding nitrogenous compounds, a TN removal efficiency of 97.72 ± 1.24% was achieved, and this was transformed into $N_2$, resulting in a $Y_{N_2}$ of 1.30 ± 0.24. A mass balance of carbonaceous and nitrogenous compounds was conducted, ruling out nitrogen and carbon accumulation from secondary metabolites of the denitrification process when fed with $CH_3COO^-$, achieving complete denitrification. Additionally, during the 100 days of UASB reactor operation, the sludge reached and maintained steady-state denitrification (SSD), as calculations of response variables such as substrate consumption efficiencies, product generation rates, and variations were less than 10%.

It should be noted that gaseous compounds such as $CH_4$, $H_2S$, and $CO_2$ were not quantified in the chromatograms, so the carbon at the inlet was transformed solely into $HCO_3^-$. This was because the reactor was operated at C/N ratios and TOC/$SO_4^{2-}$ ratios of 1.8 and 2.3, respectively. According to Lu *et al.* (2015), a TOC/$SO_4^{2-}$ ratio close to 20 promotes $CH_4$ production.

In terms of nitrogen compounds, an $E_{TN}$ of 95.68 ± 1.78% was achieved, and nitrogen in the influent of the reactor was converted to $N_2$, resulting in a $Y_{N2}$ of 1.20 ± 0.08. It is important to mention that the fed whey contained 47.12 mg $L^{-1}$ of total nitrogen, which could originate from proteins, amino acids, as well as ammonia and urea. Tsermoula *et al.* (2023) reported concentrations of up to 2576 ± 1133 and 5746 ± 371 $\mu$M of ammonia and urea in whey, respectively, as well as non-protein nitrogen compounds such as creatine, proline, creatinine, among others. Despite nitrogen in whey not being predominantly $NO_3^-$, it was converted to $N_2$, suggesting the occurrence of anammox processes during reactor operation, although denitrification was predominant (Cao *et al*., 2024). Additionally, nitrogen concentrations decreased and did not increase, ruling out the presence of nitrifying and ammonifying bacteria and indicating a predominance of denitrifying bacteria (Bucci *et al*., 2020; Chen *et al*., 2024). According to Lech & Trusek (2015), the biodegradation of organic nitrogen by bacteria is highly valuable during the biodegradation of whey. This highlights that the denitrifying respiratory process is a valuable alternative for whey biodegradation.

Similar results were reported by Xiong *et al*. (2024), achieving nitrogen and dissolved organic carbon (COD) removal efficiencies of 84 ± 3.7% and 81.4 ± 6.1%, respectively, in a partially denitrifying and anammox reactor. This reactor was fed with municipal wastewater with nitrogen and carbon loads of 155.4 ± 12.6 and 186.5 ± 25.9 mg $L^{-1}$ $d^{-1}$, respectively. The study mentions that sludge first degrades organic nitrogen followed by compounds like $NO_3^-$. This could be because the energy expenditure to convert organic nitrogen to $N_2$ is lower than reducing $NO_3^-$ to gaseous nitrogen. Thus, whey was biodegraded by denitrifying sludge in the UASB reactor. Moreover, whey has been reported as a promoter of denitrification (Margalet *et al*., 2019).

In other studies, such as that by Siqueira *et al*. (2022), organic carbon biodegradation efficiencies ranged from 69% to 81% in a methanogenic UASB reactor fed with 1834 to 7215.07 mg $L^{-1}$ of dissolved organic carbon. These results are lower than those obtained in the present study, despite Siqueira's reported hydraulic retention time of 4.4 days, twice that established in the present study. Additionally, Siqueira's study evaluated the formation of $CH_4$, $CO_2$, and $H_2S$, with a daily gas production of 3.7 liters and up to 4.88 L at best, with compositions of 66.47%, 11.43%, and 53 mg $L^{-1}$, respectively. Although methane is a gaseous biofuel, it is considered a greenhouse gas (GHG), and the presence of $H_2S$ can pose issues for the commercialization or use of this biogas (Barreto *et al*., 2024). However, in the UASB reactor with denitrifying sludge, only $N_2$ and $HCO_3^-$ were produced, considered environmentally benign, and because no gaseous metabolites like $N_2O$ accumulated, they represent a zero GHG emission option for wastewater treatment (Conthe *et al*., 2019). Mahmoudi *et al*. (2024) reported removal efficiencies of 80% for dissolved organic carbon and 90.4% for total nitrogen in an artificial wetland system using bulrushes, cattails, papyrus, and finally floating water lentils (*Lemna minor*) for whey removal. These results were lower than those reported in the present study, despite the use of three plant species in the study of Mahmoudi. This demonstrates that the denitrifying process is an efficient alternative for the degradation of whey-contaminated water, and the products generated are innocuous to the environment

# Conclusions

El denitrifying sludge achieved a degradation efficiency of 95.69 ± 0.61% and 95.68 ± 1.78% of the organic carbon and nitrogen load present in whey, respectively. The reactor products were solely $HCO_3$- and $N_2$ with yields of 0.70 ± 0.08 and 1.20 ± 0.08, respectively. Part of the carbon fed into the reactor was transformed into biomass, yielding 0.20 ± 0.03. No gaseous secondary metabolites of denitrification such as NO and $N_2O$, as well as $CH_4$, were generated. The respiratory denitrifying was not inhibited by the presence of whey. Complete denitrification was achieved in the presence of whey in the UASB reactor. The use of denitrifying sludge could be a promising future option for treating wastewater from dairy industries and even municipal wastewater.

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