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Assessment of the performance of a symbiotic microalgal-bacterial granular sludge reactor for the removal of nitrogen and organic carbon from dairy wastewater

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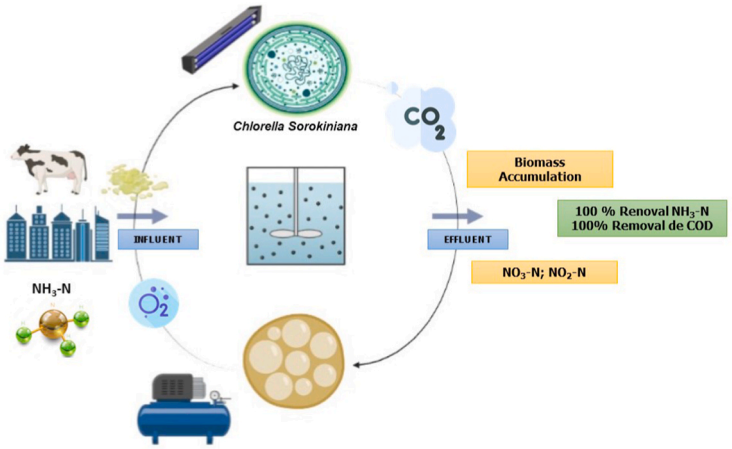
HIGHLIGHTS

- Synthetic dairy wastewaters were treated in microalgal-bacterial granular SBR (SBR_{AB}).
- Bacterial SBR (SBR_B) and microalgal-bacterial SBR (SBR_{AB}) were compared.
- Higher removal rates of cheese whey and NH_3–N were found in SBR_{AB}.
- Higher N assimilation and less SND led to higher useful biomass production in SBR_{AB}.
- SBR_{AB} microalgae growth could reduce 20% of CO_2 emissions from bacterial activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Cheese whey (CW) is a nutrient deficient dairy effluent, which requires external nutrient supplementation for aerobic treatment. CW, supplemented with ammonia, can be treated using aerobic granular sludge (AGS) in a sequencing batch reactor (SBR). AGS are aggregates of microbial origin that do not coagulate under reduced hydrodynamic shear and settle significantly faster than activated sludge flocs. However, granular instability, slow granulation start-up, high energy consumption and CO_2 emission have been reported as the main limitations in bacterial AGS-SBR. Algal-bacterial granular systems have shown be an innovative alternative to improve these limitations. Unfortunately, algal-bacterial granular systems for the treatment of wastewaters with higher organic

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loads such as CW have been poorly studied. In this study, an algal-bacterial granular system implemented in a SBR (SBR_{AB}) for the aerobic treatment of ammonia-supplemented CW wastewaters was investigated and compared with a bacterial granular reactor (SBR_B). Mass balances were used to estimate carbon and nitrogen (N) assimilation, nitrification and denitrification in both set-ups. SBR_B exhibited COD and ammonia removal of 100% and 94% respectively, high nitrification (89%) and simultaneous nitrification-denitrification (SND) of 23% leading to an inorganic N removal of 30%. The efficient algal–bacterial symbiosis in granular systems completely removed COD and ammonia (100%) present in the dairy wastewater. SBR_{AB} microalgae growth could reduce about 20% of the CO_2 emissions produced by bacterial oxidation of organic compounds according to estimates based on synthesis reactions of bacterial and algal biomass, in which the amount of assimilated N determined by mass balance was taken into account. A lower nitrification (75%) and minor loss of N by denitrifying activity (<5% Ng, SND 2%) was also encountered in SBR_{AB} as a result of its higher biomass production, which could be used for the generation of value-added products such as biofertilizers and biostimulants.

1. Introduction

In the last years, the growth of the dairy industry was relevant worldwide. As a result, large volumes of by-products such as cheese whey (CW), which is a high strength effluent, are generated. If not properly managed, CW could represent an important risk to the environment (Macwan et al., 2016). Indeed, the chemical oxygen demand (COD) of cheese whey can range from 50 to 80 g L^{-1}, while biochemical oxygen demand (BOD) varies from 40 to 60 g L^{-1} (Macwan et al., 2016). CW contains lactose 66–77% (w w^{-1}), minerals (mainly calcium and phosphorus), proteins 7–15% (w w^{-1}) as β-lactoglobulin and α-lactalbulmin and vitamins as vitamins A, D, and B5 (Fernández-Gutiérrez et al., 2017; Irkin, 2019; Mehrotra et al., 2016). In 2018, the Food and Agriculture Organization of the United Nations (FAO) estimated a global milk production of 843 million tons, with an increase of 2.2% from 2017, whereas the World trade in dairy products expanded to 75 million tons (in milk equivalents), with an increase of 2.9% from 2017 (Dairy Mark, 2018). The combination of these high organic loads, increasing CW production and the absence of sustainable dairy wastewater practices in developing countries, render CW a severe environmental problem (Macwan et al., 2016). CW wastewater has a mean COD:N:P ratio of 100:1.75:0.5, a deficient ratio for effluent treatment by aerobic biological processes. Indeed, an optimum COD:N:P ratio of 100:5:1 is widely accepted for bacterial aerobic processes based on heterotrophic growth. To overcome the above mentioned limitations, dilution of dairy wastewaters with ammonium-rich effluents could be implemented to support an effective aerobic treatment (Bucci et al., 2020).

Dairy wastewaters can be supplemented with effluents containing ammonia and micronutrients, such as landfill leachates, municipal wastewaters, and anaerobic digestion supernatant, for biological treatment, according to a report by Bucci et al., (2020). This is an intriguing approach for the treatment of dairy wastewaters.

Although anaerobic processes are widely used for treating dairy wastewaters, several disadvantages have been reported. Fats from dairy wastewaters have an inhibitory action on the anaerobic processes and operational problems such as formation of scum, sludge flotation and loss of COD removal efficiency can take place (M.C. Cammarota et al., 2001). Also, in anaerobic digestion, various microbial groups with different growth rates are involved, hence the main processes, acidogenesis and methanogenesis, are not commonly in balance (Hassan and Nelson, 2012); this imbalance among the microbial groups can lead to less methane production and process failure (Hassan and Nelson, 2012). In addition, anaerobic treatment of dairy wastewaters requires commonly an aerobic post-treatment in order to meet the effluent discharge limits ((Alayu and Yirgu, 2018).

On the other hand, aerobic processes allow achieving excellent effluent quality in terms of COD, BOD and nutrient removal. Among the aerobic processes, the SBR seems to be the most promising technology for treatment of dairy wastewaters. Using sludge granules and SBR technology, the co-treatment of dairy and ammonium-rich wastewaters represents a promising approach for the dairy industry's effluent treatment.

Several studies have indicated that dairy wastewaters can be treated using biological processes such as aerobic granular sequencing batch reactor (SBR) (Bucci et al., 2022). Aerobic granular sludge (AGS) is commonly generated in SBRs under feast/famine regime. Under these operational conditions, external organic carbon is commonly stored by heterotrophic microorganisms as intracellular polymers such as polyhydroxyalkanoates (PHA) and glycogen (Miao et al., 2016). AGS is a compact, self-immobilizing aggregate composed of microbial consortia that simultaneously remove carbon, nitrogen and other toxic pollutants in a single-stage bioreactor (Nancharaiah & Kiran Kumar Reddy, 2018). Some authors have reported that AGS is more efficient than conventional activated sludge due to its higher tolerance to toxic pollutants, lower footprint and better effluent quality (Derlon et al., 2016). However, AGS exhibits several drawbacks when implemented on an industrial scale, including granular instability and a long granulation start-up period ().

On the other hand, previous research works focused on the treatment of dairy wastewater have demonstrated that CW could be used as an energy and carbon source under mixotrophic conditions for the growth of some microalgae species. One of the genera with the widest range of applications in bioremediation is *Chlorella* (). The growth of *Chlorella* can be enhanced by utilizing free or inexpensive carbon organic substrates under mixotrophic or heterotrophic conditions, which can ultimately boost conventional wastewater treatments (Melo et al., 2018; Da Costa et al., 2017). Indeed, microalgae cultivation in wastewater can recover underutilized nutrients, remove toxic compounds and reduce wastewater treatment costs. In addition, domestic wastewater treatment based on algal-bacterial granular biomass (algal-bacterial AGS) has shown high nutrients removal efficiencies due to its superior stability and rapid granule formation (Zhang et al., 2020). Algal-bacterial granular system has achieved better removal efficiencies of total nitrogen and phosphate than those in the aerobic granular system (Liu et al., 2017). Algae can remove phosphorus and nitrogen by assimilation (Lee and Lei, 2022) using mainly inorganic nitrogen; however, organic nitrogen available in different effluents such as dairy and fish wastes can also be utilized for their growth (Vidya et al., 2021). Indeed, microalgal-bacterial aggregates allow the recovery of nutrients from wastewater, being efficient phosphorus and nitrogen sinks (Lee and Lei, 2022). Pigments, nutraceuticals, fertilizers, lipids among other value-added products can be obtained from the harvested microalgal-bacterial biomass (Quijano et al., 2017). Algal-bacterial AGS showed higher recycling value of excess biomass, through the biodiesel production, than that of the AG (Liu et al., 2017).

Microalgal-bacterial symbiotic aerobic granular sludge technology is a composite bioconcentration technology based on the coupling of microalgae and granular sludge. This symbiotic relationship is based on the photosynthetic activity of microalgae, which can provide oxygen to support the aerobic metabolism of bacterial cells using solar light. On the other hand, microalgae utilize the carbon dioxide produced by aerobic bacteria during organic matter oxidation. The combined growth of microalgae and bacteria can enhance nutrients and energy recovery from wastewaters (Ji et al., 2020; Oyserman et al., 2017). Also, this

allows combining the high biomass and high treatment efficiency of granular sludge technology with the resource recovery capacity of microalgae, while overcoming the problem of poor sedimentation of microalgae in the water treatment process. (Wang et al., 2020; Zhang et al., 2020; Brockmann et al., 2021; Guo et al., 2021; Su et al., 2012). In fact, the assimilation of nutrients by microalgae and their subsequent recovery during harvesting allow the valorization of nutrients as biofertilizer or their release for subsequent algal growth during anaerobic digestion of algal biomass. During anaerobic digestion of algal biomass, the chemical energy and carbon stored during photosynthesis are converted to methane, while the associated nitrogen and phosphorous are available in soluble phase for subsequent algal growth. Therefore, the promising role of the interaction between bacteria and microalgae during wastewater treatment can support the development of more sustainable processes for wastewater management.

Today, a large number of research studies on algae and bacteria have focused on the fundamentals of wastewater treatment using suspended cultures implemented in high rate algal ponds. In this context, the mechanisms involved in the formation of algal-bacterial AGS with stable nutrients removal and excellent settleability has been poorly investigated (Zhang et al., 2020). An increased nitrogen assimilation at the expenses of a lower nitrification-denitrification has been reported for microalgal-bacterial AGS during the treatment of toxic wastewaters containing ammonia and phenol (Bucci et al., 2023).

Accordingly, this work aims to comparing the efficiency of a conventional bacterial granular SBR and an algal-bacterial granular SBR for the simultaneous removal of organic carbon and inorganic nitrogen from synthetic chemical industry wastewater. In the present work, the contribution of the different biotic and abiotic processes for nitrogen removal was quantified using mass balances.

2. Material and methods

2.1. Microorganisms

Chlorella sorokiniana strain 211/8k was obtained from the Culture Centre of Algae and Protozoa (Cambridge, UK) and fresh inocula were prepared according to Borde et al. . In brief, *C. sorokiniana* was cultured in SK medium with a sterile solution of glucose, peptone and yeast extract at final concentrations of 3.125, 0.0625 and 0.0625 g.L^{-1}, respectively (Borde et al. .) under aseptic conditions and continuous illumination (Philips TLD 36W/840 fluorescent lamps) at approximately 2500 Lux of illuminance (Lutron LX-101 Lux Meter) for 2 weeks.

Bacterial aerobic granules fed with a synthetic wastewater containing cheese whey and ammonia were taken from a laboratory-scale wastewater treatment plant in CIDCA (Center of Research and Development in Food Cryotechnology, CONICET, UNLP, CIC, Argentina). The genomic characteristics of this granular aerobic sludge were reported in Bucci et al. (2022).

2.2. Synthetic wastewater

Cheese whey (CW) powder (LACTOFOOD, Argentina) with a COD content of 1 g COD.g CW^{-1} was used (Bucci et al., 2022). Synthetic wastewater (SWW) contained 0.963 g L^{-1} of $(NH_4)_2SO_4$, 0.219 g L^{-1} of K_2HPO_4, 0.278 g L^{-1} of KH_2PO_4, 2.048 g L^{-1} of CW and 0.75 g L^{-1} of $NaHCO_3$. Aliquots of 1 mL per liter of trace element solutions (M_1 and M_2) were also added to the SWW. M_1 contained (in g L^{-1}): $FeSO_4.7H_2O$ (15.0), $ZnSO_4.7H_2O$ (5.0), $MnSO_4.H_2O$ (3.0), $CuSO_4.5H_2O$ (0.75), CoCl2.$6H_2O$ (0.15), citric acid (6.0). M_2 showed the following composition (in g L^{-1}): (NH_4) $6Mo_7O_{24}.4H_2O$ (0.5), BO_3H_3 (0.1), IK (0.1).

2.3. Bioreactor set-ups

Two cylindrical bubble acrylic SBRs columns were used as a bacterial reactor (SBR_B) and as an algal-bacterial reactor (SBR_{AB}) (Fig. 1). The

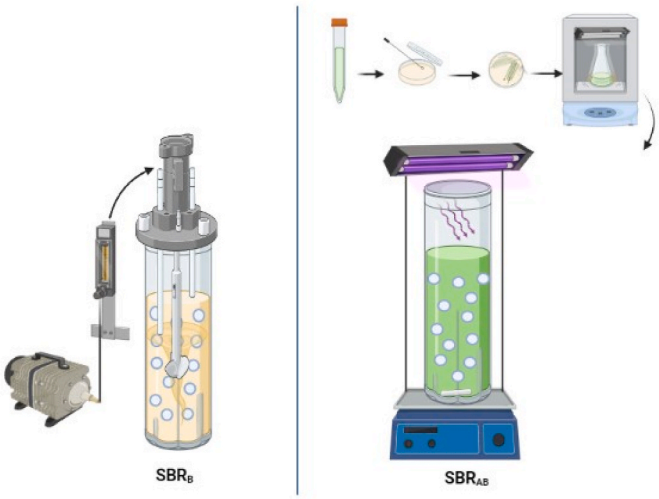

Fig. 1. Scheme of the bacterial granular SBR_B (on the left) and the algal-bacterial granular SBR_{AB} (on the right).

SBRs presented the following dimensions: internal diameter of 9.5 cm, height/diameter ratio of 3.5, and total and working volumes of 2.5 and 2 L. Aeration was introduced into the SBR_B through three stone diffusers located at the bottom of the reactor with the purpose of generating an upward culture broth flow to keep the stability of the seed bacterial granules. The air flow rate was set at 2.4 L min^{-1} with a superficial upflow air velocity of 0.9 cm s^{-1}. In the SBR_{AB} magnetic agitation at 200 rpm was applied to promote the aggregation of the seed biomass, microalgae and bacterial granules, and provide shear force to maintain stable algal-bacterial granules. The algal-bacterial system was constantly illuminated via light-emitting diodes (LED) at 200 μmol m^{-2}. s^{-1} of photosynthetic active radiation at the SBR_{AB} walls.

2.4. Inoculation and SBRs operation

AGS (500 mL) was used as seed sludge in the start-up stage of the two cylindrical reactors used: a bacterial reactor (SBR_B) and an algal-bacterial reactor (SBR_{AB}). SBR_{AB} was also seeded with a *Chlorella sorokiniana* culture. The granular sludge was obtained from an initial SBR fed with synthetic wastewater based on cheese whey and operated with an upward air flow, feast/famine regime, and a short settling time. In the initial SBR, aerobic granulation took place from activated sludge obtained from an automated laboratory-scale SBR at the Center for Research and Development in Food Cryotechnology CIDCA (CONICET, UNLP, CIC, Argentina) (Bucci et al., 2020). Thus, the acclimatization and subsequent stabilization of granular sludge in each system (AGS and algal-bacterial AGS respectively) could be investigated in SBR_B and SBR_{AB}.

SBR_B was seeded with AGS with a concentration of 1500 mg total suspended solid (TSS) L^{-1}, whereas SBR_{AB} was seeded with the same concentration of AGS and a final concentration of *Chlorella sorokiniana* of 0.2 g L^{-1}. *C. sorokiniana* was selected as model microalga because it is commonly found in wastewater treatment systems, demonstrating an excellent treatment performance and a high adaptability to complex wastewater effluents (Borde et al.).

An abiotic test to assess the stripping of NH_3–N in the mechanically aerated SBR was initially performed. The stripping experiment was carried out with 2 L of SWW at pH 7 and an aeration rate of 0.9 cm s^{-1}. The abiotic SBR was operated in the absence of biomass and sampled at 0, 2, 4, 6, and 24 h to measure NH_3 concentration.

The reactors were operated under sequential fed-batch mode with a 24 h cycle period, including 1 min of feeding, 1432 min of bioreaction, 5 min of settling and 1 min of effluent withdrawal. SBR_B was operated for

60 days until steady state was achieved, while the SBR_{AB} was operated for only 40 days because steady state was achieved in a shorter period. Both reactor columns were kept at ambient temperature (24 ± 3 °C) and were operated alternating phases of organic carbon availability (feast period) and starvation (famine period).

The volume exchange ratio was maintained at 50%, which entailed a hydraulic retention time (HRT) of 2 days, and the cellular retention time (CRT) was set at 20 days. The SBRs were fed with an organic loading rate of 1075.2 mg COD·(L day)$^{-1}$, an ammonia-nitrogen loading rate of 61.5 mg N·(L day)$^{-1}$ and a phosphorus loading rate of 35.8 mg P·(L day)$^{-1}$, which entailed a COD:TKN ratio of 13.7 (favorable for the nitrification process, and the establishment of a feast/famine regime). $NaHCO_3$ was added to maintain the pH between 7.0 and 8.0. The stability and performance of the SBRs was evaluated by periodically measuring the concentrations of (mg L^{-1}): ammonia nitrogen (NH_3–N), nitrate (NO_3^--N), nitrite (NO_2^--N), Total Kjeldahl Nitrogen (TKN), Total nitrogen (TN), nitrous oxide in the reactor headspace (N_2O), total solid (TS), total suspended solids (TSS), soluble chemical oxygen demand (COD_S). Granule size and settling properties (sludge volume index, SVI) of the biomass were periodically determined. Glycogen in the biomass was also measured. The temperature and dissolved oxygen concentration were daily measured in both SBRs.

SBR_B and SBR_{AB} were considered under stable operation when constant values (standard deviation < 15%) of the mean granular size, SVI and the specific removal rate of NH_3–N, COD_S, TNs and SVI were achieved.

2.5. Denitrification assays

The denitrification capacity of the granular sludge was studied in both SBRs. Briefly, gas-tight 120 mL glass serum bottles were filled with 80 mL of SWW (without ammonia), 1.5 g L^{-1} of granular biomass and 35 mg L^{-1} of NO_3–N as electron acceptor at a neutral pH. The serum bottles were closed with rubber septa and aluminum caps, and flushed with helium for 10 min to provide anaerobic conditions. The cultivation broths were continuously stirred under magnetic agitation (120 rpm) and 25 °C to foster the gas-liquid equilibrium and maintain the granular sludge in suspension. Gas samples of 100 μl were taken to monitor the concentration of O_2, N_2 and CO_2 in the headspace. Liquid samples of 50 mL were taken to monitor N–NH_4, N–NO_3 and N–NO_2 in the beginning and the final of the experiment. The denitrification assay was conducted in duplicate.

2.6. Calculations

The specific soluble COD uptake rate (q_{CODS}, mg COD_S·(g TSS•h)$^{-1}$), specific removal rates of ammonia ($q_{NH3\text{-}N}$, mg NH_3–N·(g TSS•h)$^{-1}$) and total soluble nitrogen (q_{TNs}, mg TN·(g TSS•h)$^{-1}$) were determined in the SBRs according to Bucci et al. (2022). Quantification of the removal efficiency of ammonia nitrogen, nitrate, nitrite and total nitrogen Kjeldahl nitrogen (TKN) was performed as reported by Bucci et al. (2022). For feast and famine periods, nitrogen assimilation defined as TKN_X was estimated as $TKN_X = TKN_W - TKN_N$, where TKN_W is the TKN for the wastewater including the TKN provided by cheese whey as N_{ORG}, and NH_3–N, and TKN_N represents the nitrified nitrogen. On the other hand, nitrogen gas (Ng) was estimated as $Ng = \Delta TKN_w - \Delta NO_x\text{-}N - \Delta TKN_X$, where ΔNO_X-N corresponds to the oxidized forms of nitrogen generated by nitrification and ΔTKN_X is the TKN used for heterotrophic growth. Simultaneous nitrification and denitrification (SND) was estimated from the difference between nitrogen nitrified and the oxidized nitrogen as nitrite and nitrate (NOx-N) throughout each operational cycle.

2.7. Analytical procedures

Liquid samples were filtered by means of cellulose acetate filters (0.45 μm) prior to the determination of soluble parameters. NH_4^+-N concentration was measured in a spectrophotometer U-200 (Hitachi, Japan) using the Nessler method at 425 nm. NO_2^--N and NO_3^--N concentrations were determined by high-performance liquid chromatography-ion conductivity (HPLC-IC) using a Waters 515 HPLC pump coupled with a Waters 432 202 IC detector and equipped with an IC-Pak Anion HC (150 mm × 4.6 mm). TSS and TS concentrations were quantified by Standard Methods (Eaton et al., 2005). Soluble TN concentrations were determined using a Shimadzu TOC-V CSH analyzer equipped with a TNM module (Japan).

Nitrous oxide (N_2O) gas concentration was measured using a Bruker Scion 436 gas chromatograph with an Electron Capture Detector (GC-ECD) (Palo Alto, USA), equipped with a HS-Q packed column (1 m × 2 mm ID × 3.18 mm OD) (Bruker, USA). Injector, detector and oven temperatures were set at 100, 300 and 40 °C, respectively. Helium was used as carrier gas at 20 mL min^{-1}. External standards of N_2O in N_2 prepared in volumetric bulbs (Sigma Aldrich, USA) were used for N_2O quantification.

The particle size distribution of the granular biomass was determined using a Mastersizer E 20003.14. The settling properties of the granular biomass were determined by means of the sludge volume index (SVI) after 5 and 30 min sedimentation (SVI_5, SVI_{30}, mL·g TS^{-1}). Temperature and dissolved oxygen (DO) concentrations in the bioreactor cultivation broths were monitored by using a ProfiLine 3320 m coupled with a sensor CellOx 325 (WTW, Germany).

The Anthrone method, a modification of the procedure proposed by Osborne and Voogt (1978), was used to determine intracellular glycogen concentration in the granular biomass.

2.8. Statistical analysis

The indicators of the process performance were statistically analyzed by means of an ANOVA at 95% of confidence level and Tukey's honest significance test, with the purpose to compare the performance of the bioreactors. Triplicates of the samples were taken in all the experiments performed. The reactors were operated under steady state from a sufficient period of time to provide a sufficiently large data set to assess the average and standard deviation of the parameters used to monitor process performance.

3. Results and discussion

3.1. Granulation, settleability and morphological changes in bacterial and algal-bacterial granular SBRs

The stability of the SBR_B based on bacterial granule size and settling properties was reached after 2 CRTs, corresponding to 40 days of operation. On the other hand, this stability of the SBR_{AB} was observed in the first 20 days of the operation. The initial TSS value was 1.5 g L^{-1} in SBR_B and 3 g L^{-1} in SBR_{AB} due to the presence of microalgae. A short settling time of 5 min and feast/famine regimes of 2/22 h were established in SBR_B and SBR_{AB}, respectively. The biomass in both reactors rapidly started to flocculate and form granules (Bucci et al. 2020, 2022). Thus, TSS concentration in the SBR_{AB} gradually increased up to day 20, reaching a stable value of 3.0 g L^{-1}, while a stable concentration of 1.5 g L^{-1} was reached in the SBR_B after about 20 days. The average size of the granules in the bacterial granular biomass and the microalgal-bacterial granular biomass increased from 0.7 to 1.2 mm and 1.8 nm (with no statistically significant difference among them ($p > 0.05$), and SVI_5 values lower than 20 and 25 mL g^{-1} were achieved in SBR_B and SBR_{AB}, respectively. In this context, Derlon et al. (2016) have reported values of SVI_5 between 30 and 80 mL g^{-1} in aerobic granules treating of 190 L bioreactor (0.25 cm of diameter and 4 m of height). This suggested that microalgae enhanced the sedimentation properties of the granular biomass, which allowed generate microalgae-bacteria flocs and granules. Magnetic agitation had no influence on the granular size in the algal-bacterial reactor, because it is only used to maintain the granules

in suspension. Thus, magnetic agitation at 200 rpm in the SBR_{AB} was applied to promote the aggregation of the seed biomass, microalgae and bacterial granules, and provide shear force to maintain stable algal-bacterial granules.

3.2. Carbon removal in bacterial and algal-bacterial SBRs

The high aeration rate used to promote granulation and maintain the granules in suspension, avoided oxygen limitation during carbon and nitrogen oxidation with an average DO concentration >7.5 mg O_2 L^{-1} in SBR_B. On the other hand, an average DO concentration of 5.7 mg O_2 L^{-1} was recorded in the SBR_{AB}.

The operational conditions were set to operate the reactors under alternating phases of external organic carbon availability (feast period) and limitation (famine period), herein referred to as feast/famine operation. It is well known that in feast phase, rapid uptake of organic carbon coupled to synthesis and accumulation of intracellular carbon and energy reserves take place. In the following famine phase, microbial maintenance and growth from endogenous organic carbon is typically expected. However, in the present study there is not a precise separation of the feast and famine phases.

Fig. 2 (A, B and S1) shows the removal of soluble COD throughout an operational cycle (24 h) of both reactors. A fast depletion of the COD present in cheese whey occurred in the first 2 h. The intracellular carbon and energy reserves (glycogen) increased from 10 to 620 mg Gly L^{-1} in SBR_B and 10–680 mg Gly L^{-1} in SBR_{AB} as the concentration of CODs

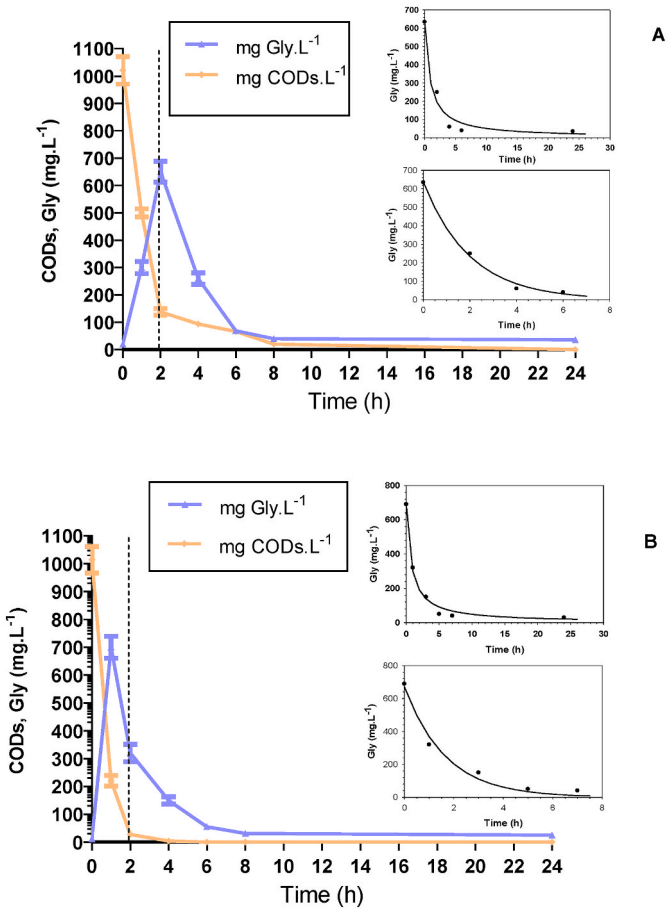

Fig. 2. Time course of COD_S and glycogen concentrations during wastewater treatment throughout an operating cycle in A) SBR_B and B) SBR_{AB}. Inserted figures: Glycogen concentration as a function of time in famine phase of SBR_B and SBR_{AB}. Upper graph: (−) Second order kinetic equation ($r^2 = 0.97$ and 0.99 for SBR_B and SBR_{AB} respectively); bottom graph: (−) first-order kinetic equation ($r^2 = 0.99$ and 0.98 for SBR_B and SBR_{AB} respectively).

significantly decreased. In SBR_B, the peak of glycogen accumulation is reached 2 h after the beginning of the cycle, and then rapid glycogen degradation and slow cheese whey consumption (CODs) simultaneously took place from 2 to 8 h. In SBR_{AB}, the maximum glycogen concentration is reached at 1 h of the cycle beginning when about 80% of the cheese whey was removed, and then rapid consumption of glycogen and cheese whey occurred between 1 and 2 h causing almost complete removal of exogenous organic carbon. The complete removal of COD from cheese whey required 8 h for SBR_B and only 2 h for SBR_{AB}. To facilitate the analysis, the separation of feast and famine phases in both reactors was set at 2 h of the beginning of the cycle, when the rate of cheese whey consumption was significantly reduced, which coincides with a removal of more than 80% and 100% of the COD_S for SBR_B and SBR_{AB} respectively (Fig. 2 A, B and S1).

To date, no previous works investigating the treatment of cheese whey in algal-bacterial systems have been identified. However, Petrini et al. (2020) and Su et al. (2012) did not observe any significant difference in COD removal performance between reactors inoculated with microalgae and activated sludge treating municipal wastewater.

The specific CODs removal rates in SBR_B and SBR_{AB} accounted for 293 ± 6.9 mg CODS·(g TSS•h)$^{-1}$ and 316 ± 3.7 mg CODS·(g TSS•h)$^{-1}$, respectively (Table 1). The initial volumetric COD_S removal rates were 439.5 ± 10.3 and 948 ± 11 mg CODS·(L•h)$^{-1}$ for SBR_B and SBR_{AB} respectively.

The glycogen synthesis rates were about 305 and 670 mg Gly·(L•h)$^{-1}$, equivalent to 361 and 794 mg COD_{Gly}·(L•h)$^{-1}$, for SBR_B and SBR_{AB}, respectively. This implies that around 82–83% of reduction equivalents from cheese whey for both reactors SBR_B and SBR_{AB} were converted into glycogen, which would allow preserving the reducing power for the denitrification process. The initial glycogen degradation rate was about 180 and 360 mg Gly·(L•h)$^{-1}$ for SBR_B and SBR_{AB}, respectively, i.e. a more rapid consumption of glycogen took place in the microalgae-bacteria reactor, which would not be favorable for the relatively slow denitrification process.

The degradation of glycogen was described by a second-order kinetic ($dGly/dt = -k.Gly^2$). This kinetic model was proposed for describing the removal rate of glycogen (fraction of glycogen of the total active biomass) in famine phase (Dircks et al., 2001). This equation satisfactorily adjusted to the experimental data by non-linear regression analysis using software Sigma-Plot 12.0 (upper graph inserted in Fig. 2 A, B). K constant (L· (mg·h)$^{-1}$) were similar: 0.0018 (SD 0.00051) and 0.0019 (SD 0.00023) for SBR_B and SBR_{AB} respectively. However, it must be considered that glycogen was estimated from measures of total carbohydrates (TC) and TC values at 24 h for both reactors corresponded to carbohydrates that constitute the biomass. In addition, it should be taken into account that glycogen was almost completely removed in

Table 1
Key performance indicators of organic carbon biodegradation and nitrogen removal in SBR_B and SBR_{AB}.

Operating cycle	Parameter	SBR_B	SBR_{AB}
24 h cycle	NH_4^+-N removal (%)	94 ± 0.6*	100 *
	Inorganic N removal (%)	30 ± 5.0*	18 ± 1.2 *
	Nitrification (%)	89 ± 3.2*	75 ± 3.5*
	TKN removal (%)	96 ± 3.3**	100**
	SND (%)	23.1 ± 3.5*	2 ± 0.7*
	Assimilation (%)	11 ± 1.9 *	25 ± 3.5 *
	N_G (mg L^{-1})	17 ± 0.2*	4 ± 0.3*
	N_G (%)	19.3 ± 0.2*	4.8 ± 0.5*
Feast Phase	q_{CODs} (mg COD_S (g TSS•h)$^{-1}$)	293.3 ± 6.9*	316.6 ± 3.7*
	NH_4^+-N removal (%)	47 ± 3.2*	71 ± 3.5*
	$q_{NH3\text{-}N}$ (mg NH_3–N (g TSS•h)$^{-1}$)	13.8 ± 2.4*	19.2 ± 1.1*
	q_{TNs} (mg TKN (g TSS•h)$^{-1}$)	6.3 ± 0.5**	6.8 ± 0.8**
Famine Phase	NH_4^+-N removal (%)	75 ± 4.4*	100*
	$q_{NH3\text{-}N}$ (mg NH_3–N (g TSS•h)$^{-1}$)	6 ± 1.9**	4.9 ± 1.9**
	q_{TNs} (mg TKN (g TSS•h)$^{-1}$)	5.6 ± 1.1 **	3 ± 0.8 **

about 6 h. Based on this analysis, a first-order kinetic (dGly/dt = -k.Gly) was proposed to describe glycogen degradation. This equation satisfactorily adjusted to the experimental data by non-linear regression analysis (lower graph inserted in Fig. 2 A, B). K constant (h^{-1}) for SBR_{AB} (0.5996, SD 0.0891) was about 20% higher than that of SBR_B (0.5007, SD 0.0391), which indicated a faster depletion of endogenous glycogen reserves in the microalgal-bacterial system as was previously explained, which would limit the denitrification process more quickly as will be explained later.

3.3. Nitrogen removal in bacterial and algal-bacterial SBRs

Ammonia (NH_3–N) is a known by-product from protein decomposition in cheese whey. Although ammonium was supplemented as $(NH_4)_2SO_4$ in this study, the concentration used was significantly lower than the toxicity levels previously reported for microalgae (100–1000 mg L^{-1}) (Xia and Murphy, 2016). NH_3–N was gradually removed during the feast and famine periods due to an active nitrification process and assimilation via microbial growth, since preliminary experiments conducted under abiotic conditions confirmed that NH_3 stripping was negligible. In SBR_B, the removal of ammonia via nitrification was correlated to the continuous generation of nitrate until the end of the cycle. Interestingly, NO_2^--N accumulated during the feast period and the first 2 h of the famine phase, and gradually disappeared after 8 h of SBR_B operation (Fig. 3A–S2). In SBR_{AB}, NO_2^--N consumption was faster and disappeared 2 h after the start of the famine period (Fig. 3B–S2). In SBR_B, the removal of NH_3–N during the feast period corresponds to about 47% of the initial ammonia concentration, while in SBR_{AB} this removal accounted for 71% (Table 1). Significant differences were found between the systems in study. In SBR_B, 89% of the remaining nitrogen from the feast period was removed during the famine period until the end of the cycle, while in SBR_{AB} this removal was completed 6 h after the end of the feast period (Fig. 3 A and B and S2). Overall, ammonia removal in SBR_B averaged 94% within 24 h at a rate of 13.8 mg NH_3–N·(g TSS•h)$^{-1}$ in the feast period, while NH_3–N was completely removed in only 8 h in SBR_{AB} at a rate of 19.2 mg NH_3–N·(g TSS•h)$^{-1}$ during feast phase (Fig. 3 A and B, S2, Table 1).

The removal of soluble TKN (TKNs), obtained from filtered samples, was similar to the removal of ammoniacal nitrogen throughout an operational cycle for SBR_B and SBR_{AB} (Fig. 4 A and B). The N_{ORG} present in cheese whey occurred mainly in particulate form, which is difficult to separate from biomass for quantification. Therefore, removal of N_{ORG} ($\Delta(N_{ORG})$) was estimated as 0.22·$\Delta(NH_3$–N) according to Bucci et al. (2020).TKN_{ML} accounts for NH_3–N, soluble and particulate N_{ORG} from the cheese whey, and the N_{ORG} corresponding to biomass. Therefore, TKN_{ML} decay throughout an operational cycle ($\Delta(TKN_{ML}) = \Delta(NH_3$–N) + $\Delta(N_{ORG})$), shown in Fig. 4 A and B, was attributed to the removal of wastewater TKN by nitrification (TKN_N). In SBR_B the nitrification process took place from the beginning of the cycle to more than 8 h, while that in the microalgal-bacterial system the nitrification occurred during a shortest period (2 h) i.e. exclusively during the feast phase (Fig. 4 A and B). Overall, the nitrifying activity in SBR_B was significantly higher than that of the SBR_{AB}. Contrary, in this last reactor, nitrogen assimilation was significantly greater than that of the SBR_B (Table 1).

In SBR_{AB}, NH_3–N removal was total and faster in comparison with SBR_B (Table 1). This finding could be probably attributed to the fact that algae provide additional oxygen to bacteria through photosynthesis and bacteria provide additional carbon source to algae by breaking down organic matter, which ultimately result in higher nitrogen assimilation. However, further studies are required to demonstrate this hypothesis. Early studies have shown that vitamins and nutrients such as nitrogen and carbon play a central role in algal and bacterial synergisms. For instance, the supply of bacterial vitamin B12 to the microalgae can stimulate microalgal metabolisms (Katam and Bhattacharyya, 2019). Similarly, Liu et al., (2017) reported that these symbiotic relationships between microalgae and bacteria can involve the transfer of nutrients, which ultimately increase nutrient removal rates.

In SBR_B, about 47% of TKN was removed in feast phase, with nitrification representing about 70% of the TNK consumption according to nitrogen balance. The remaining TKN removed in feast phase was assimilated by microbial growth, using proteins and fats as carbon and energy sources as will be explained in the following section. In SBR_{AB},

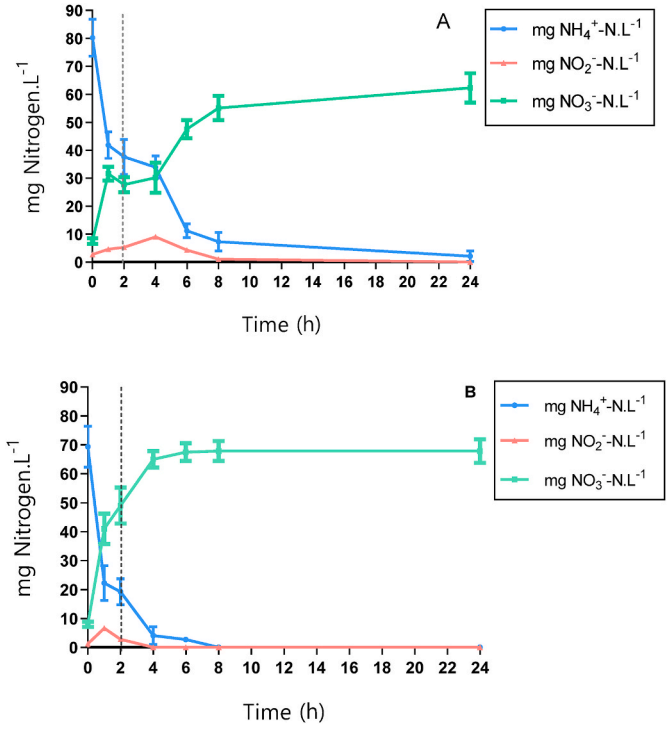

Fig. 3. Time course of the concentrations of NH4+-N (●), NO3–N (■), NO2–N (▲) during wastewater treatment throughout an operating cycle in SBRB (A) and SBRAB (B).

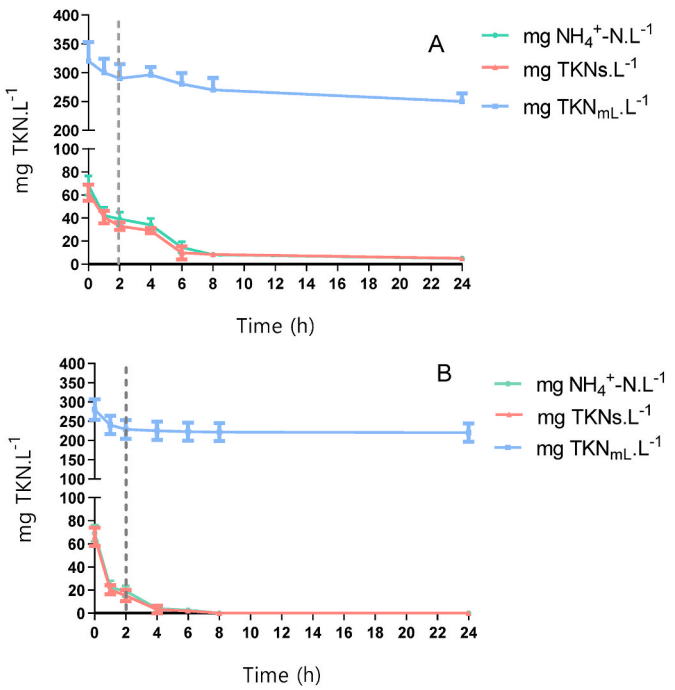

Fig. 4. Time course of TKN_{ML}, TKN_S and NH_3–N concentrations throughout an operating cycle in SBR_B (A) and SBR_{AB} (B).

the highest fraction of TKN (about 70%) was removed in the feast period and nitrification corresponded to 75% of TNK consumption. The remaining TKN, removed in feast phase, was used for growth of bacteria and microalga.

Simultaneous nitrification and denitrification (SND) represented 23% of the nitrogen removed in SBR_B but was not relevant in SBR_{AB} (Table 1). SND was estimated considering the difference between nitrogen nitrified ($\Delta(TKN_{ML})$ and the remaining oxidized nitrogen as nitrite and nitrate (NOx-N) throughout each operational cycle.

The low SND activity estimated in the microalgal-bacterial system was attributed to the following factors: a low nitrifying activity that took place only in the feast period, an elevated volumetric CODs removal rate (more than double that the corresponding to the SBR_B), and higher degradation rate of intracellular glycogen with regard to the bacterial reactor as was previously explained. Microalgae growth probably stimulated the heterotrophic bacteria activity and nitrifying bacteria were outcompeted by heterotrophs. In addition, a rapid conversion of external CODs to glycogen followed by its quick degradation cannot be coupled to the relatively slow denitrification processes. These phenomena could explain the insignificant SND process in the SBR_{AB}. Anyway, the factors responsible of the increase of the bacterial activity and substrates conversion rates in the microalgal-bacterial system should be further studied.

The specific tests carried out to study denitrification under anaerobic conditions did not show N_2 production in either of the two systems studied. Thus, SND under aerobic conditions took place in SBR_B and SBR_{AB}.

Larger TKN removal and TKN assimilation in SBR_{AB}, with regard to SBR_B, partially compensated for the lowest SND activity of the microalgal-bacterial reactor, which involves that its Ni removal performance was significantly lower than that of the bacterial system (Table 1).

Aerobic treatment using granulation technology is one of the most commercial technologies used in dairy industries due to its associated relevant economic and environmental benefits (Yukesh et al., 2020). Recent studies have proved that microalgal–bacterial symbiotic systems can support a higher biomass accumulation and a superior wastewater treatment efficiency (Sutherland et al., 2020).

Microalgae have been shown to play an important role in carbon sequestration and are also an important source of energy for consumers. Bacterial communities can perform decomposition functions by mineralizing carbon fixed through photosynthesis. The occurrence of bacteria can affect the growth of microalgae, generating symbiotic and competitive relationships between them (Li et al., 2012). Furthermore, bacteria can promote the formation of microalgae through the exchange of metabolites and promote their auto-aggregation (Li et al., 2012). The relationship between algae and bacteria consortium granules for wastewater treatment can further increase the biochemical activities of microalgae and bacteria, which is very useful for the production of microalgae biomass (Bounnit et al., 2020; Lui et al., 2017).

3.4. Estimation of the reduction of CO_2 emission in the microalgal-bacterial SBR

The following biological reactions of carbon conversion were proposed: synthesis of glycogen from carbohydrates of cheese whey (lactose), synthesis of bacterial biomass from glycogen, and protein and fat of the cheese whey. For simplification, oxygen was considered as the only electron acceptor in all reactions.

The loading rate of COD in cheese whey wastewater was 1075.2 mg COD·(L day)$^{-1}$ (corresponding to 1075.2 mg cheese way·(L day)$^{-1}$). Based on cheese whey's composition (75 % of lactose, 10% of protein and 5 % of fat), the following equivalent loads were obtained: 806.4 mg lactose·(L day)$^{-1}$ (28.27 C-mmol lactose·(L. day)$^{-1}$), 107.52 mg protein·(L day)$^{-1}$ (4.67 C-mmol protein·(L day)$^{-1}$) and 53.76 mg fat·(L day)$^{-1}$ (3.2 C-mmol fat·(L. day)$^{-1}$). Lactose, protein and fat represented about 78.2 %, 13 % and 8.8 % of the total amount of organic compounds present in the cheese whey as C-mmol (36.14 c-mmol· (L. day)$^{-1}$), respectively.

Carbohydrates are commonly stored as glycogen in SBRs operated under feast/famine regime (Serafim et al., 2008). For simplification, it was assumed that no biomass growth from lactose took place. Actually, very low fraction of glucose (2.4–5.4% C-mmol/cycle) is directly converted to biomass in aerobic SBR (Dircks et al., 2001). From the minimum formula of glycogen $CH_{1.66}O_{0.83}$ (Smolders et al., 1994) and considering the concentration of glycogen accumulated in SBR_B (22.22 $CH_{1.66}O_{0.83}$) the following reaction was adjusted:

$$28.27\ CH_{1.833}O_{0.916} + 6.04\ O_2\ 22.22\ CH_{1.66}O_{0.83} + 6.04\ CO_2 + 7.46H_2O \quad (1)$$

The following yields were estimated: $Y_{Glycogen/Lactose} = 0.78$ C-mmol Gly/C-mmol Lactose and $Y_{CO2/Lactose} = 0.21$ C-mmol CO_2/C-mmol Lactose. These values were within the range of yields reported by Dircks et al. (2001): 0.74–0.87 C-mol Gly/C-mol glucose and 0.07–0.23 C-mmol CO_2/C-mmol glucose.

Considering the TKN loading rate of the SBR_B (78.48 mg TKN·(L day)$^{-1}$), the TKN removal (96%), the TKN removal by nitrification (89%) and the nitrogen content of the biomass (11%, Table 1), the TKN assimilated was estimated 0.59 mmol TKN·(L day)$^{-1}$. Assuming a similar biomass yield for lactose, protein and fat, the N devoted to growth was estimated taking into account the C-mmol fraction of each carbon source in the cheese whey, resulting 0.461 mmol N·(L day)$^{-1}$, 0.076 mmol N·(L day)$^{-1}$ and 0.052 mmol N·(L day)$^{-1}$ respectively. Then, the following biomass synthesis reaction from glycogen is proposed based empirical data, taking into account the elemental composition of bacterial cells ($CH_{1.4}O_{0.4}N_{0.2}$, Metcalf & Eddy, 2014) and the amount of N assimilated for the case of lactose:

$$22.22\ CH_{1.66}O_{0.83} + 19.92\ O_2 + 0.461\ NH_3\ 2.30\ CH_{1.4}O_{0.4}N_{0.2} + 19.92\ CO_2 + 17.52H_2O \quad (2)$$

The following yields were obtained: $Y_{X/Glycogen} = 0.10$ C-mmol X/C-mmol Gly and $Y_{CO2/Glycogen} = 0.89$ C-mmol CO_2/C-mmol Gly. High nitrifying activity and bacterial growth from proteins/fats probably caused TKN limitation, which explains the low biomass yield from glycogen in famine phase.

The combination of the reactions of glycogen formation (1) and biomass synthesis (2) results in the following equation:

$$28.27\ CH_{1.833}O_{0.916} + 25.96\ O_2 + 0.461\ NH_3\ 2.30\ CH_{1.4}O_{0.4}N_{0.2} + 25.96\ CO_2 + 24.98H_2O \quad (3)$$

The amount of N assimilated was taken into account for biomass synthesis directly from proteins of cheese whey. For simplification, the chemical formula of casein ($C_8H_{12}O_3N_2$, Comeau, 2008) was proposed for whey proteins, which is similar to the proposal for proteins (Metcalf & Eddy, 2014). The following reaction of biomass synthesis was proposed:

$$4.67\ CH_{1.5}O_{0.375}N_{0.25} + 4.29\ O_2\ 0.38\ CH_{1.4}O_{0.4}N_{0.2} + 4.29\ CO_2 + 1.60H_2O + 1.09\ NH_3 \quad (4)$$

The following yields were obtained: $Y_{X/Protein} = 0.08$ C-mmol X/C-mmol protein and $Y_{O2/Protein} = 0.92$ C-mmol CO_2/C-mmol protein. The low yield may be due to N limitation caused by high nitrification. Similarly, the fats present in the influent cheese whey fed into the reactor at 3.2 C-mmol fat·(L. day)$^{-1}$ resulted in the formation of 0.26 C-mmol $CH_{1.4}O_{0.4}N_{0.2}$ and 2.94 C-mmol CO_2.

The total biomass production from the proposed synthesis reactions using glycogen, proteins and fat, and considering the assimilated N, was estimated at 2.94C -mmol X·(L.day)$^{-1}$. The net biomass production was also estimated from the sludge wasted from the SBR_B (6.3 C-mmol. day^{-1}), which expressed per unit volume of the reactor corresponds to 3.15 C-mmol·(L.day)$^{-1}$. The sludge present in the discharge effluent remained below the detection limits of the methodology used. As

expected, the biomass production based on synthesis reactions (2.94 C-mmol X·(L.day)$^{-1}$) was equivalent to the net biomass production quantified experimentally (3.15 C-mmol·(L.day)$^{-1}$).

It should be noted that the bacterial SBR presented a similar yield of glycogen from carbohydrate than an activated sludge culture with high capacity for glycogen accumulation (Dircks et al., 2001).

In the algal-bacterial SBR, a similar amount of glycogen was synthesized with regard to SBR_B. In addition, about 82% and 83% of reduction equivalents from cheese whey for SBR_B and SBR_{AB} respectively were converted into glycogen. Taking into account this result and considering that the systems were operated under conditions favorable for bacterial growth i.e. with COD load and without addition of CO_2, the following assumptions were proposed. The bacterial biomass did not change significantly in both systems, and the synthesis of algae occurred likely from the remaining nitrogen, not assimilated by bacteria, using mainly CO_2 generated by bacteria in the algal-bacterial SBR. The N available for microalgal growth can be estimated from the difference between the total TKN assimilated in the SBR_{AB} 1.40 mmol N·(L.day)$^{-1}$ (78.48 mg TKN·(L day)$^{-1}$ (TKN consumption) × 0.25% (assimilation), Table 1) and the N assimilated by the bacterial community resulting in 0.81 mmol N·(L day)$^{-1}$. The following synthesis reaction of microalgae biomass is proposed (Borde et al.):

$$6.75\ CO_2 + 4.79H_2O + 0.81\ NH_3\ 6.75\ CH_{1.78}O_{0.36}N_{0.12} + 7.93\ O_2 \quad (5)$$

Considering the values obtained experimentally and the balances above presented, it can be observed than microalgae could remove about 20 % of the CO_2 produced by bacteria. It shows that microalgae help in CO_2 sequestration through organic carbon absorption which is an effective method to reduce the carbon emission with producing for example of biofuels and bioenergy from microalgae biomass.

4. Conclusion

This study systematically compared a granular SBR operated with bacterial biomass and mechanical aeration, and an irradiated algal-bacterial granular SBR using a synthetic effluent of dairy industries supplemented with ammonia. The efficient algae-bacterial symbiosis in the granular systems allowed the complete elimination of COD (100%) and ammonia (100%) present in dairy wastewater. This synergism allowed a lower loss of nitrogenous nitrogen due to denitrifying activity, with a more rapid depletion of endogenous glycogen reserves and an increase in biomass that could be used to produce potential products such as biodiesel to replace fossil fuels such as mineral diesel. Also, the highest fraction of TKN was removed in the famine period and nitrification corresponded to 75% of TNK consumption in this system. Finally, lower CO_2 emissions (~20 %) occurred in the SBR_{AB} according to process stoichiometry and mass balance calculations.

CRediT authorship contribution statement

Paula Bucci: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Enrique José Marcos Montero:** Methodology, Investigation. **Octavio García-Depraect:** Conceptualization. **Noemí Zaritzky:** Writing – review & editing, Supervision, Formal analysis. **Alejandro Caravelli:** Writing – review & editing, Validation, Supervision, Investigation. **Raúl Muñoz:** Writing – review & editing, Resources, Project administration, Funding acquisition, Writing – review & editing, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141250.

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