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Comparative Assessment of Activated Sludge, Microalgae, and Microalgal–Bacterial Consortia for Dairy Wastewater Treatment Efficiency and Methane Potential of Recovered Biomass

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Abstract

Modern biological wastewater treatment technologies should not only effectively remove organic compounds and nutrients but also enable the recovery of biomass suitable for further valorization. The aim of this study was to compare activated sludge (AS), a *Chlorella* sp. monoculture (MA), and a microalgal–bacterial consortium (M-BC) for the treatment of model dairy wastewater and to assess the methane potential of the recovered biomass. AS provided the highest organic matter removal efficiency, reducing COD and BOD_5 concentrations to 141.4 ± 7.6 mg O_2/L and 32.3 ± 1.7 mg O_2/L, respectively, corresponding to removal efficiencies of 94.3 ± 0.3% and 98.1 ± 0.1%. The M-BC variant achieved the highest nitrogen and phosphorus removal efficiencies, at 90.3 ± 0.6% and 90.0 ± 0.6%, respectively. The system based solely on MA was less effective from a treatment perspective, but the resulting biomass had the highest volatile solids content and the highest methane potential. In this case, cumulative CH_4 production after 20 days was 255.1 ± 12.3 mL CH_4/g VS. The corresponding values were 230.2 ± 15.9 mL CH_4/g vs. for M-BC and 189.5 ± 6.3 mL CH_4/g vs. for AS. The Gompertz model, however, indicated the most favorable digestion kinetics for M-BC biomass, with an R_{max} of 26.8 ± 1.8 mL CH_4/g VS·d and a lag phase of 0.53 ± 0.08 d.

Keywords: activated sludge; microalgae; *Chlorella* sp.; microalgal–bacterial consortium; dairy wastewater; nutrient removal; biomass productivity; anaerobic digestion; biochemical methane potential

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1. Introduction

Increasingly stringent effluent-quality requirements, together with the need to reduce energy consumption and greenhouse gas emissions, are driving the development of technologies that combine high pollutant-removal efficiency with resource recovery [1], in line with the principles of the circular economy, bioeconomy, and zero-waste strategies [2]. Activated sludge (AS) systems remain the most mature and widely applied biological wastewater treatment technology [3]. Properly designed systems can remove more than 90–95% of organic matter, while alternating aerobic, anoxic, and anaerobic conditions also enable effective nitrogen and phosphorus removal [4]. Their major limitation is high energy

consumption [5]. Aeration accounts for 50–90% of total electricity use, while energy may represent 15–49% of wastewater treatment plant operating costs; additional energy inputs are associated with mixing, recirculation, and excess-sludge processing [6,7].

More energy-efficient anaerobic systems can achieve COD removal efficiencies exceeding 80–90% and generate biogas typically containing 55–75% CH_4, with a theoretical methane yield of approximately 0.30–0.40 m^3 CH_4/kg COD removed [8]. However, they do not provide complete nitrogen and phosphorus removal and therefore generally require further effluent polishing [9]. One of the approaches currently being developed involves the use of microalgae. Microalgae assimilate mineral forms of N and P, consume CO_2, and produce O_2, thereby potentially reducing the need for mechanical aeration [10]. Their potential for nutrient removal and recovery has also been confirmed in recent studies [11]. In wastewater containing dairy-derived constituents, a mixed microalgal culture dominated by *Spirulina* sp. and *Chlorella* sp. achieved nitrogen removal of 93.34%, compared with 92.56% and 86.85% for *Spirulina* sp. and *Chlorella* sp., respectively, while phosphorus assimilation exceeded 83% [12]. An additional advantage is the valorization potential of microalgal biomass, which may contain 20–60% protein, 10–50% lipids, and 10–40% carbohydrates on a dry-weight basis and can be further processed into biofuels, fertilizers, feeds, biostimulants, or pigments [13].

However, the technical application of microalgal monocultures (MA) is constrained by high light requirements, self-shading, reduced performance at elevated COD loads, and difficult biomass separation [14–16]. Recent studies using a native *Chlorella* strain have confirmed the feasibility of treating synthetic wastewater under both batch and continuous operation and highlighted the importance of substrate availability and operating conditions for microalgal growth [17]. When *Tetraselmis subcordiformis* was cultivated on dairy wastewater, biomass concentrations of 2.11–2.24 g/L were obtained, compared with 3.41 g/L in the control medium, while nitrogen and phosphorus removal efficiencies ranged from 74–91% and 32–75%, respectively [18]. Poor cell settleability remains an additional limitation, often necessitating coagulation, flotation, filtration, or centrifugation [19].

Microalgal–bacterial consortia (M-BC) may overcome some of these limitations through complementary metabolic functions. Bacteria degrade organic compounds, consume O_2, and release CO_2 and mineral forms of N and P, whereas microalgae use these products for growth and supply oxygen that supports COD oxidation and nitrification [20,21]. In wastewater from whey processing, microalgal oxygen production reached 84–105 mg O_2/L·d, compared with a bacterial oxygen demand of 46 mg O_2/L·d; simultaneously, COD, TN, N-NH_4, and phosphorus removal efficiencies of 93–94%, 88–90%, 88–99%, and 69–73%, respectively, were achieved, while bacterially generated CO_2 supplied up to 58% of the microalgal inorganic carbon demand [22]. M-BC can also form flocs and granules with better settling properties than dispersed microalgal cultures [23]. In pilot-scale studies, microalgal–bacterial granular sludge reached 4.8 $\pm$ 0.5 g TS/L and achieved COD and TOC removal efficiencies of 84.1 $\pm$ 5.1% and 88.2 $\pm$ 7.2%, respectively [24]. Nevertheless, this technology remains less mature than conventional AS, and its performance depends on factors such as the microalgae-to-bacteria ratio, COD loading, illumination, and the balance between oxygen production and consumption [25].

Despite the development of these technologies, AS, MA, and M-BC are still most often evaluated separately or only partially compared, frequently under different wastewater compositions and operating conditions. To the best of the authors' knowledge, studies directly comparing all three systems using the same wastewater matrix and comparable process conditions, followed by assessment of the methane potential of the recovered biomass under identical anaerobic digestion conditions, are lacking. This limits the ability to determine whether observed differences arise from biomass-specific characteristics or

from differences in experimental conditions. The novelty of the present study therefore lies in the direct comparison of AS, a *Chlorella* sp. monoculture, and M-BC within a single standardized dairy-wastewater matrix, combined with characterization of the recovered biomass and assessment of its CH_4 potential and anaerobic digestion kinetics.

The aim of this study was to compare the treatment performance of standardized model dairy wastewater using AS, MA, and M-BC and to determine the methane potential of the biomass generated in the respective variants. The evaluation included removal of organic matter and nutrients, biomass growth and composition, cumulative CH_4 production, and anaerobic digestion kinetic parameters.

2. Materials and Methods

2.1. Experimental Design

The study was divided into two experimental stages. The first stage (S1) involved the treatment of model dairy wastewater (DW), whereas the second stage (S2) concerned the assessment of the suitability of the recovered biomass as a substrate for anaerobic digestion. Each stage was divided into three variants defined by the biomass tested. Variant 1 (V1) used activated sludge (AS), variant 2 (V2) used a *Chlorella* sp. microalgal monoculture (MA), and variant 3 (V3) used a microalgal–bacterial consortium (M-BC). In S1, the variants differed not only in the type of biomass tested but also in the bioreactor operating mode appropriate to each biomass, particularly with respect to aeration, illumination, and gas exchange. Accordingly, the S1 comparison reflects the performance of the three technological configurations under their respective operating conditions rather than the isolated effect of biomass type alone. In S2, anaerobic respirometers of the same design and working volume were used in all variants. The main technological and operating parameters were also standardized, enabling comparison of the methane potential of biomass originating from the individual S1 variants. The organization of the experimental work is presented in Figure 1.

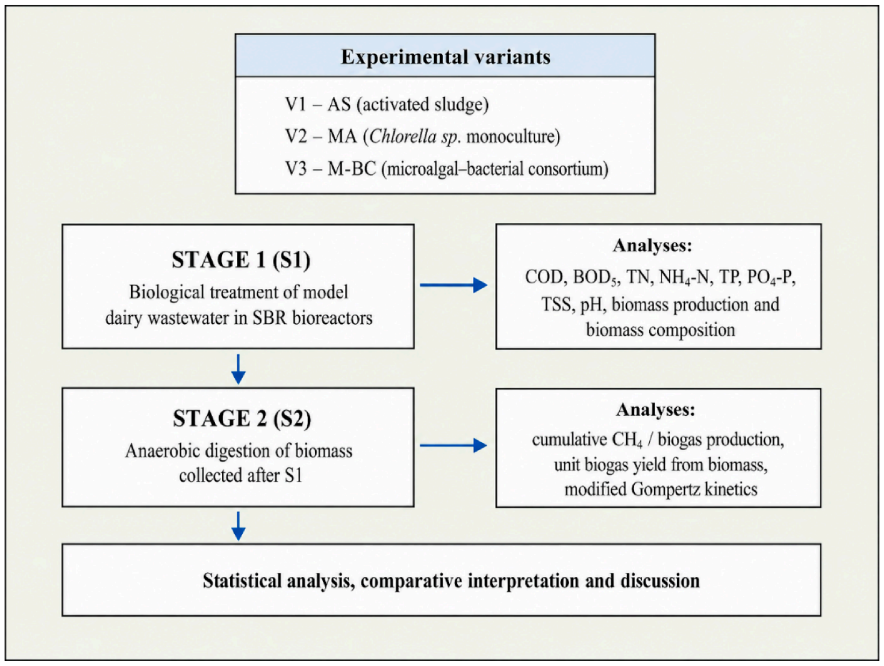

Figure 1. Experimental design and scope of experimental and analytical work.

2.2. Materials

2.2.1. Model Dairy Wastewater

Model dairy wastewater (DW) was prepared from skimmed milk powder (Mlekovita, Spółdzielnia Mleczarska Mlekovita, Wysokie Mazowieckie, Poland). A 2.0 g portion of milk powder was added to 1.0 L of distilled water (GFL 2004, Gesellschaft für Labortechnik mbH, Burgwedel, Germany). The medium was mixed for 30 min at 200 rpm and 20 ± 1 °C using a mechanical stirrer (RW 20 digital) equipped with a propeller stirrer (R 1342, IKA-Werke GmbH & Co. KG, Staufen, Germany) until a homogeneous suspension was obtained. The monitored pollution indicators were determined immediately after DW preparation. For each experimental variant, one common batch of model DW was prepared and, after homogenization, stored in a retention tank with an active volume of 5.0 L. The medium was then dosed from the tank to the operated bioreactors using peristaltic pumps (BT100-2J, Baoding Longer Precision Pump Co., Ltd., Baoding, China). The tank was equipped with an RW 20 digital vertical-axis mechanical stirrer fitted with a propeller stirrer (R 1342, IKA-Werke GmbH & Co. KG, Staufen, Germany), operated continuously to maintain suspension homogeneity. This ensured the same influent composition and a comparable initial pollutant load in all variants. DW was prepared immediately before the start of each process cycle and replaced every 24 h. Raw model DW had a COD concentration of 2470 ± 120 mg O_2/L and a BOD_5 concentration of 1710 ± 108 mg O_2/L. Total nitrogen (TN) concentration was 82.5 ± 3.8 mg N/L, whereas ammonium nitrogen (N-NH_4) concentration was 2.5 ± 0.8 mg N/L. Total phosphorus (TP) concentration was 17.0 ± 1.5 mg P/L and phosphate phosphorus (P-PO_4) concentration was 11.0 ± 1.5 mg P/L. The medium was near neutral, with a pH of 6.80 ± 0.10, while the total suspended solids (TSS) concentration was 350 ± 12 mg/L.

2.2.2. Activated Sludge (AS)

AS was collected from the aerobic chambers of the Łyna Municipal Wastewater Treatment Plant in Olsztyn, Poland (53.815153° N, 20.453615° E). The facility treats municipal wastewater conveyed through the sewerage system as well as wastewater transported from neighboring municipalities. The average plant throughput is approximately 60,000 m^3/d. The treatment train includes mechanical wastewater treatment, biological removal of organic compounds, nitrogen, and phosphorus, and biomass separation in secondary clarifiers. During sampling, the AS mixed-liquor temperature was 18 ± 2 °C, the pH was 7.1 ± 0.2, and the dissolved oxygen concentration in the aerobic zone was 2.3 ± 0.3 mg O_2/L. The biomass concentration in the activated sludge chamber was 3.5 ± 0.3 g TS/L. The hydraulic retention time was 0.8 ± 0.1 d, the sludge age was 18 ± 2 d, and the biomass loading was 0.10 ± 0.02 kg COD/kg TS·d. The sludge was transported to the laboratory in closed, aerated containers at 20 ± 2 °C. The biomass was used to inoculate the reactors within a maximum of 4 h after collection. Before inoculation, the AS was gently homogenized for 10 min at 100 rpm using an RW 20 digital mechanical stirrer equipped with a paddle stirrer (R 1313, IKA-Werke GmbH & Co. KG, Staufen, Germany). The biomass concentration in the reactors was then adjusted to 3.0 ± 0.1 g TS/L. The basic characteristics of the activated sludge are presented in Table 1.

Table 1. Characteristics of the biomass used in the individual experimental variants and anaerobic digestion tests.

Parameter	Unit	AS	MA	M-BC	AI
Volatile solids (VS)	% TS	76.0 ± 1.8	88.2 ± 1.3	82.3 ± 3.5	62.5 ± 2.8
Mineral solids (MS)	% TS	24.0 ± 1.8	11.8 ± 1.3	17.7 ± 3.5	37.5 ± 2.8
Total carbon (TC)	mg/g TS	395 ± 14	461 ± 39	455 ± 74	325 ± 18

Table 1. *Cont.*

Parameter	Unit	AS	MA	M-BC	AI
Total organic carbon (TOC)	mg/g TS	369 ± 13	423 ± 22	397 ± 70	286 ± 16
Total nitrogen (TN)	mg/g TS	38.0 ± 1.9	40.2 ± 1.8	30.6 ± 3.4	45.5 ± 2.4
C/N	–	9.7 ± 0.6	10.5 ± 0.2	14.4 ± 2.0	6.3 ± 0.4
Total phosphorus (TP)	mg/g TS	11.4 ± 0.9	18.4 ± 1.7	13.3 ± 2.9	26.8 ± 2.1
pH	–	7.18 ± 0.08	7.92 ± 0.09	7.53 ± 0.07	7.42 ± 0.09
Protein	% TS	23.8 ± 1.2	25.1 ± 2.2	24.1 ± 2.1	21.4 ± 1.5
Lipids	% TS	6.8 ± 0.6	15.2 ± 0.7	11.1 ± 1.3	7.5 ± 0.8
Carbohydrates	% TS	13.7 ± 1.4	37.2 ± 2.3	42.0 ± 4.0	10.8 ± 1.6

2.2.3. *Chlorella* sp. Biomass (MA)

The *Chlorella* sp. UTEX 636 inoculum was obtained from the Culture Collection of Algae, University of Texas, Austin, TX, USA. The biomass was propagated in a photobioreactor (PBR) installation at the Department of Environmental Engineering, University of Warmia and Mazury in Olsztyn, Poland (53.7556° N, 20.4528° E). The microalgae were cultivated in hybrid PBRs with an active volume of 2.0 m^3 [26]. Model dairy wastewater prepared from milk powder according to the procedure described above was used as the culture medium. This approach enabled prior adaptation of *Chlorella* sp. to the composition of the wastewater subsequently used in the main experiment. After cultivation, the biomass was separated from the medium using a Z41 continuous-flow centrifuge (CEPA LEA Lab, Ingersheim, Germany). Before inoculation, the biomass was gently homogenized for 10 min at 100 rpm using an RW 20 digital mechanical stirrer equipped with an R 1342 propeller stirrer (IKA-Werke GmbH & Co. KG, Staufen, Germany) to obtain a homogeneous suspension without excessive cell damage. The biomass was then used to inoculate the MA-variant reactors. The initial biomass concentration in the reactors was adjusted to 3.0 ± 0.1 g TS/L. The MA variant was inoculated with a *Chlorella* sp. monoculture, but axenic conditions were not maintained, and an accompanying bacterial population gradually developed during reactor operation. The characteristics of the *Chlorella* sp. biomass are presented in Table 1.

2.2.4. Microalgal–Bacterial Consortium (M-BC)

M-BC was obtained from mature microalgal–bacterial granular sludge (M-BGS) biomass previously developed in a hybrid PBR operated at the Łyna Municipal Wastewater Treatment Plant in Olsztyn. The system was fed with the liquid digestate fraction generated during dewatering of digested sewage sludge. Mature M-BGS biomass was obtained after 60 d of photobioreactor operation. During this period, the consortium developed a stable morphological and microbiological structure. The biomass was dominated by *Chlorella* sp. and microorganisms characteristic of activated sludge, including the filamentous bacterium *Microthrix parvicella* and types 1851 and 1701. The proportion of heterotrophic microorganisms and protozoa in the mature consortium was approximately 43 ± 9% TS [24]. Before use in the present experiment, M-BGS biomass was separated from the medium by sedimentation and drum filtration using screens with mesh sizes of 10.0 and 5.0 μm. The concentrated biomass was homogenized and used to inoculate the M-BC-variant reactors. The initial biomass concentration in the reactors was adjusted to 3.0 ± 0.1 g TS/L, as in the activated sludge and *Chlorella* sp. monoculture variants. The characteristics of M-BC are presented in Table 1.

2.2.5. Anaerobic Sludge Inoculum (AI)

The anaerobic sludge used as the anaerobic inoculum (AI) in the methane production tests was collected from closed anaerobic digesters at the Łyna Municipal Wastewater Treatment Plant in Olsztyn, Poland (53.815153° N, 20.453615° E). The digesters were operated under mesophilic conditions at approximately 38 ± 1 °C and a hydraulic retention

time of 20 ± 2 d. The organic loading rate of the digesters was 2.4 ± 0.2 kg VS/m^3·d. Before use in the respirometric measurements, the sludge was homogenized, screened through a 1.0 mm mesh to remove larger particles of undegraded organic matter, and pre-incubated in sealed containers for 5 d at 38 ± 1 °C under anaerobic conditions to reduce endogenous biogas production. The total solids content of the anaerobic inoculum was 5.0% FM (95% moisture), with vs. accounting for 62.5 ± 2.8% TS. The characteristics of the anaerobic sludge are presented in Table 1.

2.3. Wastewater Treatment Bioreactors

The experiments were conducted in vertical column reactors with an active volume of 2.0 L (Aqua Medic, Janikowo, Poland). The same reactor type was used in all variants, with only the illumination, aeration, and CO_2 supply conditions being varied. Three independent reactors were operated in parallel for each experimental variant (n = 3), resulting in a total of nine SBRs. The reactors were operated as sequencing batch reactors (SBRs) in a 24 h cycle: filling—0.25 h, aerobic/photoautotrophic phase—18.00 h, anoxic–anaerobic phase—4.00 h, sedimentation—1.50 h, and decantation—0.25 h. In each cycle, 0.25 L of medium was exchanged, corresponding to HRT = 8.0 d. Biomass concentration was monitored as TS and maintained at 3.0 ± 0.1 g TS/L by periodic withdrawal of excess biomass. The amount withdrawn was recorded for each reactor, and operational SRT was determined from the solids mass balance as the ratio of the mean TS mass in the reactor to the mean daily TS loss through biomass withdrawal and effluent discharge. At an influent COD concentration of 2470 ± 120 mg O_2/L, the volumetric loading rate was 0.30 ± 0.01 kg COD/m^3·d and the biomass loading was 0.10 ± 0.01 kg COD/kg TS·d. The temperature was maintained at 21 ± 1 °C. The configuration of the bioreactors and the main flow directions of medium, air, and recirculated gas in the individual variants are shown in Figure 2.

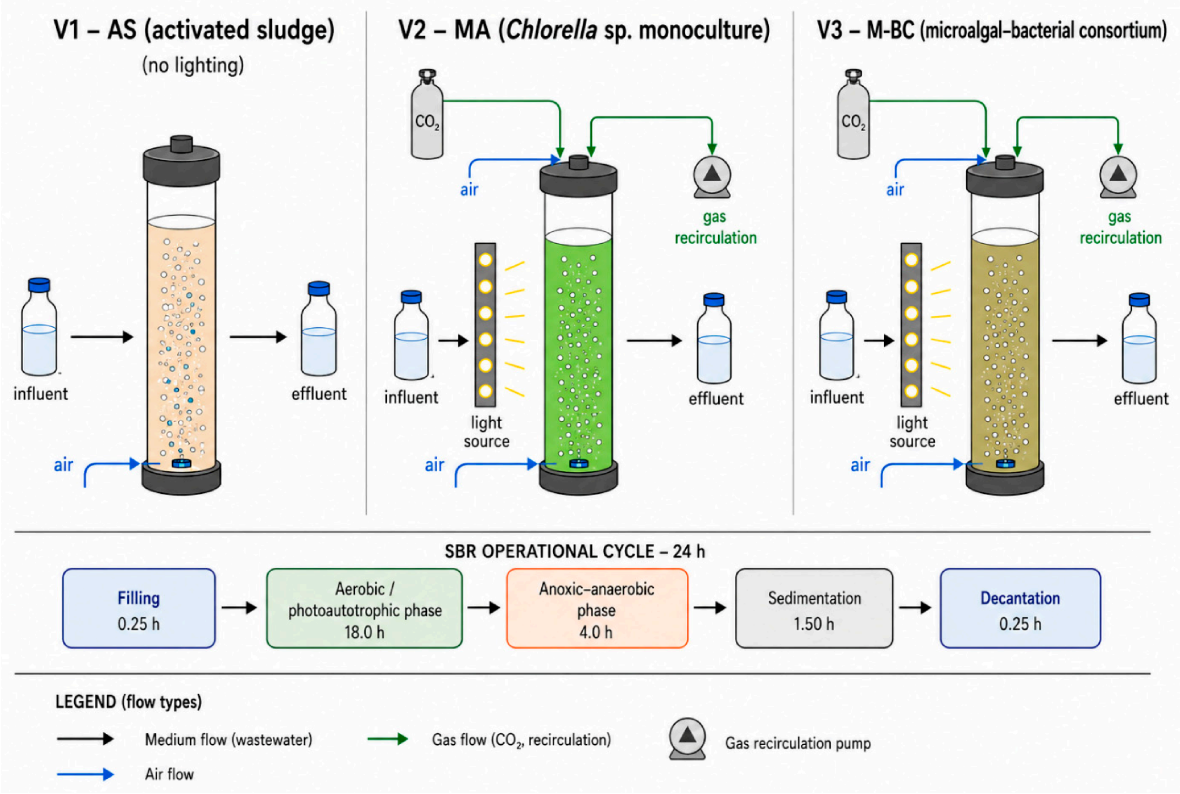

Figure 2. Schematic of the bioreactors and SBR operating phases used in S1 for the respective operating variants.

Fine-bubble aeration was applied during the filling and reaction phases using a Hailea V-10 diaphragm pump (Hailea Group, Chaozhou, China) and 30 mm Aquael Air Stone Sphere mineral diffusers (Aquael, Warsaw, Poland) positioned at the bottom of the columns. The airflow rate was 0.50 L/min per reactor and maintained dissolved oxygen (DO) at 2.5–3.0 mg O_2/L. DO concentration was monitored using a Multi 3630 IDS portable multiparameter meter equipped with an FDO 925 optical oxygen probe (WTW/Xylem Analytics, Weilheim, Germany). Measurements were performed during the reaction phase and immediately before the start of the anoxic–anaerobic phase. The measured DO values were used to adjust aeration intensity in the AS variant and to activate supplementary air supply in the M-BC variant. In the AS variant, the reactors were operated without illumination. Aeration was switched off during the anoxic–anaerobic, sedimentation, and decantation phases.

In the MA and M-BC variants, illumination was provided by 10 W LED lamps (Leddy Slim, Aquael, Warsaw, Poland), generating a luminous flux of 720 lm, a color temperature of 9000 K, and PAR of 270–300 μmol/m^2·s within the 400–700 nm range. A photoperiod of 18 h light and 6 h dark was applied. The light period included the aeration phase. In the MA variant, mixing was provided by closed-loop recirculation of the gas phase using a Hailea ACO-9601 pump (Hailea Group, Chaozhou, China) with a nominal capacity of approximately 192 L/h. The recirculated gas was introduced into the reactors through 30 mm Aquael Air Stone Sphere mineral diffusers (Aquael, Warsaw, Poland) positioned at the bottom of the columns. The actual gas flow rate was 180 ± 15 L/h per reactor. CO_2 was supplied from a gas cylinder to the gas loop and was automatically regulated based on the pH of the medium. The pH setpoint was 7.20, and the solenoid-valve hysteresis was 0.10 pH units. Gas recirculation and CO_2 dosing were switched off during the anoxic–anaerobic, sedimentation, and decantation phases.

In the M-BC variant, the same illumination, CO_2 control, gas recirculation, and diffusion system as in MA was used, but the recirculated gas flow was reduced to 150 ± 15 L/h per reactor to limit disruption of microalgal–bacterial aggregates. CO_2 originated from cylinder dosing and from the mineralization of organic compounds by heterotrophic bacteria. During the light phase, oxygen for the bacteria was supplied mainly by microalgal photosynthesis. During the dark part of the reaction, atmospheric air was supplied at 0.10 L/min per reactor only when DO fell below 1.0 mg O_2/L. The target DO range in the M-BC variant was 1.0–2.0 mg O_2/L. During the anoxic–anaerobic, sedimentation, and decantation phases, illumination, gas recirculation, CO_2 dosing, and air supply were switched off.

2.4. Anaerobic Digestion Reactors

The methane potential of the biomass recovered after completion of bioreactor operation was assessed in the second stage of the study (S2) using an automated AMPTS II respirometric system (BPC Instruments AB, Lund, Sweden). Anaerobic digestion was carried out in glass reactors with a total volume of 500 mL under mesophilic conditions at 38 ± 1 °C. For each biomass type, anaerobic digestion was performed in three independent reactors (n = 3). Each reactor was equipped with an independent vertical bladeless stirrer operated intermittently at 100 rpm for 30 s every 5 min. A schematic of the respirometric system used to assess the methane potential of the biomass in S2 is shown in Figure 3.

Each reactor was filled with 200 mL of anaerobic inoculum and 1.00 ± 0.06 g vs. of AS, MA, or M-BC biomass, corresponding to a substrate loading of 5.0 ± 0.3 g VS/L. The inoculum-to-substrate ratio (ISR) was 6.3 ± 0.5 g vs. inoculum/g vs. substrate. Before measurement, the inoculum–substrate mixture was purged with nitrogen for 3 min at a flow rate of 100 L/h to remove O_2 and establish anaerobic conditions. CH_4 production

was recorded automatically using the AMPTS II system control software. CO_2 generated during digestion was removed in an absorption module containing 100 mL of 3 M NaOH, enabling direct measurement of CH_4 volume. CH_4 volume was normalized to 101.3 kPa, 0 °C, and dry gas conditions. Measurements were continued until five consecutive daily readings differed by less than 1%, which was adopted as the criterion for completion of biodegradation of the available organic fraction. Results were expressed as specific methane production in mL CH_4/g vs. added to the reactor. BMP was defined as the cumulative specific CH_4 production measured after 20 days of digestion, whereas Vmax was estimated using the Gompertz model. Each value was corrected for endogenous CH_4 production determined in control assays containing anaerobic inoculum only [27]. CH_4 production kinetics were described using the modified Gompertz model, which characterizes the digestion process by accounting for the lag phase, the phase of intensive CH_4 production, and the stabilization phase [28]. Model parameters were estimated by nonlinear regression, and goodness-of-fit was evaluated using the coefficient of determination (R^2) and root mean square error (RMSE).

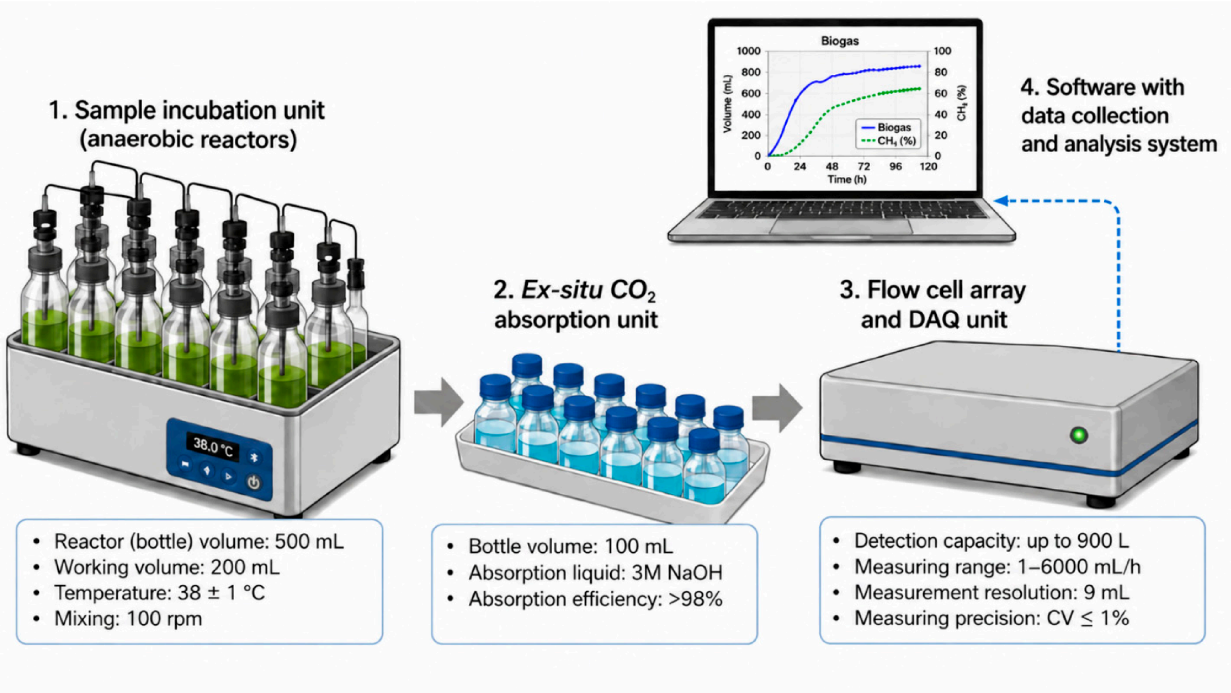

Figure 3. Schematic of the AMPTS II respirometric system used to assess the methane potential of the recovered biomass in the respective experimental variants.

2.5. Process Monitoring and Analytical, Calculation, and Statistical Methods

Influent wastewater samples were collected after preparation and homogenization of the medium, whereas effluent samples were collected after completion of the decantation phase of each SBR cycle. Separate aliquots of influent and effluent samples were used for the respective analyses. COD, BOD_5, TN, and TP were determined in unfiltered samples, whereas N-NH_4 and P-PO_4 were determined after filtration through 0.45 µm membrane filters. TSS was determined separately by filtration through pre-dried and pre-weighed Whatman GF/C glass-fiber filters with a pore size of 1.2 µm (Cytiva, Marlborough, MA, USA). Biomass samples were collected from the reaction mixture after homogenization of the reactor contents. BOD_5 was determined respirometrically using an OxiTop-IDS system (WTW/Xylem Analytics, Weilheim, Germany). TOC was measured using a TOC-L analyzer (Shimadzu, Kyoto, Japan). TSS was determined gravimetrically after filtration and drying to constant mass. Biomass concentration in the reactors was monitored as

TS and VS. TS was determined gravimetrically after drying at 105 °C, whereas vs. was determined after ignition at 550 °C. The composition of activated sludge, *Chlorella vulgaris*, and microalgal–bacterial consortium biomass was characterized based on TC, TOC, TN, TP, protein, lipids, and carbohydrates. TC, TOC, and TN were determined in dried biomass samples using a Flash 2000 elemental analyzer (Thermo Scientific, Waltham, MA, USA). TP was determined colorimetrically after sample mineralization. Protein was calculated as TN × 6.25. Lipids were determined by Soxhlet extraction, and carbohydrates by the anthrone colorimetric method. The C/N ratio was calculated as the TOC-to-TN ratio.

Reactor operation was monitored by measuring temperature, pH, dissolved oxygen (DO) concentration, and PAR intensity, as well as by controlling the alkalinity and carbonate hardness of the medium. pH was measured using a glass electrode, while DO was monitored with a Multi 3630 IDS meter equipped with an FDO 925 optical probe (WTW/Xylem Analytics, Weilheim, Germany). In the MA and M-BC variants, PAR intensity in the 400–700 nm range was monitored using an MQ-500 quantum meter (Apogee Instruments, Logan, UT, USA). Alkalinity and carbonate hardness were determined to control the stability of the buffering system and the conditions for CO_2 dosing. The morphological and taxonomic composition of the biomass was assessed by light microscopy using wet mounts and stained preparations immediately after samples were collected from the homogenized reaction mixture. Observations were performed at 100×, 400×, and 1000× magnification using a Nikon Eclipse Ci-L microscope equipped with bright-field and phase-contrast optics (Nikon Instruments Inc., Tokyo, Japan). Gram and Neisser staining were used to support the identification of filamentous bacteria and assessment of floc structure. The proportions of the main biomass components, i.e., microalgae, floc-forming bacteria, filamentous bacteria, protozoa, and the aggregate/EPS fraction, were determined semi-quantitatively based on microscopic observations and photographic documentation. The assessment was morphological and functional and did not include molecular identification of microorganisms. During bioreactor operation, the biomass concentration was maintained at 3.0 ± 0.1 g TS/L. Excess biomass was periodically removed to maintain a constant suspended-solids concentration, and the amount of recovered biomass was recorded separately for each variant.

Statistical analysis was performed using Statistica 13.3 (StatSoft, Tulsa, OK, USA). Data normality was verified using the Shapiro–Wilk test, and homogeneity of variance using Levene's test. Independent reactors were treated as experimental units and biological replicates (n = 3 per variant), whereas repeated samples and duplicate analytical determinations were not treated as independent replicates. In S2, each anaerobic digestion reactor constituted an independent experimental unit (n = 3 per biomass type). Results are presented as mean ± SD of three independent reactors. Statistical differences among variants were assessed by one-way ANOVA followed by Tukey's HSD test at $p \leq 0.05$.

Generative artificial intelligence tools were used only during manuscript preparation and not for study design, experimental work, data generation, statistical analysis, or scientific interpretation. ChatGPT (OpenAI, GPT-5.6 Sol) was used for English-language translation and linguistic editing, and OpenAI image-generation functionality was used to prepare the schematic in Figure 3 from technical specifications supplied by the authors. All generated outputs were reviewed and edited by the authors.

3. Results and Discussion

3.1. Removal of Organic Compounds

Organic matter removal performance clearly differentiated the biological systems examined. The lowest effluent COD and BOD_5 concentrations were obtained in V1, whereas the highest values of these parameters were maintained in V2 with the *Chlorella* sp. mono-

culture. This indicates that, during treatment of model DW containing a high proportion of readily biodegradable organic matter, the presence of an active heterotrophic biocenosis was crucial [29]. In V1, the mean effluent COD concentration was 141.4 ± 7.6 mg O_2/L, corresponding to a removal efficiency of 94.3 ± 0.3% (Figure 4a). These results were significantly ($p \leq 0.05$) higher than those obtained in the other variants. At the same time, BOD_5 concentration was reduced to 32.3 ± 1.7 mg O_2/L, with a removal efficiency of 98.1 ± 0.1% (Figure 4b). The high efficiency of AS resulted from the predominance of conventional heterotrophic biodegradation of organic matter by bacterial communities under intensive aeration [30]. This was probably favored by stable aerobic conditions and the flocculent structure of the sludge, which may have facilitated adsorption of colloidal and suspended fractions followed by their biodegradation [31]. The very high BOD_5 removal suggests that the readily biodegradable fraction was almost completely removed in V1, whereas the small residual COD may have consisted of more slowly degradable compounds or fine organic particles.

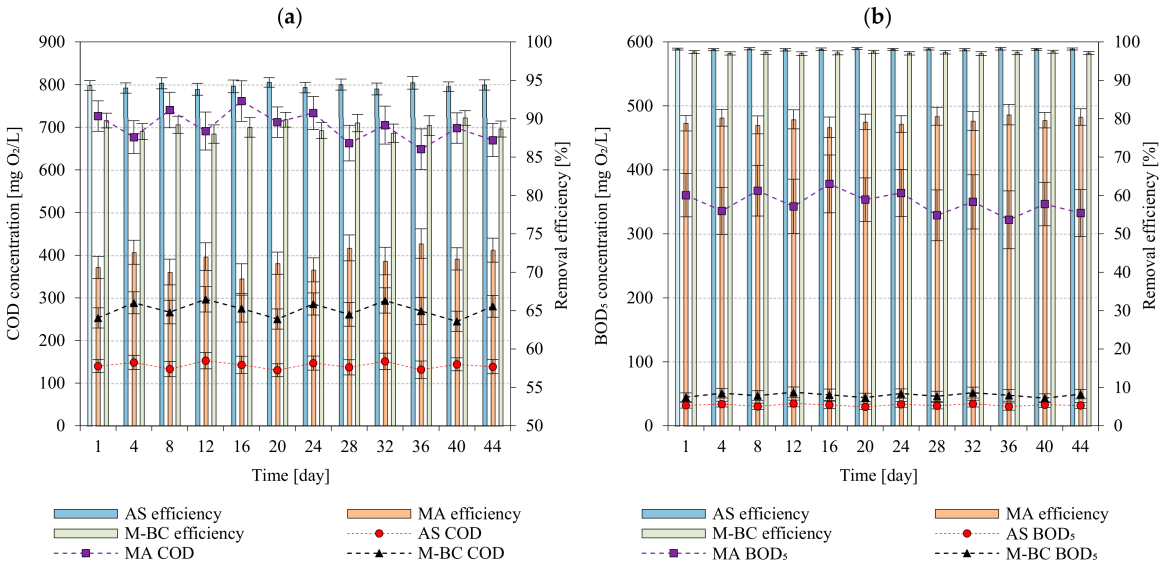

Figure 4. Changes in the concentration and removal efficiency of organic compounds: (**a**) COD and (**b**) BOD_5 during treatment of model DW.

For organic matter removal, intermediate performance was observed in V3 (M-BC). Effluent COD and BOD_5 concentrations were 272.0 ± 17.4 mg O_2/L and 48.0 ± 3.1 mg O_2/L, respectively, corresponding to removal efficiencies of 89.0 ± 0.7% and 97.2 ± 0.2% (Figure 4a,b). Comparative studies indicate that co-cultivation of microalgae and activated sludge can provide dissolved organic carbon removal comparable to AS, together with faster nutrient removal [32]. These effects have been suggested to result from cooperation between the two groups of microorganisms. Heterotrophs are responsible for the direct degradation of organic compounds, whereas microalgae supply O_2 during the light phase to support aerobic bacterial metabolism [33,34]. Microalgae may also release organic exometabolites that are available to bacteria [35]. At the same time, microalgae assimilate CO_2 and mineral nutrient forms, further supporting the treatment process [36]. In the present study, this effect may have been partially limited by the gentler gas recirculation and lower aeration applied to protect M-BC aggregates.

Significantly ($p \leq 0.05$) lower organic matter removal efficiencies were recorded in V2 (MA) than in the other variants. The mean effluent COD concentration was 702.1 ± 34.2 mg O_2/L, and the removal efficiency was only 71.6 ± 1.4% (Figure 4a). For BOD_5, the corresponding values were 348.5 ± 17.0 mg O_2/L and 79.5 ± 1.0% (Figure 4b).

This resulted from the metabolic characteristics of microalgae and the operating limitations of the PBR. Microalgae can assimilate some organic compounds and nutrients; however, at high COD loading, rapid activity of heterotrophic bacteria is crucial [37]. In the *Chlorella* sp. monoculture, the contribution of this function was limited. High concentrations of dispersed material in the medium and increasing biomass, including partly competing bacterial biomass, intensified self-shading and reduced photosynthetic activity [38].

3.2. Removal of Nitrogen Compounds

A different pattern was observed for nitrogen transformations. The best TN and N-NH_4 removal results were obtained in the M-BC system. In V3, the mean effluent TN concentration was 8.0 ± 0.5 mg N/L, corresponding to a removal efficiency of 90.3 ± 0.6% (Figure 5a). The observed treatment effects were significantly ($p \leq 0.05$) higher than in the other experimental systems. The use of M-BC resulted in the lowest effluent N-NH_4 concentration, at 0.32 ± 0.02 mg N/L. This corresponded to a N-NH_4 removal efficiency of 87.2 ± 0.9% (Figure 5b). In microalgal–bacterial consortia, nitrogen removal may proceed through multiple pathways, involving both assimilation of mineral N forms by microalgae and conventional bacterial transformations [39].

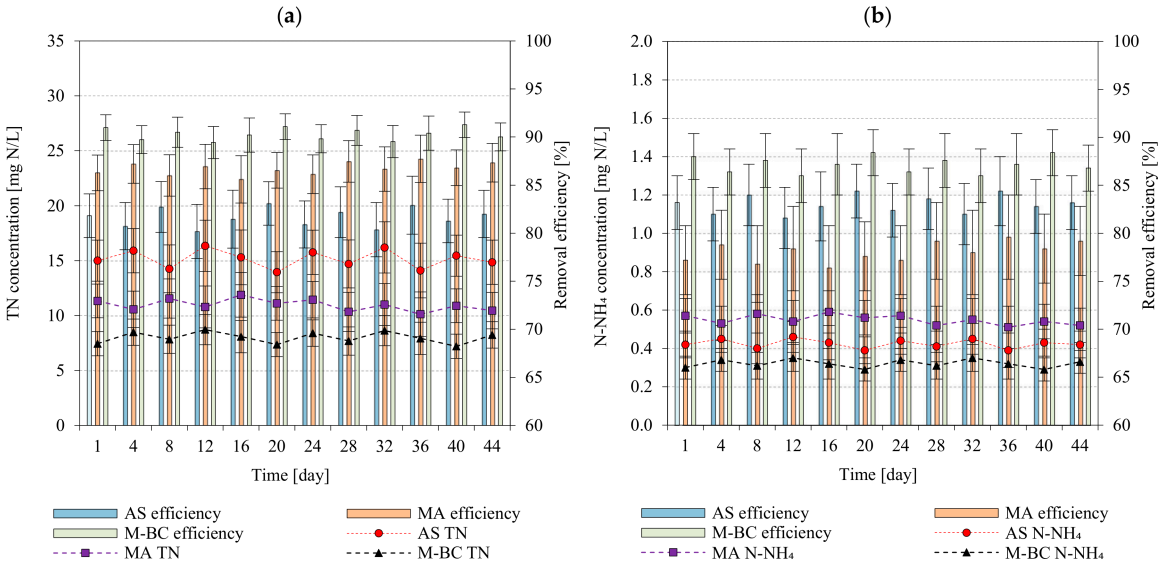

Figure 5. Changes in the concentration and removal efficiency of nitrogen compounds: (**a**) TN and (**b**) N-NH_4 during treatment of model DW.

The mean effluent TN and N-NH_4 concentrations observed in V1 (AS) were 15.2 ± 0.8 mg N/L and 0.42 ± 0.02 mg N/L, respectively. The corresponding removal efficiencies were 81.6 ± 1.0% and 83.0 ± 0.9% (Figure 5a,b). Thus, V1 effectively reduced N-NH_4 in the tested wastewater, whereas TN removal efficiency was significantly ($p \leq 0.05$) lower than in M-BC. The lower TN removal in AS than in M-BC may be associated with the strongly aerobic operating regime, which favored ammonium oxidation [40]. Subsequent denitrification may have been limited by the depletion of readily biodegradable COD during the aerobic phase [41]. The higher TN removal in M-BC may have resulted partly from the contribution of microalgal assimilation to nitrogen removal [42]. More variable oxygen conditions may also have supported complementary bacterial nitrogen transformations within the consortium [43]. However, the individual nitrogen-transformation pathways were not quantified directly, and these mechanisms should therefore be regarded as plausible explanations of the observed results.

In V2 (MA), the mean effluent TN concentration was 11.0 ± 0.5 mg N/L, with a removal efficiency of 86.7 ± 0.7% (Figure 5a). This treatment effect was higher than in V1 (AS) but significantly ($p \leq 0.05$) lower than in M-BC. The effluent N-NH_4 concentration was the highest among the variants, at 0.55 ± 0.03 mg N/L, and only 78.1 ± 1.0% of this nitrogen form was removed (Figure 5b). The higher residual N-NH_4 concentration in MA may reflect a greater contribution of nitrogen assimilation by microalgae and a lower contribution of bacterial nitrification. However, nitrifying populations and individual nitrogen-transformation pathways were not quantified, and this interpretation should therefore be considered a hypothesis rather than a directly demonstrated mechanism. These results indicate that microalgal biomass-based systems can be regarded as effective for nutrient capture but less complete in terms of controlling individual mineral nitrogen forms. Overall, M-BC showed the most favorable nitrogen-removal performance, with the lowest effluent TN and N-NH_4 concentrations among the investigated configurations.

3.3. Removal of Phosphorus Compounds

The results for phosphorus removal confirmed a significant ($p \leq 0.05$) advantage of the M-BC system over the other experimental variants. In V3, the mean effluent TP and P-PO_4 concentrations were 1.70 ± 0.11 mg P/L and 0.75 ± 0.05 mg P/L, respectively, corresponding to removal efficiencies of 90.0 ± 0.6% and 93.2 ± 0.4% (Figure 6a,b). The higher P removal in M-BC may have been partly associated with phosphorus assimilation into biomass [44]. Sorption onto aggregate surfaces may also have contributed to phosphorus removal [45]. Retention within the EPS-rich extracellular matrix represents another possible pathway [46]. However, the contribution of the individual mechanisms was not determined directly.

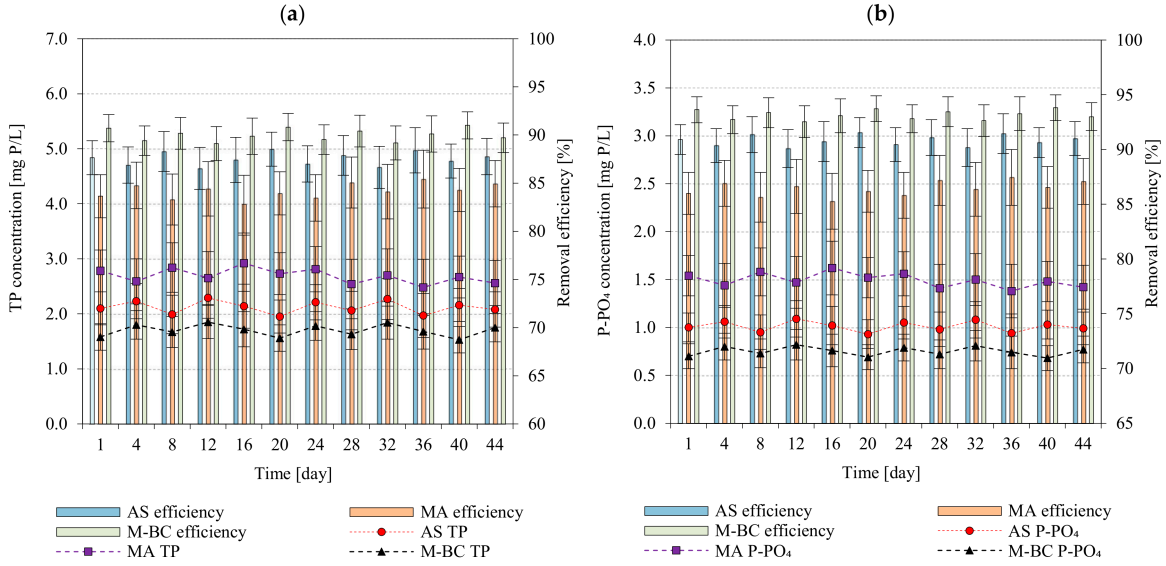

Figure 6. Changes in the concentration and removal efficiency of phosphorus compounds: (**a**) TP and (**b**) P-PO_4 during treatment of model DW.

In V1 (AS), the final TP concentration was 2.12 ± 0.12 mg P/L, with a removal efficiency of 87.5 ± 0.7% (Figure 6a). For P-PO_4, the corresponding values were 1.01 ± 0.05 mg P/L and 90.8 ± 0.5% (Figure 6b). In AS, biological incorporation of P into biomass and its sorption onto sludge flocs may have played an important role [47]. The SBR cycle applied included aerobic and anoxic–anaerobic phases, which may also have supported phosphorus transformations characteristic of biological nutrient removal systems [48]. At the same time, it cannot be stated unequivocally that the full enhanced biological

phosphorus removal (EBPR) mechanism occurred, because cyclic phosphate release and uptake and the abundance of polyphosphate-accumulating organisms were not measured directly. In MA, the mean effluent TP concentration was significantly ($p \leq 0.05$) higher than in the other variants, at 2.69 ± 0.13 mg P/L, with a removal efficiency of 84.2 ± 0.8% (Figure 6a). For P-PO_4, values of 1.49 ± 0.07 mg P/L and 86.4 ± 0.7% were obtained (Figure 6b). These results show that the *Chlorella* sp. monoculture removed phosphorus, but less efficiently than M-BC and AS. In MA, phosphorus assimilation by microalgae represents a plausible contribution to the observed P removal [12].

3.4. Total Suspended Solids and pH

The greatest differences among the variants were observed for TSS, directly reflecting the ability of the respective biomass types to settle and be retained in the reactor (Figure 7a). The lowest effluent TSS concentration was obtained in M-BC, at 24.0 ± 1.5 mg/L, with a removal efficiency of 93.1 ± 0.4%. In AS, the mean TSS concentration was 34.3 ± 1.8 mg/L, with an efficiency of 90.2 ± 0.5%. By far the highest TSS concentration was recorded in MA, where the mean effluent concentration reached 104.6 ± 5.1 mg/L, with a removal efficiency of 70.1 ± 1.5% (Figure 7a). Differences among all variants were statistically significant ($p \leq 0.05$).

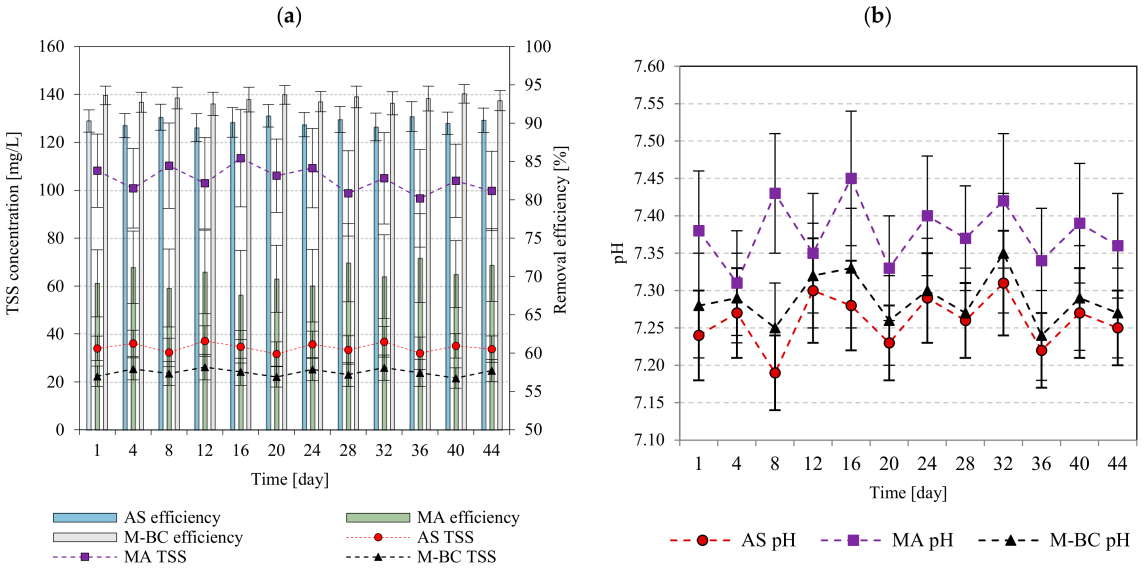

Figure 7. Changes in (**a**) total suspended solids (TSS) concentration and (**b**) pH in treated DW.

These results indicate that effluent quality depended strongly not only on the efficiency of biochemical transformations but also on the physical properties of the biomass, particularly its ability to aggregate, flocculate, and settle rapidly. The low TSS concentration in AS resulted from the presence of conventional activated-sludge flocs, which readily separated from the liquid phase during sedimentation [49]. However, the even lower TSS concentrations in M-BC indicate that the microalgal–bacterial aggregates had very good separation properties. It may be assumed that the presence of bacteria, microalgae, and extracellular polymeric substances (EPS) promoted the formation of more compact structures that retained cells more effectively in the reactor [50]. The high TSS concentration in MA resulted from operation with dispersed microalgal biomass. *Chlorella* sp. cells are small, have low settling velocities, and readily remain suspended. Without flocculation, granulation, or another aggregation mechanism, their separation during the conventional SBR sedimentation phase is difficult [19]. In practice, this means that using a microalgal

monoculture as a stand-alone treatment stage would require an additional separation process, such as coagulation, flotation, filtration, or centrifugation [51].

Effluent pH remained stable in all variants. Mean pH values were 7.25 ± 0.04 in AS, 7.34 ± 0.06 in MA, and 7.27 ± 0.05 in M-BC (Figure 7b). The slightly higher pH in MA was consistent with photosynthetic CO_2 uptake by *Chlorella* sp. and the associated shift in carbonate equilibrium. The similar pH values in AS and M-BC may reflect a balance between photosynthetic CO_2 uptake, bacterial mineralization, and pH-controlled CO_2 dosing [21]. From a technological perspective, M-BC provided the most favorable final profile because it combined low TSS, stable pH, and the highest nutrient removal efficiency. This indicates that M-BC may represent a favorable compromise between the metabolic activity of microalgae and bacteria and the separation properties of the biomass.

3.5. Biomass Growth and Morphological–Taxonomic Composition

Biomass growth and its morphological–taxonomic composition clearly depended on the biological system applied. In all variants, the initial biomass concentration in the reactors was maintained at 3.0 ± 0.1 g TS/L, while excess biomass was periodically removed to maintain stable operating conditions. The lowest biomass productivity was recorded in V1 (AS), at 0.078 ± 0.006 g TS/L·d, corresponding to 0.62 ± 0.05 kg TS/m^3 DW. In V2 (MA), productivity was higher at 0.096 ± 0.010 g TS/L·d, equivalent to 0.77 ± 0.08 kg TS/m^3 DW. The highest biomass productivity was observed in V3 (M-BC), at 0.105 ± 0.009 g TS/L·d, corresponding to 0.84 ± 0.07 kg TS/m^3 DW (Table 2). When expressed as the organic fraction, the corresponding values were 0.061 ± 0.005 g VS/L·d for AS, 0.083 ± 0.009 g VS/L·d for MA, and 0.085 ± 0.008 g VS/L·d for M-BC (Table 2). The higher biomass productivity in MA and M-BC probably resulted from simultaneous nutrient assimilation, CO_2 utilization, and incorporation of part of the organic carbon into new biomass [52,53]. In AS, growth was lower because a larger fraction of readily biodegradable COD was mineralized to CO_2 under intensive aeration [5].

Table 2. Biomass productivity and observed biomass yield coefficient in the AS, MA, and M-BC variants during treatment of model DW.

Variant	TS Productivity [g TS/L·d]	VS Productivity [g VS/L·d]	TS Production [g TS/L_DW]	VS Production [g VS/L_DW]	Y_{obs} [g TS/g $COD_{removed}$]
AS	0.078 ± 0.006	0.061 ± 0.005	0.62 ± 0.05	0.49 ± 0.04	0.27 ± 0.02
MA	0.096 ± 0.010	0.083 ± 0.009	0.77 ± 0.08	0.67 ± 0.07	0.43 ± 0.04
M-BC	0.105 ± 0.009	0.085 ± 0.008	0.84 ± 0.07	0.68 ± 0.06	0.38 ± 0.03

In V1 (AS), a typical activated-sludge biocenosis was observed, dominated by heterotrophic floc-forming bacteria accounting for approximately 55–65% of the observed biomass. They were accompanied by filamentous bacteria, which accounted for approximately 8–12% (Table 3). The filamentous forms were dominated by morphotypes characteristic of municipal activated sludge, including structures resembling *Microthrix parvicella*, types 1701 and 021N, and individual *Thiothrix*-like forms. The higher-organism fraction, consisting mainly of sessile and crawling ciliates such as forms resembling *Vorticella*, *Epistylis*, *Aspidisca*, and *Euplotes*, accounted for approximately 5–8% of the observed biocenosis (Table 3). Rotifers and amoebae were also occasionally present, but their proportion did not exceed 1–2%. This composition indicates mature, stable activated sludge adapted to aerobic degradation of readily biodegradable organic compounds and partial nitrogen transformations [54]. Thus, in the AS system, biomass structure was favorable for conventional wastewater treatment because activated-sludge flocs provided good sedimentation, adsorption of the colloidal fraction, and intensive COD biodegradation [49].

Table 3. Approximate morphological–taxonomic structure of the biomass during the final phase of reactor operation.

Organism Group/Biomass Component	AS [%]	MA [%]	M-BC [%]
Chlorella sp.	–	85–88	50–57
Heterotrophic floc-forming bacteria	55–65	8–10	25–32
Filamentous bacteria	8–12	<1	6–10
Nitrifying and other specialized bacteria	5–8	1–3	3–6
Protozoa and small higher organisms	5–8	<1	4–7
EPS, detritus, and the organic–mineral fraction associated with aggregates	10–15	2–5	10–15

In V2 (MA), the inoculum initially consisted of a *Chlorella* sp. monoculture, but the system did not remain axenic during reactor operation. At the beginning of operation, *Chlorella* sp. cells accounted for more than 98% of the observed biological fraction, and bacteria were present only in trace amounts. During reactor operation with model DW, however, the system did not retain complete monoculture purity. In the final phase of the experiment, the proportion of accompanying bacteria stabilized at approximately 12–15%, whereas *Chlorella* sp. remained the dominant biomass component, accounting for approximately 85–88% of the observed biocenosis (Table 3). The development of accompanying bacteria should be regarded as expected because the model DW contained readily biodegradable organic compounds that favored heterotrophic growth [55]. The accompanying bacteria were observed mainly as small forms associated with microalgal cells and suspended organic matter. Based on the wastewater composition and available literature, their potential functions may have included the utilization of readily biodegradable organic compounds and microalgal exometabolites [35]. Similar microalgal wastewater-treatment systems have been reported to contain representatives of Proteobacteria, Bacteroidota, and Actinobacteriota [56]. However, molecular analyses were not performed in the present study; therefore, taxonomic and functional assignments based on microscopic observations should be regarded as tentative.

In V3 (M-BC), biomass composition remained the most stable during operation. The consortium was dominated by *Chlorella* sp. cells and microorganisms typical of activated sludge, forming compact microalgal–bacterial aggregates. The microalgal fraction remained at approximately 50–57%, whereas heterotrophic microorganisms, filamentous bacteria, and protozoa together accounted for approximately 40–45%, consistent with the characteristics of the mature consortium used to inoculate the reactors (Table 3). The M-BC structure contained floc-forming and filamentous bacteria, including morphotypes resembling *Microthrix parvicella* and types 1851 and 1701, as well as small numbers of protozoa characteristic of stable activated-sludge systems.

3.6. Biomass Composition Characteristics

After completion of bioreactor operation, biomass composition differed from that of the inoculum, indicating adaptation of the respective biological systems to the model DW (Table 4). From the perspective of anaerobic digestion, the most important changes involved the vs. fraction, TOC, TN, C/N, and the protein, lipid, and carbohydrate fractions, because these parameters determine biomass susceptibility to hydrolysis, potential CH_4 yield, and the risk of nitrogen-compound accumulation. In V1 (AS), the vs. fraction increased from 76.0 ± 1.8 to 78.4 ± 2.0% TS, accompanied by a decrease in MS from 24.0 ± 1.8 to 21.6 ± 2.0% TS. This was accompanied by an increase in TOC from 369 ± 13 to 386 ± 15 mg/g TS and in TN from 38.0 ± 1.9 to 44.8 ± 2.3 mg/g TS. Protein content increased from 23.8 ± 1.2 to 28.0 ± 1.4% TS, while C/N decreased from 9.7 ± 0.6 to 8.6 ± 0.5

(Tables 1 and 4). These changes indicate enrichment of AS biomass in an organic cellular fraction with a higher proportion of nitrogen-containing compounds.

Table 4. Characteristics of the biomass after completion of bioreactor operation.

Parameter	Unit	AS	MA	M-BC
Volatile solids (VS)	% TS	78.4 ± 2.0	86.7 ± 1.6	81.1 ± 3.2
Mineral solids (MS)	% TS	21.6 ± 2.0	13.3 ± 1.6	18.9 ± 3.2
Total carbon (TC)	mg/g TS	408 ± 16	452 ± 34	462 ± 58
Total organic carbon (TOC)	mg/g TS	386 ± 15	414 ± 25	420 ± 52
Total nitrogen (TN)	mg/g TS	44.8 ± 2.3	45.6 ± 2.1	36.8 ± 3.1
C/N	–	8.6 ± 0.5	9.1 ± 0.4	11.4 ± 1.5
Total phosphorus (TP)	mg/g TS	14.2 ± 1.1	21.6 ± 1.9	16.8 ± 2.5
pH	–	7.22 ± 0.07	7.58 ± 0.10	7.34 ± 0.08
Protein	% TS	28.0 ± 1.4	28.5 ± 1.3	23.0 ± 1.9
Lipids	% TS	8.1 ± 0.7	13.4 ± 0.9	12.0 ± 1.4
Carbohydrates	% TS	17.2 ± 1.8	34.6 ± 2.5	45.8 ± 4.3

In V2 (MA), the final biomass retained the highest vs. fraction among the variants despite a slight decrease from 88.2 ± 1.3 to 86.7 ± 1.6% TS. At the same time, TOC decreased slightly from 423 ± 22 to 414 ± 25 mg/g TS, and the contents of the main carbon-rich storage fractions declined, with lipids decreasing from 15.2 ± 0.7 to 13.4 ± 0.9% TS and carbohydrates from 37.2 ± 2.3 to 34.6 ± 2.5% TS. These changes were accompanied by increases in TN from 40.2 ± 1.8 to 45.6 ± 2.1 mg/g TS, TP from 18.4 ± 1.7 to 21.6 ± 1.9 mg/g TS, and protein from 25.1 ± 2.2 to 28.5 ± 1.3% TS (Table 1, Table 4). Consequently, the C/N ratio decreased from 10.5 ± 0.2 to 9.1 ± 0.4, indicating a shift in biomass composition toward a more nitrogen-rich fraction together with a lower contribution of carbon-rich storage compounds. This compositional shift is consistent with preferential biomass growth and protein synthesis rather than lipid and carbohydrate accumulation [57]. From the perspective of anaerobic digestion, MA biomass remained favorable because of its highest vs. fraction and relatively high lipid and protein contents; however, the low C/N ratio may increase the importance of mineralization of organic nitrogen to N-NH_4 [58].

In V3 (M-BC), the biomass had the highest carbohydrate content, distinguishing it from the more protein-rich AS and MA biomass. The vs. fraction decreased slightly from 82.3 ± 3.5 to 81.1 ± 3.2% TS, whereas TOC increased from 397 ± 70 to 420 ± 52 mg/g TS. At the same time, TN increased from 30.6 ± 3.4 to 36.8 ± 3.1 mg/g TS, but its final level remained lower than in AS and MA. Consequently, C/N decreased from 14.4 ± 2.0 to 11.4 ± 1.5 but remained higher than in the other variants (Table 1, Table 4). This may be associated with the aggregated structure of M-BC, in which EPS polysaccharides and structural carbohydrates, rich in carbon but relatively poor in nitrogen, probably constituted an important fraction of the organic matter [59]. TP content increased from 13.3 ± 2.9 to 16.8 ± 2.5 mg/g TS, indicating an increase in the phosphorus content of M-BC biomass during reactor operation. Particularly notable was the increase in carbohydrate content from 42.0 ± 4.0 to 45.8 ± 4.3% TS, which may be associated with EPS polysaccharides stabilizing microalgal–bacterial aggregates [50]. For anaerobic digestion, M-BC may be a favorable substrate because of its relatively high vs. content, large carbohydrate fraction, moderate protein content, and better biomass separability compared with a microalgal monoculture [60].

3.7. Anaerobic Digestion

Anaerobic digestion performance clearly differed among the biomass types obtained in the individual experimental variants. The highest BMP after 20 days was obtained in V2

(MA), at 255.1 ± 12.3 mL CH_4/g VS, followed by V3 (M-BC), at 230.2 ± 15.9 mL CH_4/g VS, and V1 (AS), at 189.5 ± 6.3 mL CH_4/g vs. (Figure 8a, Table 5). One-way ANOVA followed by Tukey's HSD test showed that AS had a significantly lower BMP than both MA and M-BC ($p \leq 0.05$), whereas the difference between MA and M-BC was not statistically significant ($p > 0.05$). The BMP of *Chlorella* sp. biomass was approximately 34.6% higher than that of AS and approximately 10.8% higher than that of M-BC. A similar ranking was confirmed by V_{max} values determined using the Gompertz model, which were 268.0 ± 13.0 mL CH_4/g vs. for MA, 237.3 ± 17.0 mL CH_4/g vs. for M-BC, and 196.7 ± 6.5 mL CH_4/g vs. for AS (Table 5).

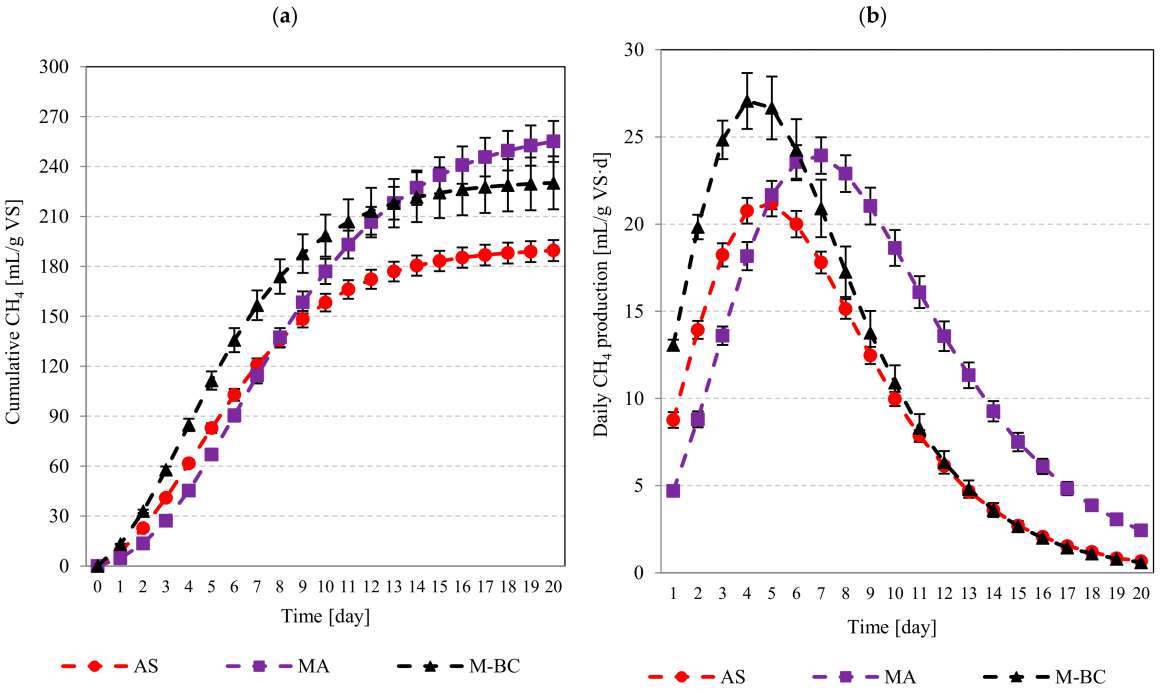

Figure 8. Cumulative CH_4 production (**a**) and daily CH_4 production (**b**) during anaerobic digestion of biomass obtained in the respective experimental variants.

Table 5. CH_4 production parameters and anaerobic digestion kinetics of biomass obtained in the respective experimental variants.

Variant	BMP on Day 20 [mL CH_4/g VS]	V_{max} [mL CH_4/g VS]	R_{max} [mL CH_4/g VS·d]	λ [d]
AS	189.5 ± 6.3	196.7 ± 6.5	21.2 ± 0.8	0.82 ± 0.08
MA	255.1 ± 12.3	268.0 ± 13.0	24.1 ± 1.0	2.12 ± 0.08
M-BC	230.2 ± 15.9	237.3 ± 17.0	26.8 ± 1.8	0.53 ± 0.08

The highest BMP obtained for MA biomass should be interpreted in relation to both the amount and composition of its organic fraction. MA had the highest vs. content (86.7 ± 1.6% TS) and the lowest mineral-solids fraction (13.3 ± 1.6% TS), together with relatively high protein (28.5 ± 1.3% TS) and lipid contents (13.4 ± 0.9% TS) and a carbohydrate content of 34.6 ± 2.5% TS. In comparison, M-BC contained 81.1 ± 3.2% VS, 18.9 ± 3.2% mineral solids, 23.0 ± 1.9% protein, 12.0 ± 1.4% lipids, and the highest carbohydrate fraction (45.8 ± 4.3% TS), whereas AS had the lowest vs. content (78.4 ± 2.0% TS), the highest mineral-solids fraction (21.6 ± 2.0% TS), and markedly lower lipid (8.1 ± 0.7% TS) and carbohydrate contents (17.2 ± 1.8% TS) (Table 4). Thus, the higher BMP of MA cannot be attributed to vs. content alone, but is consistent with its high organic fraction and favorable biochemical composition, particularly its relatively high lipid content. Differences

in methane yield may also reflect the accessibility and biodegradability of these fractions rather than their abundance alone [58,61]. At the same time, the daily CH_4 production curve indicates that MA biomass degraded more slowly than M-BC. The maximum daily production occurred later, and the λ parameter was the highest among the tested variants, at 2.12 ± 0.08 d (Table 5). This may suggest that, despite the high methane potential, the accessibility of the organic fraction in *Chlorella* sp. cells was partly limited by the cell-wall structure, delaying hydrolysis and shifting the intensive methanogenic phase to a later time [62].

Digestion of M-BC biomass followed a different pattern. Although BMP was lower than for MA, the microalgal–bacterial consortium achieved the highest maximum CH_4 production rate (R_{max} = 26.8 ± 1.8 mL CH_4/g VS·d) and the shortest lag phase (λ = 0.53 ± 0.08 d) (Table 5). This kinetic pattern is consistent with greater accessibility of part of the organic fraction during the initial stage of AD. This may be associated with the high carbohydrate content of M-BC, 45.8 ± 4.3% TS (Table 4), and with the presence of extracellular polymeric substances and the aggregate matrix, which may have provided more readily available substrates for hydrolytic and fermentative bacteria [63]. At the same time, the lower final CH_4 production compared with MA may have resulted from the lower lipid fraction, lower vs. content, and the presence of a more structural or partly resistant EPS fraction [64]. M-BC therefore showed more favorable initial digestion kinetics, but did not achieve the highest BMP.

The lowest BMP was obtained for AS biomass. Both the BMP of 189.5 ± 6.3 mL CH_4/g vs. and the V_{max} of 196.7 ± 6.5 mL CH_4/g vs. were the lowest among the tested variants (Figure 8a, Table 5). AS biomass contained fewer lipids and carbohydrates than MA and M-BC and had the lowest C/N ratio, 8.6 ± 0.5 (Table 4). This indicates a greater contribution of protein- and nitrogen-rich fractions and a lower contribution of high-energy carbon compounds. In addition, activated-sludge biomass is usually partly stabilized under aerobic conditions, which may limit its subsequent susceptibility to anaerobic degradation [7]. In this context, the lower methane potential of AS may reflect not only its lower vs. content but also differences in organic-matter composition and previous aerobic stabilization [61].

The use of model dairy wastewater reduced influent variability and enabled a more controlled comparison of the three treatment systems. This approach is commonly applied in comparative studies because it allows the composition of the medium to be controlled and limits the influence of wastewater variability on the assessment of treatment performance [65]. However, real dairy wastewater may vary considerably depending on the type of production, the technological process, and the cleaning procedures applied, including differences in fat content, organic loading, nutrient ratios, turbidity, pH, and the presence of residual cleaning agents [66]. Such variability may affect the relative performance of AS, MA, and M-BC, particularly by modifying light availability and the balance between heterotrophic and photoautotrophic processes. From a practical perspective, the obtained results enable a comparative assessment of the technological performance of the individual systems and may support the selection of a configuration suited to a specific treatment objective, such as maximizing organic matter removal, nutrient removal, reduction of suspended solids in the effluent, or biomass recovery. The parameters obtained in the present experiment may also serve as a reference point for further studies using real dairy wastewater with comparable influent characteristics and under similar process conditions. Accordingly, direct generalization of the present findings to full-scale industrial dairy wastewater should be made with caution.

An important limitation of the study is that the three variants were operated under different process conditions. Differences in aeration, illumination, gas recirculation, and CO_2 supply were necessary because AS, MA, and M-BC require different operating en-

vironments to maintain biological activity and treatment functions. The observed effects therefore reflect the combined influence of biomass characteristics and the operating conditions required by each system, and the comparison should be interpreted at the level of complete technological configurations rather than as an isolated effect of biomass type. At the same time, this approach has practical relevance because it allows assessment not only of treatment performance and biomass recovery, but also of the suitability of the recovered biomass for subsequent anaerobic digestion. The CH_4 production performance and digestion kinetics of the recovered biomass may directly influence the potential energy and economic balance of the overall system. A comprehensive assessment of these relationships, however, requires consideration of the actual energy demand for aeration, illumination, gas recirculation, CO_2 dosing, and biomass separation. Therefore, a reliable techno-economic evaluation should be performed at pilot scale under conditions representative of practical operation.

4. Conclusions

Under the operating conditions applied in this study, AS was the most effective system for removing organic compounds, achieving COD and BOD_5 reductions of 94.3 ± 0.3% and 98.1 ± 0.1%, respectively. This confirms the high efficiency of a conventional heterotrophic biocenosis in biodegrading readily degradable organic matter present in dairy wastewater. M-BC provided the best removal of nutrients and suspended solids. MA was the least favorable as a stand-alone wastewater treatment system, mainly because of lower COD removal and high effluent TSS.

At the same time, MA biomass had the highest BMP, at 255.1 ± 12.3 mL CH_4/g VS, which was associated with its high vs. fraction and relatively high lipid and protein contents. M-BC biomass achieved a lower BMP of 230.2 ± 15.9 mL CH_4/g vs. but showed more favorable anaerobic digestion kinetic parameters, including the highest R_{max} of 26.8 ± 1.8 mL CH_4/g VS·d and the shortest lag phase, λ = 0.53 ± 0.08 d. This indicates greater availability of readily biodegradable fractions during the initial stage of AD. AS biomass showed the lowest BMP, reaching 189.5 ± 6.3 mL CH_4/g VS, consistent with its lower lipid and carbohydrate contents, low C/N ratio, and partly stabilized sludge characteristics.

Under the laboratory conditions tested, M-BC combined high nitrogen and phosphorus removal efficiency, low effluent TSS, high biomass productivity, good separability, and favorable CH_4 production kinetics. However, these results demonstrate comparative laboratory-scale performance rather than establish the technological superiority of any configuration. Validation under continuous pilot-scale operation using real dairy wastewater, including assessment of seasonal variability, biomass harvesting, long-term anaerobic digestion stability, and net energy performance, is required before selecting a preferred configuration for integrated wastewater treatment and energy recovery.

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References

1. Biliani, S.E.; Manariotis, I.D. High-rate algal ponds in wastewater treatment: A critical look at recent developments. *Environ. Sci. Water Res. Technol.* **2026**, *12*, 1359–1373. [CrossRef]
2. Chhandama, M.V.L.; Rai, P.K.; Lalawmpuii. Coupling bioremediation and biorefinery prospects of microalgae for circular economy. *Bioresour. Technol. Rep.* **2023**, *22*, 101479. [CrossRef]
3. Dueholm, M.K.D.; Nierychlo, M.; Andersen, K.S.; Rudkjøbing, V.; Knutsson, S.; MiDAS Global Consortium; Arriaga, S.; Bakke, R.; Boon, N.; Bux, F.; et al. MiDAS 4: A global catalogue of full-length 16S rRNA gene sequences and taxonomy for studies of bacterial communities in wastewater treatment plants. *Nat. Commun.* **2022**, *13*, 1908. [CrossRef]
4. Ramsuroop, J.; Gutu, L.; Ayinde, W.B.; Basitere, M.; Manono, M.S. A Review of Biological Processes for Dairy Wastewater Treatment and the Effect of Physical Parameters Which Affect Their Efficiency. *Water* **2024**, *16*, 537. [CrossRef]
5. Kadam, R.; Khanthong, K.; Park, B.; Jun, H.; Park, J. Realizable wastewater treatment process for carbon neutrality and energy sustainability: A review. *J. Environ. Manag.* **2023**, *328*, 116927. [CrossRef]
6. Drewnowski, J.; Remiszewska-Skwarek, A.; Duda, S.; Łagód, G. Aeration Process in Bioreactors as the Main Energy Consumer in a Wastewater Treatment Plant Review of solutions and methods of process optimization. *Processes* **2019**, *7*, 311. [CrossRef]
7. Appels, L.; Baeyens, J.; Degrève, J.; Dewil, R. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog. Energy Combust. Sci.* **2008**, *34*, 755–781. [CrossRef]
8. Demirel, B.; Yenigun, O.; Onay, T.T. Anaerobic treatment of dairy wastewaters: A review. *Process Biochem.* **2005**, *40*, 2583–2595. [CrossRef]
9. Kisielewska, M.; Dębowski, M.; Zieliński, M.; Kazimierowicz, J.; Quattrocelli, P.; Bordiean, A. Effects of Liquid Digestate Treatment on Sustainable Microalgae Biomass Production. *BioEnergy Res.* **2022**, *15*, 357–370. [CrossRef]
10. Abdelfattah, A.; Ali, S.S.; Ramadan, H.; El-Aswar, E.I.; Eltawab, R.; Ho, S.-H.; Elsamahy, T.; Li, S.; El-Sheekh, M.M.; Schagerl, M.; et al. Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. *Environ. Sci. Ecotechnol.* **2023**, *13*, 100205. [CrossRef]
11. Popa, M.D.; Simionov, I.-A.; Petrea, S.M.; Georgescu, P.-L.; Ifrim, G.A.; Iticescu, C. Efficiency of Microalgae Employment in Nutrient Removal (Nitrogen and Phosphorous) from Municipal Wastewater. *Water* **2025**, *17*, 260. [CrossRef]
12. Hajri, A.K.; Alsharif, I.; Albalawi, M.A.; Alshareef, S.A.; Albalawi, R.K.; Jamoussi, B. Utilizing Mixed Cultures of Microalgae to Up-Cycle and Remove Nutrients from Dairy Wastewater. *Biology* **2024**, *13*, 591. [CrossRef]
13. Suparmaniam, U.; Lam, M.K.; Uemura, Y.; Lim, J.W.; Lee, K.T.; Shuit, S.H. Insights into the microalgae cultivation technology and harvesting process for biofuel production: A review. *Renew. Sustain. Energy Rev.* **2019**, *115*, 109361. [CrossRef]
14. Kant Bhatia, S.; Ahuja, V.; Chandel, N.; Mehariya, S.; Kumar, P.; Vinayak, V.; Saratale, G.D.; Raj, T.; Kim, S.-H.; Yang, Y.-H. An overview on microalgal-bacterial granular consortia for resource recovery and wastewater treatment. *Bioresour. Technol.* **2022**, *351*, 127028. [CrossRef]
15. Hammond, C.R.; Loge, F.J. Wastewater treatment with microalgal-bacterial aggregates: The tradeoff between energy savings and footprint requirements. *Bioresour. Technol.* **2024**, *395*, 130270. [CrossRef]
16. Cheah, W.Y.; Ling, T.C.; Show, P.L.; Juan, J.C.; Chang, J.-S.; Lee, D.-J. Cultivation in wastewaters for energy: A microalgae platform. *Appl. Energy* **2016**, *179*, 609–625. [CrossRef]
17. Lima, S.; Cosenza, A.; Caputo, G.; Grisafi, F.; Scargiali, F. Utilization of native Chlorella strain in laboratory-scale raceway reactor for synthetic wastewater treatment: A study in batch and continuous modes with multi-substrate modeling. *J. Water Process Eng.* **2024**, *60*, 105145. [CrossRef]
18. Dudek, M.; Dębowski, M.; Kazimierowicz, J.; Zieliński, M.; Quattrocelli, P.; Nowicka, A. The Cultivation of Biohydrogen-Producing Tetraselmis subcordiformis Microalgae as the Third Stage of Dairy Wastewater Aerobic Treatment System. *Sustainability* **2022**, *14*, 12085. [CrossRef]

19. Deepa, P.; Sowndhararajan, K.; Kim, S. A Review of the Harvesting Techniques of Microalgae. *Water* **2023**, *15*, 3074. [CrossRef]
20. Su, Y.; Zhu, X.; Zou, R.; Zhang, Y. The interactions between microalgae and wastewater indigenous bacteria for treatment and valorization of brewery wastewater. *Resour. Conserv. Recycl.* **2022**, *182*, 106341. [CrossRef]
21. Casagli, F.; Rossi, S.; Steyer, J.P.; Bernard, O.; Ficara, E. Balancing Microalgae and Nitrifiers for Wastewater Treatment: Can Inorganic Carbon Limitation Cause an Environmental Threat? *Environ. Sci. Technol.* **2021**, *55*, 3940–3955. [CrossRef]
22. Marazzi, F.; Bellucci, M.; Fantasia, T.; Ficara, E.; Mezzanotte, V. Interactions between Microalgae and Bacteria in the Treatment of Wastewater from Milk Whey Processing. *Water* **2020**, *12*, 297. [CrossRef]
23. Hammond, C.R.; Gradilla-Hernández, M.S.; Loge, F.J. Microalgal-bacterial aggregates for wastewater treatment: Origins, challenges, and future directions. *Water Environ. Res.* **2025**, *97*, e70018. [CrossRef]
24. Kazimierowicz, J.; Dębowski, M.; Zieliński, M. Taxonomic Structure Evolution, Chemical Composition and Anaerobic Digestibility of Microalgae-Bacterial Granular Sludge (M-BGS) Grown during Treatment of Digestate. *Appl. Sci.* **2023**, *13*, 1098. [CrossRef]
25. Zhang, J.-T.; Wang, J.-X.; Liu, Y.; Zhang, Y.; Wang, J.-H.; Chi, Z.-Y.; Kong, F.-T. Microalgal-bacterial biofilms for wastewater treatment: Operations, performances, mechanisms, and uncertainties. *Sci. Total Environ.* **2024**, *907*, 167974. [CrossRef]
26. Dębowski, M.; Zieliński, M.; Kisielewska, M.; Kazimierowicz, J.; Dudek, M.; Świca, I.; Rudnicka, A. The Cultivation of Lipid-Rich Microalgae Biomass as Anaerobic Digestate Valorization Technology—A Pilot-Scale Study. *Processes* **2020**, *8*, 517. [CrossRef]
27. Holliger, C.; Alves, M.; Andrade, D.; Angelidaki, I.; Astals, S.; Baier, U.; Bougrier, C.; Buffière, P.; Carballa, M.; de Wilde, V.; et al. Towards a standardization of biomethane potential tests. *Water Sci. Technol.* **2016**, *74*, 2515–2522. [CrossRef]
28. Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; Van't Riet, K. Modeling of the Bacterial Growth Curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875–1881. [CrossRef]
29. Bucci, P.; Marcos Montero, E.J.; García-Depraect, O.; Zaritzky, N.; Caravelli, A.; Muñoz, R. Assessment of the performance of a symbiotic microalgal-bacterial granular sludge reactor for the removal of nitrogen and organic carbon from dairy wastewater. *Chemosphere* **2024**, *351*, 141250. [CrossRef]
30. Tocchi, C.; Federici, E.; Fidati, L.; Manzi, R.; Vincigurerra, V.; Petruccioli, M. Aerobic treatment of dairy wastewater in an industrial three-reactor plant: Effect of aeration regime on performances and on protozoan and bacterial communities. *Water Res.* **2012**, *46*, 3334–3344. [CrossRef]
31. Lu, J.; Lu, Q.; Hu, Q.; Qiu, B. Recovery of organic matters by activated sludge from municipal wastewater: Performance and characterization. *Environ. Res.* **2024**, *252*, 118829. [CrossRef]
32. Maulana, A.; Mori, K.; Toyama, T. Comparative evaluation of microalgae-activated sludge cocultivation for municipal wastewater treatment using four microalgal species under nonaerated batch conditions. *Water Sci. Technol.* **2026**, *94*, 338–350. [CrossRef]
33. Flores-Salgado, G.; Thalasso, F.; Buitrón, G.; Vital-Jácome, M.; Quijano, G. Kinetic characterization of microalgal-bacterial systems: Contributions of microalgae and heterotrophic bacteria to the oxygen balance in wastewater treatment. *Biochem. Eng. J.* **2021**, *165*, 107819. [CrossRef]
34. Holmes, B.; Paddock, M.B.; VanderGheynst, J.S.; Higgins, B.T. Algal photosynthetic aeration increases the capacity of bacteria to degrade organics in wastewater. *Biotechnol. Bioeng.* **2020**, *117*, 62–72. [CrossRef]
35. Perera, I.A.; Abinandan, S.; Subashchandrabose, S.R.; Venkateswarlu, K.; Naidu, R.; Megharaj, M. Combined inorganic nitrogen sources influence the release of extracellular compounds that drive mutualistic interactions in microalgal-bacterial co-cultures. *J. Appl. Phycol.* **2022**, *34*, 1311–1322. [CrossRef]
36. Ji, B.; Zhang, M.; Gu, J.; Ma, Y.; Liu, Y. A self-sustaining synergetic microalgal-bacterial granular sludge process towards energy-efficient and environmentally sustainable municipal wastewater treatment. *Water Res.* **2020**, *179*, 115884. [CrossRef]
37. Nguyen, T.-T.-D.; Nguyen, T.-T.; An Binh, Q.; Bui, X.-T.; Ngo, H.H.; Vo, H.N.P.; Andrew Lin, K.-Y.; Vo, T.-D.-H.; Guo, W.; Lin, C.; et al. Co-culture of microalgae-activated sludge for wastewater treatment and biomass production: Exploring their role under different inoculation ratios. *Bioresour. Technol.* **2020**, *314*, 123754. [CrossRef]
38. Shi, Y.; Du, C.; Chen, B.; Ding, B.; Li, A.; Ji, B. Evaluating the performance and stability of microalgal-bacterial granular sludge in municipal wastewater treatment plants. *J. Environ. Manag.* **2025**, *373*, 123912. [CrossRef]
39. Jin, Y.; Zhan, W.; Wu, R.; Han, Y.; Yang, S.; Ding, J.; Ren, N. Insight into the roles of microalgae on simultaneous nitrification and denitrification in microalgal-bacterial sequencing batch reactors: Nitrogen removal, extracellular polymeric substances, and microbial communities. *Bioresour. Technol.* **2023**, *379*, 129038. [CrossRef]
40. Hao, Z.-L.; Ali, A.; Ren, Y.; Su, J.-F.; Wang, Z. A mechanistic review on aerobic denitrification for nitrogen removal in water treatment. *Sci. Total Environ.* **2022**, *847*, 157452. [CrossRef]
41. Cydzik-Kwiatkowska, A.; Rusanowska, P.; Zielińska, M.; Bernat, K.; Wojnowska-Baryła, I. Structure of nitrogen-converting communities induced by hydraulic retention time and COD/N ratio in constantly aerated granular sludge reactors treating digester supernatant. *Bioresour. Technol.* **2014**, *154*, 162–170. [CrossRef]
42. Clagnan, E.; Petrini, S.; Pioli, S.; Piergiacomo, F.; Chowdhury, A.A.; Brusetti, L.; Foladori, P. Conventional activated sludge vs. photo-sequencing batch reactor for enhanced nitrogen removal in municipal wastewater: Microalgal-bacterial consortium and pathogenic load insights. *Bioresour. Technol.* **2024**, *401*, 130735. [CrossRef]

43. Tang, L.; Zhang, Y.; Gao, M.; Wang, X. Simultaneous Nitrification–Endogenous Denitrification with Phosphorus Removal Sustained by Aeration-Free Filamentous Microalgal–Bacterial Granular Sludge for Low C/N Wastewater Treatment. *ACS ES&T Eng.* **2025**, *5*, 1160–1170. [CrossRef]
44. Zhao, Z.; Liu, S.; Yang, X.; Lei, Z.; Shimizu, K.; Zhang, Z.; Lee, D.-J.; Adachi, Y. Stability and performance of algal-bacterial granular sludge in shaking photo-sequencing batch reactors with special focus on phosphorus accumulation. *Bioresour. Technol.* **2019**, *280*, 497–501. [CrossRef]
45. Zhang, J.-T.; Wang, J.-X.; Liu, Y.; Wang, J.-H.; Chi, Z.-Y. Effects of stratified microbial extracellular polymeric substances on microalgae dominant biofilm formation and nutrients turnover under batch and semi-continuous operation. *Bioresour. Technol.* **2025**, *420*, 132120. [CrossRef]
46. Chen, X.; Wang, J.; Wang, Q.; Yuan, T.; Lei, Z.; Zhang, Z.; Shimizu, K.; Lee, D.-J. Simultaneous recovery of phosphorus and alginate-like exopolysaccharides from two types of aerobic granular sludge. *Bioresour. Technol.* **2022**, *346*, 126411. [CrossRef]
47. Cheng, H.; Zhang, H.; Zhao, Z.; Zou, L.; Chen, Y.; Ai, H.; He, Q. Optimal low-frequency mechanical vibration enhances extracellular polymeric substances-mediated phosphorus removal in biological systems. *Water Res.* **2025**, *287*, 124494. [CrossRef]
48. Lee, C.; Truong, H.; Ngo, K.N.; AlSayed, A.; Kin, E.K.; Fuentes, S.; Chen, X.; Clippeleir, H.D.; Wells, G. Integrating Biological Phosphorus Removal with High-Rate Activated Sludge for Enhanced Settleability and Nutrient Management at Short Solids Retention Times. *ACS ES&T Eng.* **2025**, *5*, 377–388. [CrossRef]
49. Li, X.; Yang, S. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res.* **2007**, *41*, 1022–1030. [CrossRef]
50. Liu, X.; Ji, B.; Li, A. Enhancing biolipid production and self-flocculation of Chlorella vulgaris by extracellular polymeric substances from granular sludge with CO2 addition: Microscopic mechanism of microalgae-bacteria symbiosis. *Water Res.* **2023**, *236*, 119960. [CrossRef]
51. de Morais, E.G.; Sampaio, I.C.F.; Gonzalez-Flo, E.; Ferrer, I.; Uggetti, E.; García, J. Microalgae harvesting for wastewater treatment and resources recovery: A review. *N. Biotechnol.* **2023**, *78*, 84–94. [CrossRef]
52. Daneshvar, E.; Zarrinmehr, M.J.; Koutra, E.; Kornaros, M.; Farhadian, O.; Bhatnagar, A. Sequential cultivation of microalgae in raw and recycled dairy wastewater: Microalgal growth, wastewater treatment and biochemical composition. *Bioresour. Technol.* **2019**, *273*, 556–564. [CrossRef]
53. Kusmayadi, A.; Huang, C.-Y.; Kit Leong, Y.; Lu, P.-H.; Yen, H.-W.; Lee, D.-J.; Chang, J.-S. Integration of microalgae cultivation and anaerobic co-digestion with dairy wastewater to enhance bioenergy and biochemicals production. *Bioresour. Technol.* **2023**, *376*, 128858. [CrossRef]
54. Karczmarczyk, A.; Kowalik, W. Combination of Microscopic Tests of the Activated Sludge and Effluent Quality for More Efficient On-Site Treatment. *Water* **2022**, *14*, 489. [CrossRef]
55. Petrini, S.; Foladori, P.; Beghini, F.; Armanini, F.; Segata, N.; Andreottola, G. How inoculation affects the development and the performances of microalgal-bacterial consortia treating real municipal wastewater. *J. Environ. Manag.* **2020**, *263*, 110427. [CrossRef]
56. Wirth, R.; Pap, B.; Böjti, T.; Shetty, P.; Lakatos, G.; Bagi, Z.; Kovács, K.L.; Maróti, G. Chlorella vulgaris and Its Phycosphere in Wastewater: Microalgae-Bacteria Interactions During Nutrient Removal. *Front. Bioeng. Biotechnol.* **2020**, *8*, 557572. [CrossRef]
57. Liu, T.; Chen, Z.; Xiao, Y.; Yuan, M.; Zhou, C.; Liu, G.; Fang, J.; Yang, B. Biochemical and Morphological Changes Triggered by Nitrogen Stress in the Oleaginous Microalga Chlorella vulgaris. *Microorganisms* **2022**, *10*, 566. [CrossRef]
58. Magdalena, J.A.; Ballesteros, M.; González-Fernandez, C. Efficient Anaerobic Digestion of Microalgae Biomass: Proteins as a Key Macromolecule. *Molecules* **2018**, *23*, 1098. [CrossRef]
59. Fard, M.B.; Wu, D. Potential interactive effect on biomass and bio-polymeric substances of microalgal-bacterial aerobic granular sludge as a valuable resource for sustainable development. *Bioresour. Technol.* **2023**, *376*, 128929. [CrossRef]
60. Arcila, J.S.; Buitrón, G. Microalgae–bacteria aggregates: Effect of the hydraulic retention time on the municipal wastewater treatment, biomass settleability and methane potential. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 2862–2870. [CrossRef]
61. Angelidaki, I.; Sanders, W. Assessment of the anaerobic biodegradability of macropollutants. *Rev. Environ. Sci. Biotechnol.* **2004**, *3*, 117–129. [CrossRef]
62. Passos, F.; Uggetti, E.; Carrère, H.; Ferrer, I. Pretreatment of microalgae to improve biogas production: A review. *Bioresour. Technol.* **2014**, *172*, 403–412. [CrossRef]
63. Zou, J.; Cai, L.; Lin, J.; Wang, R.; Li, J.; Jia, M. Anaerobic fermentation of aerobic granular sludge: Insight into the effect of granule size and sludge structure on hydrolysis and acidification. *J. Environ. Manag.* **2023**, *343*, 118202. [CrossRef]
64. Godvin Sharmila, V.; Kumar, G.; Sivashanmugham, P.; Piechota, G.; Park, J.-H.; Adish Kumar, S.; Rajesh Banu, J. Phase separated pretreatment strategies for enhanced waste activated sludge disintegration in anaerobic digestion: An outlook and recent trends. *Bioresour. Technol.* **2022**, *363*, 127985. [CrossRef]

65. Dębowski, M.; Dudek, M.; Nowicka, A.; Quattrocelli, P.; Kazimierowicz, J.; Zieliński, M. Suitability of pre-digested dairy effluent for mixotrophic cultivation of the hydrogen-producing microalgae Tetraselmis subcordiformis. *Environ. Technol.* **2024**, *45*, 471–482. [CrossRef]
66. Zieliński, M.; Dębowski, M.; Kazimierowicz, J. Microwave Radiation Influence on Dairy Waste Anaerobic Digestion in a Multi-Section Hybrid Anaerobic Reactor (M-SHAR). *Processes* **2021**, *9*, 1772. [CrossRef]

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