Performance of CO₂ concentrations on nutrient removal and biogas upgrading by integrating microalgal strains cultivation with activated sludge

Shiqing Sun^a, Zhigang Ge^a, Yongjun Zhao^{a*}, Changwei Hu^b, Hui Zhang^a, Lifeng Ping^c

^aCollege of Biological Chemical Science and Engineering, Jiaxing University, Jiaxing 314001, P.R. China

^bShandong Provincial Key Laboratory of Water and Soil Conservation and Environmental Protection, Linyi University, Linyi 276005, China

^cInstitute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

*Corresponding author

Prof. Yongjun Zhao

College of Biological Chemical Science and Engineering, Jiaxing University, Jiaxing 314001, P.R. China

Email: zyjun2007@163.com

Tel.: +86-573-83647467

Fax: +86-573-83647467

ABSTRACT

In this work, a cooperative algal-bacterial system that efficiently upgrade biogas, simultaneously reduce the biogas slurry nutrient, and exhibits high biomass productivity, was developed. The method about removing H₂S and CO₂ from biogas by three microalgal strains (*Chlorella vulgaris*, *Scenedesmus obliquus*, and *Neochloris oleoabundans*) mixed with activated sludge using biogas slurry as nutrient medium.

A CO₂ concentration of 45% (v/v) was considered optimum to support CO₂ and H₂S removals of 74.11%–80.57% and 99.04%–99.42%, respectively. At the CO₂ concentration of 45%-55%, the mixed culture containing *S. obliquus* and *C. vulgaris* efficiently removed COD and TP, respectively. The mixed culture containing *S. obliquus* demonstrated high N removal efficiency at CO₂ concentration of 45%. Biomass productivity increased at increasing CO₂ concentration in a certain range, with a maximum of 0.177 g L⁻¹ d⁻¹ at CO₂ concentration of 45% (v/v) for the mixed culture containing *S. obliquus*, whereas the C, N, and P biomass contents remained constant at 46.73%–52.31%, 7.59%–9.08%, and 0.91%–1.08%, respectively. This study showed the potential of the combination of alga and bacteria to serve as a treatment for nutrient removal and biogas upgrading in algal-bacterial processes.

Keywords: Algal-bacterial processes, CO₂ removal, H₂S removal, Macromolecular and elemental composition, Nutrient removal

Nomenclature

AD	anaerobic digestion	CO ₂	carbon dioxide
CH ₄	methane	LED	light-emitting diode
COD	chemical oxygen demand	TN	total nitrogen
TP	total phosphorus	TOC	total organic carbon
TSS	total suspended solids	H ₂ S	hydrogen sulfide

PBRb	photobioreactor bag	O ₂	oxygen
N ₂	nitrogen	RE	removal efficiency
DO	dissolved oxygen	NH ₄ ⁺	ammonium nitrogen
NO ₃ ⁻	nitrate nitrogen	NO ₂ ⁻	nitrite nitrogen
H ₂	hydrogen		

1. Introduction

T The challenges associated with the availability of fossil fuels in the past decades intensified the search for alternative energy sources, based on an ever-increasing demand for energy [1]. In the last 70 years, microalgae have been widely studied because of their metabolic versatility and capacity for carbon dioxide mitigation. Recent studies have shown the potential of microalgae for the production of a variety of active compounds, such as vitamins, pigments, carotenoids, proteins, aminoacids, lipids, polysaccharides, biofuels and others [2]. However, the industrial cultivation of microalgae faces an economic feasibility issue because of the high price of artificial medium and the low levels of biomass production. Biogas slurry contains high concentrations of essential nutrients (carbon, nitrogen, and phosphorus) for microalgal growth and can serve as an inexpensive nutrient medium [3]. Thus, the utilization of biogas slurry is an interesting alternative that possesses the added benefit of associating microalgal production with wastewater treatment and biogas upgrading. This would bring advantages such as cost effectiveness, low energy requirements, reduction in sludge formations, greenhouse gas mitigation and the production of useful microalgal biomass. Zhao et al. [4] have studied the biomass growth, nutrient recovery, and biogas upgrade of green algae *Chlorella sp.* in a photobioreactor by using light-emitting diodes (LEDs); these groups observed the high removal efficiency (RE) of the organisms for main nutrients and CO₂ and concluded that microalgae may be utilized for biogas slurry treatment and CH₄ enrichment. Thus, the utilization of biogas slurry is an interesting alternative that possesses the added benefit

of associating microalgal production with wastewater treatment and biogas upgrading. This would bring advantages such as cost effectiveness, low energy requirements, reduction in sludge formations, greenhouse gas mitigation and the production of useful microalgal biomass.

Recent developments are increasing our knowledge about biogas upgrading by microalgae growth. Theoretical calculations as well as bottle and photobioreactor bag experiments have shown the great potential of different microalgae for biogas upgrading which can be further increased CH_4 amount (v/v) in the upgraded biogas[5,6].

The application of anaerobic digestion (AD) as a core treatment technology in biogas plants should be highlighted, since this process combines the pollution control of wastewaters and the generation of bioenergy, based on the conversion of the organic fraction to biogas, a methane-rich gaseous mixture that may supply the energetic demands in biogas plants [7].

Crude biogas from AD is typically composed of CH_4 (40%–75%), CO_2 (25%–60%), H_2S (0.005%–2%), and H_2 , O_2 , or N_2 at trace levels [8]. The above-listed composition of crude biogas shows a very low heating value because only a biogas CH_4 concentration higher than 90% meets the requirement of high-efficiency combustion in burners or boiler units [9]. Although biogas is an environment-friendly fuel, it must be upgraded before use [10]. After upgrading, the final product is referred to as ‘biomethane’, typically containing 95-97% CH_4 and 1-3% CO_2 . Biomethane can be used as an alternative for natural gas. In general, the type of end use of the biogas

sets its quality demands [8]. CO₂ removal from biogas reduces compression and transportation costs, while increasing the calorific value of the crude biogas [10, 11]. Similarly, H₂S removal is also recommended because its hazards and toxicity are connected with the corrosion of engines, pipelines, and biogas storage facilities [12].

Numerous methods such as physical absorption, chemical conversion, membrane separation, pressure swing adsorption, and cryogenic separation have been utilized to upgrade biogas [13]. However, these methods consume a large amount of energy, auxiliary materials, and chemicals as well as generate wastes that require further treatment [14]. Among the various strategies for biogas upgrading by CO₂ removal, the biological sequestration of CO₂ using photosynthetic microalgae has been receiving considerable attention because the CO₂ fixation capability of microalgae is high given their biomass [15]. Moreover, continuous cultivation of some specific microalgal species (e.g., *Chlorella sp.*) yields value-added products, such as proteins, fatty acids, and dietary supplements from biomass [10]. Thus, the removal of CO₂ from biogas by culturing microalgae with biogas slurry is a potential method to upgrade biogas applications. Recently, the potential value of algal-bacterial processes has been proposed for simultaneous biogas slurry nutrient, CO₂, and H₂S removal [10]. The biogas slurry serves as an inexpensive nutrient medium that can enhance microalgal and bacterial growth, thereby reducing costs and environmental damages [16]. Studies have clearly shown that CO₂ is converted to microalgae biomass in the presence of light by photosynthesis, and H₂S is oxidized to sulfate by sulfur-oxidizing bacteria using O₂ produced from microalgal photosynthesis in algal-bacterial

processes [17]. This novel biotechnology can be improved using waste CO₂ as the carbon source and wastewater as the nutrient source to support the growth of microalgae and bacteria, with environmental benefits associated with reduction of CO₂ and the mitigation of the eutrophication potential of wastewaters from biogas slurry [15, 18]. Therefore, the development of cost-effective and environment-friendly biotechniques for integral biogas slurry nutrient removal and biogas upgrading is mandatory [19].

Most previous studies related to the cultivation of microalgae for the production of bioproducts or biodiesel and the upgrade of biogas have only focused on their growth and hydrocarbon/lipid/protein contents under low CO₂ concentrations or ambient air [20, 21]. The CH₄ concentration in biogas varies depending on its source [8]. Crude biogas, which contains 25% to 55% (v/v) CO₂, may be used as a carbon source for the cultivation of microalgae and bacteria. Most algae cultivating operations use a photobioreactor with CO₂ addition to prevent carbon limitation and pH inhibition of algal growth [22,23]. Yan and Zheng [24] studied the biomass growth, nutrient removal, and biogas upgrading of green algae *Chlorella* sp. in a photobioreactor bag (PBRb) using light-emitting diodes (LEDs). These research groups observed that the removal efficiency (RE) of the main nutrients and CO₂ were affected by the CO₂ concentration and microalgal species and concluded that the successful mitigation of CO₂ and production of renewable energy (bioethanol, biogas, biodiesel, and biohydrogen) biodiesel using microalgae require sorting of microalgae according to their growth rate, lipid content, and tolerance to high levels of CO₂ [25, 26]. The

treatment performance of a bioreactor depends on various operational factors that are related to microalgal strain and biogas composition [3]. In this context, the cost-effective conversion of biogas to biomethane via CO₂ and H₂S removal by optimal microalgal species is crucial to promote biogas applications. Additionally, the microalgal-bacterial biomass produced during nutrient removal and biogas upgrading can also be used as a feedstock for the production of renewable energy or commercial bioproducts [27]. Unfortunately, the number of studies on the integral upgrading of biogas coupled with biogas slurry treatment in algal-bacterial photobioreactors is limited, with a knowledge gap on the influence of operational conditions on the biomass composition of different microalgal species. In a preliminary study, Yan and Zheng [6] recorded CO₂ removal from simulated biogas (using *Chlorella* sp. and various LED light wavelength treatments) of 75.5%–85.3% during the treatment of biogas slurry nutrients and biogas upgrading in PBRb. Despite these promising results, the potential of this novel biotechnology to simultaneously treat biogas and biogas slurry can be further boosted by optimizing CO₂, H₂S, and nutrient removal, while selecting or adjusting the composition of the algal-bacterial biomass to provide cost-effective biomass valorization [10].

This work studies the potential of different microalgae for use in the promotion of biogas slurry treatment with COD, nitrogen and phosphorus removal, which is associated with biogas upgrading through high algal biomass productivity. This study mainly aimed to investigate the influence of CO₂ concentration from simulated biogas on the macromolecular and elemental composition of the algal-bacterial biomass

produced from three microalgal strains during simultaneous biogas upgrading and biogas slurry nutrient reduction in a 96 L PBRb. The influence of the four CO₂ concentrations from simulated biogas (25%, 35%, 45%, and 55%) on the removal of CO₂ and H₂S and on the O₂ and CH₄ contents of the upgraded biogas was also determined. The mechanism by which the treatment performance of the three microalgal strains is affected by different CO₂ concentrations in the algal-bacterial processes was also determined. Moreover, the potential carbon and nutrient removal and optimal CO₂ concentration for algal-bacterial growth were also investigated.

2. Materials and methods

2.1. Microalgae strain, microorganisms, and culture conditions

We selected three algal strains, namely, *Chlorella vulgaris* FACHB-31, *Scenedesmus obliquus* FACHB-416, and *Neochloris oleoabundans* UTEX-1185, on the basis of their high biogas tolerance and rapid growth in high-strength wastewater [28, 29]. All of these strains were obtained from the Institute of Hydrobiology in the Chinese Academy of Sciences. The strains were preserved in BG11 medium [30]. The cultivating conditions were as follows: cool-white LED light with $100 \pm 8 \mu\text{mol m}^{-2} \text{s}^{-1}$, 25 ± 0.5 °C, light: dark periods of 16:8 h, and artificial intermittent shaking for three times a day (8:00 a.m., 2:00 p.m., and 8:00 p.m.) at approximately 200 rpm.

The biogas slurry was inoculated with 1L of a 0.75 g total suspended solid (TSS) L⁻¹ for each microalgal strain culture and 200 mL of a 4.14 g TSS L⁻¹ nitrifying–denitrifying activated sludge from wastewater treatment plant of Nanjing, Jiangsu, China. The initial analyzed TSS concentration in the cultivation broth of the

biogas slurry was 0.06 g L^{-1} . The amount of bacteria inoculated in the biogas slurry was twice higher than that of microalgae to ensure a rapid biodegradation start-up [10].

2.2 Biogas and biogas slurry

Four synthetic biogas mixtures were used in accordance with a typical simulated biogas composition for biogas upgrading. Biogas mixture 1 (BM1) was composed of CO_2 (25%), H_2S (0.5%), and CH_4 (74.5%); biogas mixture 2 (BM2) was composed of CO_2 (35%), H_2S (0.5%), and CH_4 (64.5%); biogas mixture 3 (BM3) was composed of CO_2 (45%), H_2S (0.5%), and CH_4 (54.5%); and biogas mixture 4 (BM4) was composed of CO_2 (55%), H_2S (0.5%), and CH_4 (44.5%). Biogas slurry was collected from an anaerobic digester in Nanjing, Jiangsu Province, China. The biogas slurry was pretreated using an ultraviolet sterilizer (KCJ-10W, Konche Water Treatment Co., Ltd., PR China) for 2 min and then filtered using a glass microfiber filter (GF/C, Whatman, USA) to prevent interference from sediments and other microorganisms [24]. Table 1 lists the characteristics of biogas slurry prior to pretreatments. After pretreatment, the pH, total organic carbon (TOC), chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) levels were 7.37 ± 0.18 , 407.52 ± 13.84 , 979.22 ± 29.45 , 245.38 ± 12.17 , and $12.19 \pm 1.84 \text{ mg L}^{-1}$, respectively. These values were only slightly reduced or virtually unchanged after filtration and sterilization. Therefore, pretreatment did not significantly affect the characteristics of the biogas slurry, and these characteristics can be represented by the filtrate.

[Table 1 inserted here]

2.3. PBRb

A transparent polyethylene bag ($80 \text{ cm} \times 60 \text{ cm} \times 20 \text{ cm}$; 96 L) was used as the

photobioreactor in this study. The sampling hole consisted of a plug and rubber gasket. The PBRb was pretreated by washing with distilled water for three times and then sterilized using an ultraviolet sterilizer for 2 min (KCJ-10W, Konche Water Treatment Co., Ltd., PR China) [22]. The LED used in this study was purchased from Canal Optoelectronic Technology Co., Ltd., P. R. China.

2.4. Experimental setup

A PBRb contained 16 L of biogas slurry culture and 80 L of simulated biogas (the initial TSS concentration in the biogas slurry was $135 \pm 9 \text{ mg L}^{-1}$ across all samples). Illumination of all treatments was conducted using 16:8 h light: dark cycles at $105 \pm 10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ during the illuminated period (8:00–24:00) with 30 fluorescent bulbs and 15 Gro-lux fluorescent lamps. The optimal mixture of biogas was analyzed by evaluating the CO_2 , biogas slurry nutrient RE, and CH_4 contents (% , v/v). The system was operated for 10 d at $25 \pm 0.5 \text{ }^\circ\text{C}$. All the treatments were carried out in duplicate, and average values are reported as results.

Gas samples (100 μL) were drawn daily at the outlet of sampling to monitor CO_2 , H_2S , CH_4 , and O_2 concentrations. The initial CO_2 and H_2S concentrations were also measured to accurately determine CO_2 and H_2S removal. Similarly, 50 mL liquid samples were drawn from the biogas slurry daily to monitor COD, TN, and TP concentrations. Finally, the elemental (C, N, and P) and macroscopic (carbohydrates, proteins, lipids, and ash) biomass compositions were determined at the end and at the start of the experiment.

2.5. Analytical procedures

The concentrations (v/v) of CO₂, H₂S, CH₄, and O₂ in the biogas were measured with a circulating gas analyzer [24]. TP was measured using a segmented flow analysis (Automated Chemistry Analyzer, England), and TN using liquor TOC II (Germany). All analyses, including COD and TSS, were carried out according to Standard Methods [31]. The biogas slurry was sampled through the sampling hole of the photobioreactor bag by using a 50 mL syringe. pH (oxidation–reduction potential) was recorded using a portable pH meter equipped with microprobes (LA-pH10, Hach, Loveland, CO, USA). Nutrient CO₂ and H₂S removal efficiency was determined based on the following equation:

$$R = \left(1 - \frac{X_e}{X_i} \right) \times 100, \quad (1)$$

where X_e and X_i are the final and initial nutrient concentrations (mg L⁻¹), respectively. Culture growth was experimentally estimated by measuring DW in each treatment [32]. Biomass productivity (P) was calculated according to the following formula (2):

$$P = (DW_i - DW_0) / (t_i - t_0), \quad (2)$$

where DW_i and DW_0 are the dry weights (g L⁻¹) at time t_i and t_0 (initial time), respectively. The harvested biomass was dried for 24 h at 105 °C in a muffle furnace. The carbohydrate content was spectrophotometrically determined using the Dubois method, and the biomass protein content was determined using the Lowry method [33]. The lipid content was gravimetrically determined following biomass extraction with chloroform: methanol (2:1 v/v) [34]. The ash content was determined according to APHA [28]. The C and N contents of the algal-bacterial biomass were determined using LECO CHNS-932, whereas the phosphorus content was analyzed

spectrophotometrically after acid digestion in a microwave [10].

2.6. Statistical treatment

Statistical analyses were performed by ANOVA with Fisher's least significant difference test using a 95% confidence level. Differences in related parameters among the CO₂ concentrations treatments were tested using one-way ANOVA. Duncan's multiple range tests were conducted to assess significant differences among CO₂ concentrations for the same microalgal strain.

3. Results and discussion

3.1. Biomass productivity

Biomass productivity is an important parameter used to evaluate the potential for CO₂ removal in algal-bacterial processes. Table 2 shows the mean daily productivity obtained under the four CO₂ concentrations from synthetic biogas treatments. The BM3 and BM4 treatments yielded higher growth rates than the BM1 and BM2 treatments. This result was confirmed by the mean daily productivity data (Table 2). Statistical data analysis showed that biomass productivity increased when the CO₂ concentrations were augmented ($P < 0.05$). Therefore, CO₂ concentrations of 45%–55% are more appropriate than 25%–35% for microalgal growth [3, 6]. However, when the CO₂ concentration increased from 45% (Table 2) to 55% (Fig. 1) with the same microalgal strains, a slight decrease in biomass productivity from 0.165 g L⁻¹ d⁻¹ to 0.153 g L⁻¹ d⁻¹, 0.177 g L⁻¹ d⁻¹ to 0.168 g L⁻¹ d⁻¹, and 0.149 g L⁻¹ d⁻¹ to 0.138 g L⁻¹ d⁻¹ was achieved for the mixed culture containing *C. vulgaris*, *S. obliquus*, and *N. oleoabundans*, respectively. These results suggested that,

under the conditions studied, the CO₂ concentration in the simulated biogas may be the limiting factor. Previous studies showed that CO₂ above 55% can be harmful to microalgal cells and inhibit the growth of *Chlorella* sp. [35]. Zhao et al. [3] obtained optimal growth in the biogas slurry and significantly enhanced growth in synthetic biogas with 50% CO₂ with *Nannochloropsis* sp. In the present study, biomass production was approximately 19%–25% higher in the mixed culture containing *S. obliquus* than that in *N. oleoabundans*, and the corresponding biomass production for the mixed culture containing *N. oleoabundans* was approximately 87–138 mg L⁻¹ d⁻¹ for all treatments. The highest biomass production rate was recorded at BM3, followed by BM4, BM2, and BM1 for *S. obliquus*. Considering these findings, the CO₂ concentration of 45% was chosen as the most effective. Maybe, the higher CO₂ concentration in the supplied biogas promoted the photosynthetic efficiency of the microalgae to reproduce within a shorter time; thus more microalgae biomass were achieved [36]. In addition, microalgae was reported to tolerate high CO₂ concentration by possibly adjusting its structural anatomy and redistributing certain cellular organelles [37]. In general, the biomass production values of the mixed culture containing *S. obliquus* were higher than most of those reported for *C. vulgaris* (Table 2) strains, which suggested the potential of *S. obliquus* for capturing CO₂ from biogas and producing biomass or biofuel. However, biomass production varies with operational factors, such as light intensity, pH, working volume of the photobioreactor, and initial CO₂ concentration in the simulated biogas [10, 33, 35]. Moreover, according to biomass production reported in their work for different microalgal strains

(0.058–0.140 g L⁻¹ d⁻¹), *Scenedesmus* species can be ranked as one of the most productive strains [26].

[Table 2 inserted here]

3.2. CO₂ and H₂S RE and O₂ and CH₄ amounts in the upgraded biogas

Complete H₂S removal (98.03%–99.51% RE) was obtained within the range of the CO₂ concentrations studied (Figures 1a, 1b, and 1c). The results obtained also showed that CO₂-RE and the O₂ concentration in the upgraded biogas increased from BM1 to BM3 (Figures 2a, 2b, and 2c). Yan and Zheng [35] also reported increasing CO₂-RE at increasing CO₂ concentration in the synthetic biogas using *Chlorella* sp. However, for all the strains, CO₂-RE under the four different CO₂ concentration dilution ratios showed no significant ($P < 0.05$, data not shown) differences, and the maximum CO₂-RE was obtained at BM3 and did not depend on the inlet CO₂ concentration. Moreover, no significant differences were found on H₂S-RE in all treatments ($P < 0.05$, data not shown), likely because the removal process was always operated at a higher O₂ concentration. H₂S-REs were lower than that reported by Serejo et al. [10], who achieved a percentage of 100% for the mixed culture containing *C. vulgaris*. This result suggested that complete H₂S removal in our experiments was probably limited by the relatively low O₂ concentration (<1%) in the upgraded biogas. In the final phase of the experiment, the outlet H₂S content was 0.029%–0.048% (v/v) for the mixed culture containing *S. obliquus*, which constituted a concentration of <50 ppm (v/v). Moreover, O₂ in biogas remained almost unchanged in the four CO₂ concentration treatments during the experimental period (Figures. 3).

In particular, O₂ (v/v) was maintained between 0.28±0.02% and 0.87±0.04%, which constitutes the lower explosive limit for methane/O₂ mixtures [8, 38]. The presence of O₂ in biogas was not expected to have any significant negative effect toward microalgal growth, given that the biogas is always saturated with water; moreover, O₂ can be used for microalgal photosynthesis [39]. Considering the simultaneous removal of CO₂ and H₂S, the mixed culture containing *S. obliquus* contributed to a higher CO₂ and H₂S-REs than *C. vulgaris* and *N. oleoabundans* in the present study. Therefore, using a given microalgal strain can be an alternative method to increase CO₂ and H₂S-REs (Fig. 1).

In the upgraded biogas during the experiment, the CO₂ amount (v/v) decreased as the CH₄ amount (v/v) increased. In this study, the effect of biogas upgrading agreed with the variation trends of microalgal dry biomass weight (Table. 2) because approximately half of the microalgal dry weight was CO₂-derived carbon [27]. The microalgae developed the CO₂-concentrating mechanism to adapt to changes in the CO₂ concentration. In the present study, the CO₂ were consumed during the microalgal photosynthesis when microalgae were cultured in the biogas slurry. In all treatments for *S. obliquus*, the CH₄ amount (v/v) was approximately 88.13%–93.14%, which was slightly higher than that for the mixed culture containing *C. vulgaris* (86.49%–92.47%) and *N. oleoabundans* (85.42%–91.32%). Meanwhile, only the treatment with 25%–35% CO₂ achieved the biogas grade required for combustion (i.e., a CH₄ concentration higher than 90% v/v) [8] with values of 91.36%-93.14%, 90.58%-92.47%, and 90.09%-91.32% for *S. obliquus*, *C. vulgaris* and *N.*

oleoabundans (Fig. 3). No significant differences in biogas CH₄ amount among the three microalgal strains ($P > 0.05$, data not shown) were found at the same CO₂ concentration treatments. This phenomenon was consistent with the results of previous studies by Zhao et al. [3], who found that different microalgal strains have minimal effects on the CH₄ amount (v/v) and CO₂ concentration condition. Also, the impact of the growth medium on the resulting CH₄ amount (v/v) in the upgraded biogas was shown to be significant, independent of the microalgal strains. Therefore, the three microalgal strains could all be selected for biogas upgrading in algal-bacterial processes in the engineering application.

[Fig. 1, Fig. 2, and Fig. 3 inserted here]

3.3. Biogas slurry nutrient removal

The variations in the nutrient REs (COD, TN, and TP) of the three microalgal strains with time in the different CO₂ concentration treatments for the 10 d batch culture are presented in Table 2. In these treatments, 45%–55% CO₂ achieved a considerably higher COD-RE than the other mixed ratio treatments. Multiple comparisons detected significantly higher COD removal rates in the BM4 treatment than those in the other treatments ($P < 0.05$, Table 2). Based on the results, the COD-RE (%) was approximately 56.35%–69.78% for the mixed culture containing *S. obliquus*, which was higher than that for *C. vulgaris* (52.71%–62.16%) and *N. oleoabundans* (47.09%–59.48%). Carbon is a fundamental element in microalgal cells, accounting for approximately 50% of microalgal biomass [40]. A carbon mass balance calculation over the entire experimental period revealed that assimilation into

biomass was the main C removal mechanism [10]. Some species of microalgae can utilize organic carbon for heterotrophic or mixotrophic growth. The three microalgal strains can change their metabolic pathway according to the supply of organic substrates, such as organic acids or glucose, suggesting that they can sustain heterotrophic growth in addition to common autotrophic growth using CO₂ as the sole carbon source [41]. The experiments revealed that the COD-RE in the biogas slurry was strongly dependent on the species. Therefore, the mixed culture containing *S. obliquus* growth in algal-bacterial processes was considered to be the most effective microalgal species to remove organic matter from biogas slurry. Serejo et al. [10] found lower COD-REs of 31%–51% for the mixed culture containing *C. vulgaris* in diluted biogas slurry, which possibly resulted from different influent CO₂ concentrations, operational time, and bioreactor types in their work compared with this study. Similarly, our result was higher than that of Yan and Zheng [6], who reported COD-REs of 48%–56% by cultivating *Chlorella* sp. alone with the same PBRb under a CO₂ concentration of 35%–55%. These results indicated that adding activated sludge could be a simple and effective operational strategy to increase COD-REs.

In this context, TN decreased at the end of the culture in every microalgal group under the four CO₂ concentration treatments. However, the TN-REs greatly varied among the different microalgal groups. The highest TN-RE was obtained in BM3 for the mixed culture containing *C. vulgaris*, *S. obliquus*, and *N. oleoabundans*, reaching 61.49±5.48%, 63.13±6.21%, and 55.26±3.09%, respectively. Too little or too much

addition of CO₂ into the biogas caused a decline in TN-RE associated with low biomass production in BM1 and BM4. The mixed culture containing *S. obliquus* demonstrated high N removal efficiency in BM3. Likewise, assimilation into biomass was the principal mechanism of N removal in the study. In BM1, the pH remained at 7.13–7.21 because of the low CO₂ concentration. However, pH at approximately 7.30 (in BM4) is optimum for freshwater microalgae cultivation, as well as preventing ammonia toxicity and phosphate precipitation [42]. Therefore, the pH and CO₂ concentrations exerted a significant effect on TN-RE [43]. Nitrogen was successfully removed in the 10 d cultivation, most likely by the microalgae. The presence of microalgae may have a positive effect on the NH₄⁺, NO₂⁻, and NO₃⁻ assimilation rates, which were higher than those in activated sludge samples without microalgal inoculation [44]. Among the microalgal cultures, *S. obliquus* was found to be better in the removal of TN. In agreement with the findings, *S. obliquus* also predominated in TN-REs in the algae-inoculated cultures [26]. Therefore, the appropriate CO₂ concentration of 45% in the biogas using the mixed culture containing *S. obliquus* in BM3 was the most appropriate for nitrogen removal in biogas slurry.

Similar to nitrogen removal, the amounts of phosphorus removal in BM3 and BM4 were slightly higher than those in BM1 and BM2. The highest TP-REs in BM3 for the mixed culture of activated sludge and *S. obliquus*, *C. vulgaris*, and *N. oleoabundans* were 74.11±6.89%, 69.28±5.19%, and 66.59±5.39%, respectively, during the operation. In this context, while the pH influence on TP-RE was negligible in the tested range, the use of CO₂ from the biogas exhibited a competitive advantage

in terms of TP-REs [26]. This advantage could have mediated higher microalgal and bacterial activity because of the absence of inhibitory DO concentrations in the biogas slurry and a higher CO₂ availability that likely favored the higher average TP removals [17]. In all treatments, 0.91%–1.01%, 0.97%–1.08%, and 0.93%–0.97% of the TP removed (independent of the influent CO₂ concentrations) from the biogas slurry was recovered in the harvested biomass for the mixed culture containing *S. obliquus*, *C. vulgaris*, and *N. oleoabundans*, respectively. Therefore, assimilation into biomass was not the main phosphorus removal mechanism in this study. Indeed, the presence of Ca²⁺ and Mg²⁺ in the biogas slurry and the alkaline conditions caused by microalgal growth promoted phosphorus precipitation. Notably, the formed deposits were helpful for phosphorus removal from biogas slurry [44, 45].

In this study was based on batch operation, which lasted for 10 days. This mode may be caused by a limited capacity and ability to treat biogas slurry and biogas in practice. According to the data from our results, thus we suggest that the semi-continuous operation was first performed in batch mode for 5 days, and then in semi-continuous mode with 50%–60% algae culture solution and 40%–50% harvested biogas, and the same amount of fresh wastewater and crude biogas were replenished every 24 h for 10 days.

In summary, the use of algal-bacterial cultures increased the COD, TN, and TP-REs in the mixed cultures containing the three tested green algae, and CO₂ concentration is a significant factor for increasing nutrient removal.

3.4. Macromolecular and elemental composition

The algal-bacterial C, N, and P contents (on a dry weight basis) remained constant at 46.73%–52.31%, 8.78%–9.18%, and 0.91%–1.08%, respectively, regardless of the microalgal strain and CO₂ concentration (Table 2). The elemental composition of the biomass obtained in this study (C/N biomass ratio of 5.15–5.70) was in agreement with previous literature findings (C/N = 5.6±0.2) [10]. The algal-bacterial consortium used for PBRb inoculation exhibited carbohydrate, protein, lipid, and ash concentrations of 66.01%–75.24%, 13.48%–16.46%, 7.19%–8.14%, and 3.39%–8.78%, respectively (Figure 4). A similar carbohydrate content (68±8%) was found by Serejo et al. [10] in the mixed culture of *Chlorella* sp. and activated sludge in diluted biogas slurry for synthetic biogas mixture treatments. Overall, the carbohydrate content of the algal-bacterial biomass recorded in this study was superior to that typically reported for microalgae (8%–35%) [46]. The carbohydrate content increased to 75.24%, along with a decrease in lipid content to 7.53% under the BM4 treatment for the mixed culture containing *S. obliquus*. The carbohydrate content was approximately 3%–7% higher in the mixed culture containing *S. obliquus* than that in *N. oleoabundans*, and the corresponding lipid content for the mixed culture containing *N. oleoabundans* was approximately 0.4%–1.2% higher in *S. obliquus* for all mixed biogas treatments. High carbohydrate content in microalgae has been associated with phosphorus limitation [11, 47]. Furthermore, the gradual increase in CO₂ concentrations from 25% (BM1) to 55% (BM4) (resulting in an increase in the C/N ratio from 5.34 to 5.70 for the mixed culture containing *S. obliquus*) induced a

decrease in the protein content, concomitant with an increase in the carbohydrate content. Similar data have been found for the mixed culture containing *C. vulgaris* and *N. oleoabundans* treatments. These results suggested that the algal-bacterial biomass accumulated carbohydrates because of a potential carbon limitation below the optimum C/N ratio (5.4), concomitant with a limitation in P supply [48]. The low recorded protein content of the algal-bacterial biomass in all treatments compared with the values of $20\pm1\%$ reported by Serejo et al. [10] was found for the mixed culture containing *Chlorella* sp. under a CO₂ concentration of 29.5% (Table 2). This result may be attributed to the different microalgal strains and CO₂ concentrations compared in this study. By contrast, high ash content was found in all mixed samples for the mixed culture containing *N. oleoabundans*, and the values varied among the different mixed biogas treatments. The ash content observed in this work was lower than that reported by Serejo et al. [10], who reported an ash content of up to 8% in the mixed culture containing *Chlorella* sp. under a biogas flow rate of $1.2\text{ m}^3\text{ m}^{-2}\text{ h}^{-1}$. This result may be related to the CO₂ amount and microalgal strain in the photobioreactor used. In this context, the potential biotechnology to produce high-value added products from biogas upgrading and nutrient removal was recently reported by Bahr and co-workers in a pilot high-rate algal pond interconnected via liquid recirculation with an external absorption column in algal-bacterial processes [17].

[Table 3 and Fig. 4 inserted here]

4. Conclusions

A specific algal-activated sludge mixture -based photobioreactor bag experimental design was developed to remove biogas slurry nutrients and upgrade biogas effectively and simultaneously. Three strains survived under all mixed conditions with CH_4 , CO_2 , and H_2S and showed high COD, TN, and TP REs. Among the three strains, *S. obliquus* achieved the highest growth rate in 45% CO_2 . Biomass growth was always directly related to the nutrient removal and CH_4 content in the biogas. The mixed culture containing *S. obliquus* in 45% CO_2 presented the highest CO_2 and H_2S removals of 80% and 99%, respectively. The best range of influent CO_2 concentration for simultaneous biogas upgrading and biogas slurry nutrient removal was determined to be between the moderate and high influent CO_2 concentration treatments, i.e., at approximately 45% to 55% influent CO_2 concentration. Maybe, appropriate selection of microalgal strains and control of the influent CO_2 concentration can achieve the optimal effect of nutrients removal and biogas upgrading. In developing new technologies for the microalgae-based photobioreactor experimental design to removing slurry nutrients and upgrade biogas effectively and simultaneously, there are many residual issues to be investigated. Many of these problems can be examined using laboratory scale (small scale) experiments effectively, rapidly and cheaply. The areas where such experiments can be useful include: microalgae production, screening of microalgae with potential for biogas upgrading, reactor efficiency, CO_2 removal and examination of major metabolites of activated sludge.

Acknowledgments

This study was supported by the National Science Foundation of China (Grant No. 31370520), the Zhejiang Provincial Natural Science Foundation (Grant No. LY16C030003), and the Public Science and Technology Projects of Zhejiang Province (Analytical Test No. 2014C37049).

Reference

- [1] Fuess LT, Garcia ML. Bioenergy from stillage anaerobic digestion to enhance the energy balance ratio of ethanol production. *J Environ Manage* 2015; 162: 102-14.
- [2] Sydney EB, da Silva TE, Tokarski A., Novak AC, de Carvalho JC, Woiciechowski AL, Larroche C, Soccol CR. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. *Appl Energy* 2011; 88: 3291-94.
- [3] Zhao YJ, Sun SQ, Chang WH, Zhang H, Xu J, Ping LP. Performance of three microalgal strains in biogas slurry purification and biogas upgrade in response to various mixed light-emitting diode light wavelengths. *Bioresour Technol* 2015; 187: 338-45.
- [4] Zhao YJ, Wang J, Zhang H, Yan C, Zhang YJ. Effects of various LED light wavelengths and intensities on microalgae-based simultaneous biogas upgrading and biogas slurry nutrient reduction process. *Bioresour Technol* 2013; 136: 461–68.
- [5] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol Adv* 2009; 27:409-16.
- [6] Yan C, Zheng Z. Performance of mixed LED light wavelengths on biogas upgrade and biogas fluid removal by microalga *Chlorella sp.* *Appl Energy* 2014; 113:

1008-14.

- [7] Fuess LT, Garcia ML. Anaerobic digestion of stillage to produce bioenergy in the sugarcane-to-ethanol industry. *Environ Technol* 2014; 35:333-9.
- [8] Ryckebosch E, Drouillon MH, Vervaeren H. Techniques for transformation of biogas to biomethane. *Biomass Bioenerg* 2011; 35: 1633-45.
- [9] Tippayawong N, Thanompongchart P. Biogas quality upgrade by simultaneous removal of CO₂ and H₂S in a packed column reactor. *Energy* 2010; 35: 4531-5.
- [10] Serejo ML, Posadas E, Boncz MA, Blanco S, García-Encina P, Muñoz R. Influence of Biogas Flow Rate on Biomass Composition During the Optimization of Biogas Upgrading in Microalgal-Bacterial Processes. *Environ Sci Technol* 2015; 49: 3228-36.
- [11] Markou G, Chatzipavlidis I, Georgakakis D. Carbohydrates Production and bio-flocculation characteristics in cultures of *Arthrospira (Spirulina) platensis*: Improvements through phosphorus limitation process. *Bioenergy Res.* 2012; 5: 915-25.
- [12] Kao CY, Chiu SY, Huang TT, Dai L, Hsu LK, Lin CS. Ability of a mutant strain of the microalga *Chlorella sp.* to capture carbon dioxide for biogas upgrading. *Appl Energy* 2012; 93: 176-83.
- [13] Deng L, Hagg MB. Techno-economic evaluation of biogas upgrading process using CO₂ facilitated transport membrane. *Int J Greenhouse Gas Control* 2010; 4:638-46.
- [14] Raab K, Lamprecht M, Brechtel K, Scheffknecht G. Innovative CO₂ separation of

- biogas by polymer resins: operation of a continuous lab-scale plant. *Eng Life Sci* 2012; 3:327-35.
- [15] Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM. Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresour Technol* 2010; 101: S71-4.
- [16] Uggetti E, Sialve B, Latrille E, Steyer J. Anaerobic digestate as substrate for microalgae culture: the role of ammonium concentration on the microalgae productivity. *Bioresour Technol* 2014; 152: 437-43.
- [17] Bahr M, Díaz I, Dominguez A, González Sánchez A, Muñoz R. Microalgal-biotechnology as a platform for an integral biogas upgrading and nutrient removal from anaerobic effluents. *Environ. Sci. Technol.* 2014; 48: 573-81.
- [18] Mussnug JH, Klassen V, Schlüter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J Biotechnol* 2010; 150: 151-56.
- [19] Alcántara C, García-Encina R, Muñoz R. Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. *Chem Eng J* 2013; 221: 238-46.
- [20] Pulz O, Gross W. Valuable products from biotechnology of microalgae. *Appl Microbiol Biotechnol* 2004; 65: 635-48.
- [21] Skjanes K, Lindblad P, Muller J. BioCO₂ -a multidisciplinary, biological approach using solar energy to capture CO₂ while producing H₂ and high value

- products. *Biomol Eng* 2007; 24: 405-13.
- [22] Woertz I, Feffer A, Lundquist T, Nelson Y. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *J Environ Eng* 2009; 135: 1115-22.
- [23] Park JBK, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. *Bioresour Technol* 2011; 102: 35-42.
- [24] Yan C, Zheng Z. Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella sp.* *Bioresour Technol* 2013; 139: 292-99.
- [25] Jacob-Lopes E, Revah S, Hernandez S, Shirai K, Franco TT. Development of operational strategies to remove carbon dioxide in photobioreactors. *Chem Eng J* 2009;153: 120-26.
- [26] Ho SH, Chen CY, Lee DJ, Chang JS. Perspectives on microalgal CO₂-emission mitigation systems- A review. *Biotechnol Adv* 2011; 29: 189-98.
- [27] Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* 2008; 26: 126-31.
- [28] Li B. A research for purifying biogas slurry and upgrading biogas by *Chlorella sp.*. Master Dissertation, Fudan University, Shanghai, P.R. China 2012. (in Chinese)
- [29] Li B, Yan C, Wang D, Qian JC, Nie E, Zheng Z. A method for purifying biogas slurry and upgrading biogas by *Chlorella sp.*. *Chinese J Environ Eng* 2013; 6: 2396-2400. (in Chinese)

- [30] Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure culture of cyanobacteria. J Gen Microbiol 1979; 111: 1–61.
- [31] APHA, AWWA, WEF. Standard methods for the examination of water and wastewater. 19th ed. Washington, DC: American Public Health Association; 1998.
- [32] Li Y, Chen Y, Chen P, Min M, Zhou W, Martinez B, Zhu J, Ruan R. Characterization of a microalga *Chlorella sp.* well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour Technol 2011; 102: 5138-44
- [33] Toledo-Cervantes A, Morales M, Novelo E, Revah S. Carbon dioxide fixation and lipid storage by *Scenedesmus obtusiusculus*. Bioresour Technol 2013; 130: 652-58.
- [34] Kochert G.. Handbook of Phycological Methods; Cambridge University Press: London; 1978.
- [35] Yan C, Zhang L, Luo XZ, Zheng Z. Influence of influent methane concentration on biogas upgrading and biogas slurry purification under various LED light wavelengths using *Chlorella sp.* Energy 2014; 69:419–26.
- [36] Lam MK, Lee KT, Mohamed AR. Current status and challenges on microalgae-based carbon capture. Int J Greenhouse Gas Control 2012; 10: 456-69.
- [37] Hessen DO. Excess carbon in aquatic organisms and ecosystems: physiological,

- ecological, and evolutionary implications. *Limnol Oceanogr* 2008; 53:1685-96.
- [38] Leis J, Member S, Buttsworth D, Snook C, Holmes G. Detection of Potentially Explosive Methane Levels Using a Solid-State Infrared Source. *IEEE Trans Instrum Meas.* 2014; 12: 3088-95.
- [39] Kubler JE, Johnston AM, Raven JA. The effects of reduced and elevated CO₂ and O₂ on the sea-weed *Lomentaria articulata*. *Plant Cell Environ* 1999; 22:1303-10.
- [40] Singh M, Das K. Low Cost Nutrients for Algae Cultivation, *Algal Biorefineries*. Springer 2014; pp 69-82.
- [41] Tan X, Chu H, Zhang Y, Yang L, Zhao F, Zhou X. *Chlorella pyrenoidosa* cultivation using anaerobic digested starch processing wastewater in an airlift circulation photobioreactor. *Bioresour Technol* 2014; 170: 538-48.
- [42] Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour Technol* 2011; 102:17-25.
- [43] González C, Marciniak J, Villaverde S, García-Encina P, Muñoz R. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. *Appl Microbiol Biotechnol* 2008; 80: 891-8.
- [44] Posadas E, García-Encina P A, Domínguez A, Díaz I, Becares E, Blanco S, Muñoz R. Enclosed tubular and open algalbacterial biofilm photobioreactor for carbon and nutrient removal from domestic wastewaters. *Ecol Eng* 2014; 67: 156-64.
- [45] Ruiz-Marin A, Mendoza-Espinosa LG, Stephenson T. Growth and nutrient

removal in free and immobilizer green algae in batch and semi-continuous culture treating real wastewater. *Bioresour Technol* 2010; 101: 58-64.

[46] Markou G, Georgakakis D. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Appl Energy* 2011; 88: 3389–401.

[47] Margarites ACF, Costa JAV. Increment of carbohydrate concentration of *Chlorella minutissima* microalgae for bioethanol production. *Int J Eng Res Ind Appl* 2014; 4: 80-6.

[48] Möllers KB, Cannella D, Jørgensen H, Frigaard NU. Cyanobacterial biomass as carbohydrate and nutrient feedstock for bioethanol production by yeast fermentation. *Biotechnol Biofuels* 2014; 7:1-11.

Figure Captions

Figure 1. CO₂ (□) and H₂S (■) removal efficiency (%) in the biogas upgraded in the photobioreactor bag at the four CO₂ concentration levels treatment.

(a) *C. vulgaris*, (b) *S. obliquus*, and (c) *N. oleoabundans*.

Figure 2. H₂S (■) and O₂ (■) concentrations (% v/v) in the biogas upgraded in the photobioreactor bag at the four CO₂ concentration levels treatment.

(a) *C. vulgaris*, (b) *S. obliquus*, and (c) *N. oleoabundans*.

Figure 3. CO₂ (□) and CH₄ (■) concentrations (% v/v) in the biogas upgraded in the photobioreactor bag at the four CO₂ concentration levels treatment.

(a) *C. vulgaris*, (b) *S. obliquus*, and (c) *N. oleoabundans*.

Figure 4. Carbohydrates, proteins, lipids, and ash concentrations (%) in the harvested biomass under steady state at the four CO₂ concentration levels treatment.

Fig.1

Fig.2

Fig.3

Fig.4

Table 1**The characteristics of biogas slurry.**

Parameter	Original concentration/ pH
pH	7.43±0.11
TOC (mgL ⁻¹)	413.78±18.52
COD(mgL ⁻¹)	998.58±37.66
TN(mgL ⁻¹)	256.91±16.79
TP(mgL ⁻¹)	14.06±2.08

Table 2. COD, TN, and P removal efficiencies, TSS concentration, biomass productivity and pH under steady state during the operational period evaluated.

Three microalgae strains The biogas mixture	COD Removal (%)	TN Removal (%)	TP Removal (%)	Mean daily productivity (gL ⁻¹ d ⁻¹)	pH
<i>C. vulgaris</i> + activated sludge					
BM1	52.71 ^b ±4.11	49.93 ^b ±4.79	61.58 ^b ±5.84	0.097 ^b ±0.008	7.13 ^a ±0.11
BM2	54.83 ^b ±4.79	51.15 ^b ±5.18	68.26 ^a ±5.43	0.112 ^b ±0.006	7.24 ^a ±0.19
BM3	56.29 ^b ±3.19	61.49 ^a ±5.48	69.28 ^a ±5.19	0.165 ^a ±0.007	7.37 ^a ±0.21
BM4	62.16 ^a ±4.34	53.22 ^b ±4.23	65.21 ^a ±4.78	0.153 ^a ±0.005	7.04 ^a ±0.15
<i>S. obliquus</i> + activated sludge					
BM1	56.35 ^b ±2.97	51.74 ^c ±5.75	64.23 ^b ±5.17	0.103 ^c ±0.012	7.21 ^a ±0.16
BM2	59.38 ^b ±4.79	57.29 ^b ±6.37	67.19 ^{ab} ±5.83	0.131 ^b ±0.014	7.33 ^a ±0.29
BM3	64.74 ^{ab} ±6.05	63.13 ^a ±6.21	74.11 ^a ±6.89	0.177 ^a ±0.011	7.24 ^a ±0.16
BM4	69.78 ^a ±3.77	59.34 ^{ab} ±5.27	69.98 ^a ±7.62	0.168 ^a ±0.013	7.11 ^a ±0.14
<i>N. oleoabundans</i> + activated sludge					
BM1	47.09 ^b ±4.91	47.36 ^b ±4.61	57.92 ^b ±5.13	0.087 ^b ±0.006	7.18 ^a ±0.24
BM2	51.02 ^b ±5.87	49.87 ^b ±5.18	61.62 ^{ab} ±4.35	0.095 ^b ±0.008	7.23 ^a ±0.13
BM3	53.82 ^b ±4.32	55.26 ^a ±3.09	66.59 ^a ±5.39	0.149 ^a ±0.005	7.30 ^a ±0.19
BM4	59.48 ^a ±5.23	52.14 ^{ab} ±3.87	64.05 ^a ±4.37	0.138 ^a ±0.008	7.12 ^a ±0.25

Table 3. Carbon (C), nitrogen (N), and phosphorus (P) concentrations in the harvested biomass under steady state during the operational period evaluated.

Three microalgae strains /the biogas mixture	C (%)	N (%)	P (%)
<i>C. vulgaris</i> + activated sludge			
BM1	46.78 ^a ±2.09	8.97 ^a ±0.18	0.91 ^a ±0.11
BM2	47.41 ^a ±1.16	8.89 ^a ±0.21	0.95 ^a ±0.11
BM3	49.39 ^a ±1.93	8.95 ^a ±0.24	0.99 ^a ±0.12
BM4	50.39 ^a ±1.77	8.84 ^a ±0.27	1.01 ^a ±0.11
<i>S. obliquus</i> + activated sludge			
BM1	48.27 ^a ±2.05	9.04 ^a ±0.13	1.03 ^a ±0.11
BM2	49.23 ^a ±1.78	8.91 ^a ±0.16	0.98 ^a ±0.13
BM3	51.14 ^a ±1.28	8.99 ^a ±0.14	0.97 ^a ±0.10
BM4	52.31 ^a ±2.14	9.18 ^a ±0.21	1.08 ^a ±0.12
<i>N. oleoabundans</i> + activated sludge			
BM1	46.02 ^a ±2.14	8.93 ^a ±0.19	0.95 ^a ±0.12
BM2	46.73 ^a ±1.43	9.03 ^a ±0.22	0.97 ^a ±0.10
BM3	48.15 ^a ±1.57	8.78 ^a ±0.29	0.93 ^a ±0.09
BM4	49.28 ^a ±2.25	8.87 ^a ±0.17	0.96 ^a ±0.09