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Biogas upgrading and valorization to single-cell protein in a bioinorganic electrosynthesis system

Mingyi Xu, Dan Zhao, Xinyu Zhu, Yanyan Su, Irini Angelidaki, Yifeng Zhang *

Department of Environmental Engineering, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark

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ABSTRACT

Valorization of crude biogas to value-added products is the key step toward techno-economic biogas business. Besides, the increasing world population puts heavy pressure on food supply and will double protein demand in the coming decades. In this context, we propose a bioinorganic electrosynthesis process for the integration of biogas upgrading and edible single-cell protein production, which could be an alternative solution to address these challenges. With a biogas inflow of 70%CH_4/30%CO_2 at 50 mL·d^{-1} and an applied voltage of 3.0 V, the protein concentration of 472.04 ± 22.05 mg·L^{-1} was achieved with the "CO_2-to-CH_4" bioconversion efficiency of 92.97 ± 5.61%. Higher CO_2 content in the biogas resulted in a comparatively lower protein concentration. The system was tested resilient to the toxic H_2S in biogas (up to 5000 ppm). It was possible to improve the protein yield three times by scaling up the fermenter from 100 mL to 1 L, with a "CH_4-to-SCP" fermentation efficiency of 70.67 ± 2.37%. The methanotrophic biomass produced in the system was found rich in protein with a total amino acids mass-content of over 62.8%. The outcomes of this study will offer a new solution for sustainable protein production, biogas upgrading and valorization, which are perfectly in line with the United Nations Sustainable Development Goals.

1. Introduction

Biogas, produced by Anaerobic digestion (AD) of organic materials, has a long tradition of being utilized as a sustainable energy alternative against conventional fossil fuels [1,2]. There are currently at least 18,943 biogas plants in Europe producing 167 TWh or 15.8 bcm of biogas, with the ambitious goal of fulfilling a 100% renewable energy system [3,4]. Though well-promoted, several challenges are still needed to be addressed in developing the biogas business. Firstly, to enhance the usability of biogas for multiple applications (e.g., to the natural gas grid), cleaning and upgrading of crude biogas to remove or reduce the component of CO_2 (30 ~ 40%) and H_2S (500 ~ 5000 ppm) are necessary. However, this step is currently still energy-intensive and costly [5–7]. Secondly, the extensive development of biogas plants could mainly be attributed to political stimulation, such as the strong policies for decentralized combined heat and power production, which relies heavily on government subsidies to stay profitable [3,7,8]. Considering that it is an inevitable trend that the government gradually reduces or cancels the financial support for biogas plants, the profitability of future biogas business is challenged by high initial capital costs and market barriers such as inadequate institutional framework and infrastructure [9–11]. Thus, alternative technology for cost-effective biogas upgrading and value-added product production is urgently needed.

Microbial electrosynthesis or electro-fermentation is a representative green technology that can potentially produce multiple bio-commodities from organics or inorganics (e.g., CO_2) with the assistance of microbial catalysts and electricity [12,13]. Significantly, the application of microbial electrosynthesis for biogas upgrading has been demonstrated with high CH_4 content in the treated biogas (over 97%) and acceptable energy/economic benefits [14,15]. Though promising, the end-product (biomethane) is still low-value and cannot contribute to a considerable profit margin. Therefore, more advanced products should be pursued to make this application more competitive in both scientific and socio-economic contexts.

Recently, Single-cell protein (SCP), which refers to edible unicellular microorganisms, has been widely studied because it offers a sustainable solution to protein production challenges [16–18]. Especially, Methane-oxidizing bacteria (MOB), which can utilize methane as the sole carbon and energy source, is received as a promising SCP alternative in light of its high and fast yield, broad substrates availability, and high protein

* Corresponding author.
E-mail address: yifz@env.dtu.dk (Y. Zhang).

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content (50 ~ 80%) [19,20]. Besides, MOB has also been approved by the EU for the nutrition of humans and animals and has been demonstrated on an industrial scale [19]. Though promising, the feedstock of MOB today is still limited to unrenewable and expensive natural gas, which essentially hinders its wide application. Biogas derived from organic wastes could be a renewable source of methane that can reduce the production costs and climate impact for the SCP production. Nevertheless, to reduce the carbon losses (e.g., CO_2 for 30–50%) and the negative effect from the toxic gases such as H_2S on SCP fermentation, biogas upgrading is required before using it as the feedstock of MOB [21]. In this context, the combination of microbial electrosynthesis, biogas upgrading, and MOB fermentation for SCP production could provide synergistic benefits and meet the challenges encountered by each individual process.

In this study, we propose a Bioinorganic electrosynthesis (BIES) system to realize the engagement of upstream biogas upgrading and downstream SCP production. The performance of "CO_2-to-CH_4" bioconversion, MOB growth, and SCP yield were systematically evaluated. Besides, the effects of different CO_2 content and H_2S content in biogas on system performance were investigated individually. Finally, the scalability of the BIES system was explored in terms of amino acid profile and microbial composition.

2. Materials and methods

2.1. Strain, medium, and substrates

The mixed methanotrophic seed was derived from a lab-scale fermenter, dominated by *Methylophilus* and *Methylomonas* [22]. Before being inoculated to the system, the seed was cultivated and subcultured in a chemostat incubator at 37.0 ± 0.5℃ for two months of acclimatization. During the cultivation, the methane content in the gas phase was maintained at 10 ~ 30% by the intermittent supply of the feeding gas with 60%CH_4/40%O_2. The medium for the growth of MOB (based on dAMS medium) included 0.1 g·L^{-1} NH_4Cl, 0.2 g·L^{-1} $MgSO_4{\cdot}7H_2O$, 0.03 g·L^{-1} $CaCl_2{\cdot}2H_2O$, trace element (500 μg·L^{-1} FeNaEDTA, 50 μg·L^{-1} $Na_2EDTA{\cdot}2H_2O$, 20 μg·L^{-1} $FeSO_4{\cdot}7H_2O$, 3 μg·L^{-1} H_3BO_3, 2 μg·L^{-1} $CoCl_2{\cdot}6H_2O$, 1 μg·L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.3 μg·L^{-1} $MnCl_2{\cdot}4H_2O$, 0.3 μg·L^{-1} $Na_2MoO_4{\cdot}2H_2O$, 0.2 μg·L^{-1} $NiCl_2{\cdot}6H_2O$, 2.5 μg·L^{-1} $CuSO_4{\cdot}5H_2O$), and PBS buffer (1.79 g·L^{-1} $Na_2HPO_4{\cdot}12H_2O$, 0.68 g·L^{-1} KH_2PO_4) for pH control at 7.0.

In the BIES system, the anolyte was 10 mmol·L^{-1} Na_2SO_4. The catholyte consisted of 1.91 g·L^{-1} $(NH_4)_2HPO_4$ and PBS solution (5.74 g·L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 2.01 g·L^{-1} $NaH_2PO_4{\cdot}H_2O$, 0.15 g·L^{-1} KH_2PO_4). Besides, 12.5 mL·L^{-1} vitamin solution and 2.5 mL·L^{-1} trace element solution were added with the recipe described in the previous study [14,23]. The anaerobic granular sludge (AnGS) was used as the biocatalyst for electromethanogenesis in the system, which was collected from a wastewater treatment plant (Colsen, Netherlands) [14,23]. In our previous study, AnGS was considered as a high-efficient and robust biocatalyst for cathodic electromethanogensis because it has the merits of richness in methanogens, high biomass level, rapid settling capacity, and the unique layered structure [14,23]. The dominant microbes in raw AnGS were identified as *Methanobacterium* (14.7 ± 0.7%) and *Methanosaeta* (14.2 ± 0.5%) [14]. The abundance of the hydrogenotrophic methanogens *Methanobacterium* increased to 28.5 ~ 37.7% after the bioelectrochemical acclimatization [14].

2.2. Experimental setup

2.2.1. System configuration and assembly

The graphical system setup is shown in Fig. 1. The BIES includes two parts. In part I, an anode chamber for O_2 evolution and a cathode chamber for biogas upgrading (Polycarbonate, working volume of each chamber 5 × 5 × 8 cm^3) were separated by a cation exchange membrane (CEM, CMI-7001, Membranes International Inc., New Jersey, USA) [24]. The anodic electrode was made of titanium alloy mesh with IrO_2 coated (Magneto Special Anodes B.V., Schiedam, the Netherlands). The cathodic electrode was made of titanium-woven wire mesh, wrapping the AnGS in. The electrodes were connected to the external power supply (HQ PS3003, Helmholt Elektronik A/S, Denmark). The Ag/AgCl electrode (Sat. KCl, +0.197 V vs SHE, Sensortechnik Meinsberf, Germany) was used as the reference electrode for cathodic potential recording [25]. The synthetic biogas was continuously provided to the cathode chamber via a peristaltic pump (BT100-2 J, Longer Precision Pump Co., Ltd, Baoding, China). In part II, an SCP fermenter was employed to receive O_2 and upgraded biogas for MOB fermentation. The gas effluent from the system was collected in a gas collecting bag. The whole system was operated in a microbiological incubator (Thermo Scientific Heraeus®, VWR International, Denmark) at 37.0 ± 0.1 °C.

2.2.2. System operation and experimental tests

The system was operated in batch mode. The catholyte was previously flushed with pure N_2 to ensure an anaerobic environment before each batch. The inoculum size for each batch was 5% (v/v). The effects of CO_2 and H_2S content in the inflow raw biogas on system performance

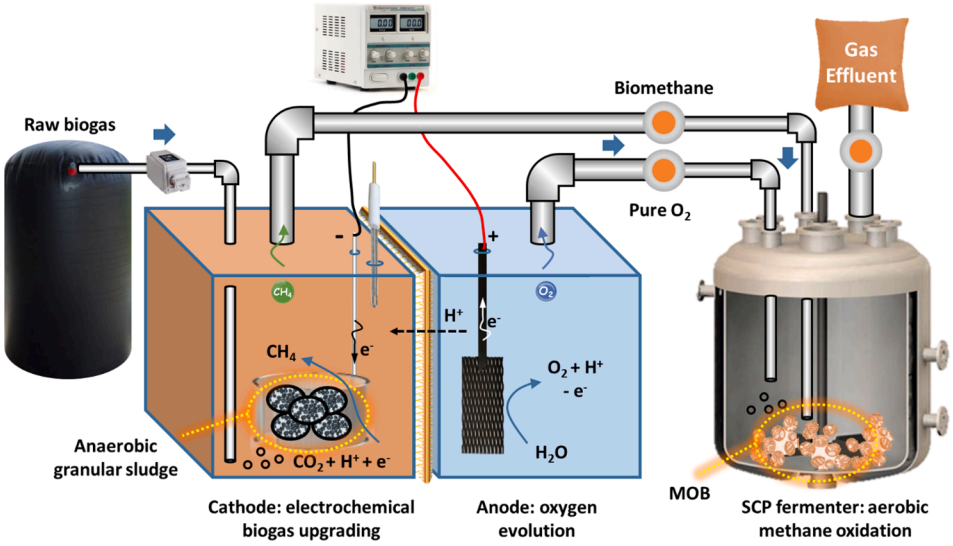

Fig. 1. Schematic illustration of the proposed BIES for crude biogas upgrading and SCP production.

were individually studied. Besides, a scale-up SCP fermenter was applied to the system to evaluate the system productivity further. The external voltage supply, biogas flow rate, biogas composition, H_2S content in the biogas, and SCP fermenter working volume for each group were summarized in Table 1.

2.3. Analytical methods

The gas samples were taken individually from the headspace of the recirculation bottle (cathode off-gas) and the gas collection bag connected to the SCP-fermenter (gas effluent) every two days. The content of CH_4, O_2, H_2, CO_2, and H_2S in the gas samples was determined by gas chromatography (GC-TRACE 1310, Thermo Scientific®, Micolab A/S, Denmark) and calibrated by the standard gases. A data acquisition system (2701 Ethernet Multimeter, Keithley Instruments LLC, USA) was employed for current real-time recording. The liquid samples from the catholyte and the SCP fermenter in the BIES system were taken every two days. pH was measured by a PHM 210 pH meter (Radiometer). Total inorganic carbon (TIC) was measured by a Total Organic Carbon Analyzer (Shimadzu® TOC-V WP, Holm & Halby, Denmark) together with an Autosampler (Shimadzu® ASI-V, Holm & Halby, Denmark). The OD_{600} (optical density at the wavelength of 600 nm) was adopted to indicate the growth of MOB and determined by UV–Visible spectrophotometer (Varian Cary® 50 Bio). The concentration of sulfate and sulfide in the liquid sample was quantified by the sulfate test kit (No. 102537, Spectroquant®, Merck KGaA, Germany) and Methylene Blue Kit (HACH®, USA) individually, using a portable spectrophotometer (Model: DR3900, HACH Lange®) at a wavelength of 525 nm (SO_4^{2-}-S) and 665 nm (S^{2-}-S) [21]. The gaseous H_2S content was detected by a portable gas analyzer (Geotech BIOGAS 5000, QED Environmental Systems Ltd., UK). At the end of each batch, the residual MOB biomass was collected to analyze the biomass concentration and amino acid profile through the pretreatment approaches reported in the previous study [21].

The community composition of SCP bacteria was characterized by 16 s amplicon sequencing analysis. Specifically, genomic DNA of SCP bacteria was extracted with PowerSoil® DNA Isolation Kit (QIAGEN Bioinformatics, Germany) with additional phenol cleaning steps [26]. The amplification of the prokaryotic16s ribosomal RNA gene was performed with PCR using 515F/806R primers (V4 region). The gene amplicons were sequentially sequenced by the Illumina MiSeq platform. The raw sequencing data were analyzed using CLC Workbench software (V.8.0.2) with a Microbial genomics module plug-in (QIAGEN) [27]. The represented sequences of abundance OTUs (higher than 1%) were blasted against the NCBI 16S rRNA database for taxonomy assignment. The raw sequences were deposited in Sequence Read Archive (SRA) database with the accession BioProject number PRJNA543037.

3. Results and discussions

3.1. Effect of different CO_2 content in biogas on system performance

Synthetic biogas with different CO_2 content was fed into the BIES system for a proof-of-concept test. Fig. 2a exhibits the performance of the SCP fermentation in terms of final biomass concentration and the accumulated amount of effluent gases. It was observed that the final SCP concentration raised with the increase of CH_4 content in the feeding gas. The final SCP concentration was 136.32 ± 11.35 (BG-$1_{100\%CO2}$), 360.01 ± 17.58 (BG-$2_{50\%CO2/50\%CH4}$), and 472.04 ± 22.05 mg·L^{-1} (BG-$3_{30\%CO2/70\%CH4}$), which showed the good potential of biogas valorization into SCP. The results indicate that the sufficiency of CH_4 supply was the key limiting factor for the yield of MOB-based SCP [28,29]. The residual CO_2 in the gas effluent was 50.97 ± 2.24 (BG-1), 47.71 ± 1.24 (BG-2), and 45.54 ± 0.39 mL (BG-3), implying the potential of BIES for CO_2 capture and utilization. The detailed carbon balance analysis for the two stages of the BIES system was recorded in Table 2. In Stage I, the "CO_2-to-CH_4" conversion efficiency in the cathode increased from 65.54 ± 5.32% to 92.97 ± 5.61% when the initial CO_2 content in the biogas was decreased from 100% to 30%. This efficiency was comparable with previous studies on microbial electrosynthesis of methane [14,15,30]. The change of catholyte pH could be one reason for the different "CO_2-to-CH_4" efficiency during the tests. The reported optimum pH for electromethanogensis was 7.0 ~ 7.5 [31,32]. As Fig. S1 shows, the cathodic pH in Group BG-1 decreased to 6.81 ± 0.03 as a result of CO_2 dissolution, which could contribute to the relatively lower CO_2 conversion efficiency than the other two groups. In Stage II, along with the higher SCP concentration, the CH_4 consumption and the CO_2 generation from the aerobic methane oxidation process were correspondingly higher in Group BG-3 than the other two groups. Also, higher "CH_4-to-SCP" efficiencies of 22.89 ± 0.26% was achieved in this group.

Fig. 2b shows the growth performance of MOB in the BIES system. The highest OD_{600} of 1.22 ± 0.09 was obtained in Group BG-$3_{30\%CO2/70\%CH4}$ at the end of the batch run, which was in line with SCP yield. No obvious lag phase was observed, indicating the better robustness and flexibility of mixed MOBs for SCP production than pure MOB cultures [22,33]. Along with the MOB growth, the pH of the medium in the SCP-fermenter gradually dropped from 7.01 ± 0.01 to 5.86 ± 0.11 due to the proton donation from the ammonium metabolism by MOBs [34,35].

Fig. 2c presents the change of cathodic off-gas content, indicating the biogas upgrading performance in Stage I. In Group BG-$1_{100\%CO2}$, the CH_4 content in the cathodic off-gas raised to 62.95 ± 1.18%, and the CO_2 content declined to 9.74 ± 0.16% on the 8th day. While in Group BG-$2_{50\%CO2/50\%CH4}$ and BG-$3_{30\%CO2/70\%CH4}$, higher CH_4 content of 81.03 ± 2.77% and 94.11 ± 2.07% was achieved in the cathodic off-gas, which was comparable with other studies on electromethanogensis for biogas upgrading [36,37]. No H_2 gas was found (<0.01%) in the cathodic off-gas, which indicated no H_2 was wasted, and the H_2 partial pressure was well controlled. Fig. 2d shows the results of system effluent gas content. The residual O_2 was the main content in all three groups on the 2nd day (around 59.03 ~ 64.66%), but it gradually decreased afterward due to the consumption by MOBs and the ascending of CH_4. The residual CO_2 content was limited to 7.33 ± 0.51% (BG-1), 2.81 ± 0.16% (BG-2), and 1.56 ± 0.17% (BG-3) at the end of the batch. Besides, the average ratio of CH_4/O_2 in the headspace of SCP-fermenter was calculated as 1:3.10 (BG-1), 1:1.67 (BG-2), and 1:1.33 (BG-3), respectively. An efficient and safe supply of CH_4 and O_2 appears a bottleneck for high-rate methanotrophs cultivation [21,38]. The closer CH_4/O_2 ratio in Group BG-3 to the theoretical stoichiometric value (1:1.45) of aerobic methane

Table 1
System operational conditions in experimental tests.

Experiment	Group	Voltage V	Biogas flow rate mL·d^{-1}	Biogas composition	H_2S content in biogas ppm	SCP fermenter working volume Ml
Effect of CO_2 in biogas	BG-1	3	50	100%CO_2	0	100
	BG-2			50%CO_2/50%CH_4		
	BG-3			30%CO_2/70%CH_4		
Effect of H_2S in biogas	S-1	3	50	30%CO_2/70%CH_4	1000	100
	S-2		50		5000	
	S-3		200		5000	
Scaled-up SCP fermenter	–	3	50	30%CO_2/70%CH_4	0	1000

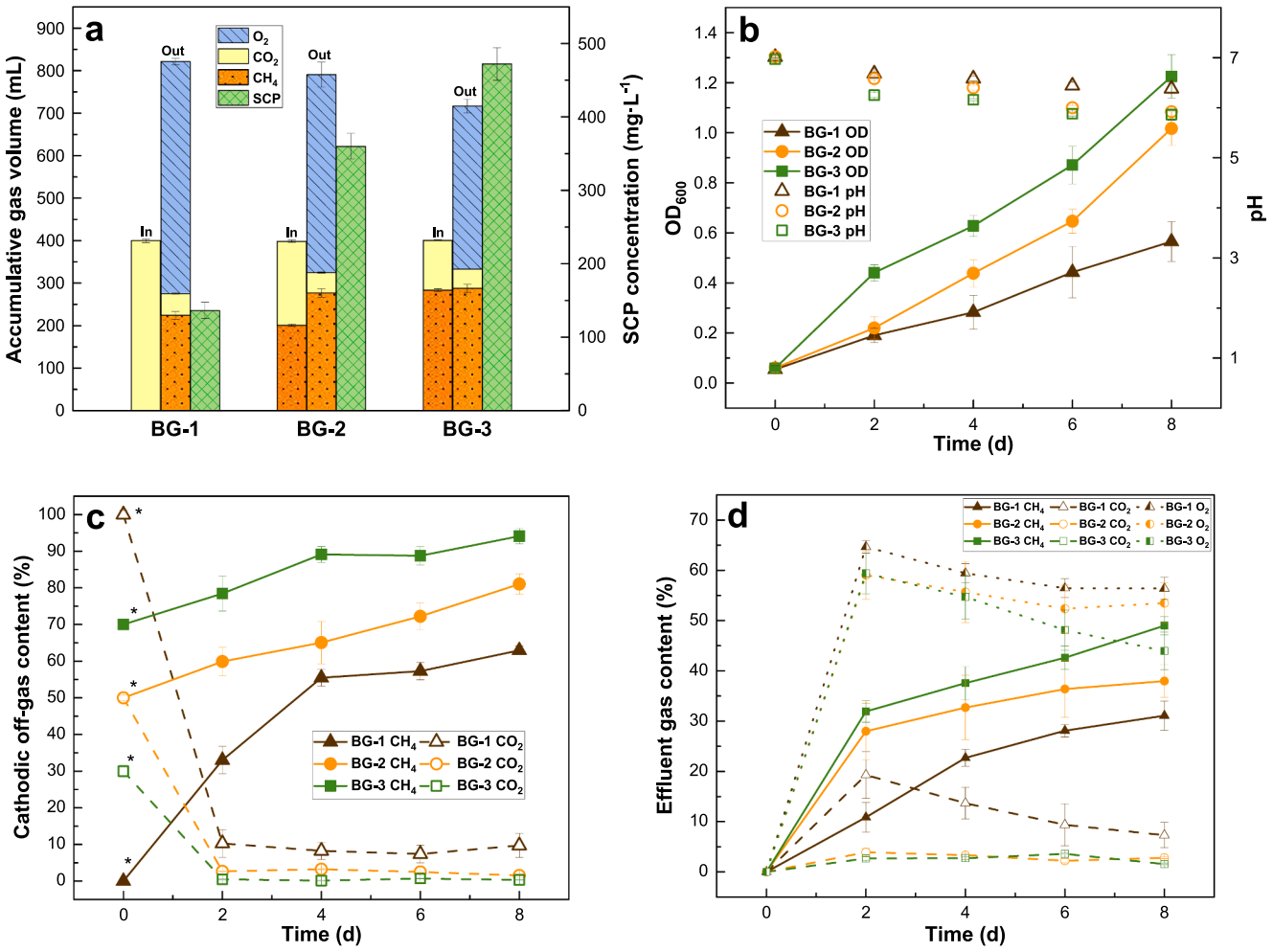

Fig. 2. Effect of CO_2 content in synthetic biogas on BIES system performance, including 100%CO_2 (Group BG-1), 50%CO_2/50%CH_4 (Group BG-2), and 30%CO_2/70% CH_4 (Group BG-3): a) final SCP concentration and accumulative effluent gas amount at the end of the batch run; b) change of MOB's growth and medium pH over time; c) change of cathodic off-gas content over time (* the results of day 0 represent the inflow gas content); d) change of system effluent gas content over time (in gas collecting bag and the headspace of SCP fermenter).

Table 2
Carbon conversion efficiency analysis on the BIES system feeding with different synthetic biogas including 100%CO_2 (Group BG-1), 50%CO_2/50%CH_4 (Group BG-2), 30%CO_2/70%CH_4 (Group BG-3), and 30%CO_2/70%CH_4 with a scaled-up fermenter.

Stage I: Biogas upgrading				Stage II: SCP fermentation				
Group	Generated CH_4 mL	Dissolved CO_2 mL	CO_2-to-CH_4 efficiency%	SCP yield mg·L^{-1}	Consumed CH_4 mL	Generated CO_2 mL	Dissolved CO_2 mL	CH_4-to-SCP efficiency%
BG-1	262.18 ± 21.28	95.39 ± 13.42	65.54 ± 5.32	136.32 ± 11.35	25.97 ± 2.16	12.46 ± 1.04	3.92 ± 0.72	9.90 ± 0.40
BG-2	160.27 ± 11.35	22.46 ± 3.44	80.13 ± 5.67	360.01 ± 17.58	68.58 ± 3.35	32.92 ± 1.61	2.48 ± 0.14	19.03 ± 0.22
BG-3	108.77 ± 6.56	4.58 ± 0.99	92.97 ± 5.61	472.04 ± 22.25	89.92 ± 4.24	43.16 ± 2.03	1.27 ± 0.03	22.89 ± 0.26
Scaled-up fermenter	106.35 ± 5.79	6.12 ± 1.44	90.90 ± 3.89	144.82 ± 9.47	275.88 ± 18.88	132.42 ± 8.20	89.54 ± 5.18	70.67 ± 2.37

Note: No TOC was found in the catholyte and the medium in SCP fermenter (protein filtered). Carbon balance analysis was summarized in Table S1.

oxidation could be a reason for its higher SCP production [39,40]. It was aware that the flammable gas mixture of CH_4 and O_2 might lead to safety issues. Thus, it is essential to keep the CH_4 content in the SCP fermenter out of the explosive range during the operation by controlling the gas supply process. Gas diffusion via hydrophobic hollow fiber membranes could also be a feasible solution to solubilize CH_4 in the liquid phase without compromising safety issues.

3.2. *Effect of H_2S in biogas on SCP production*

Fig. 3a exhibits the growth performance of MOB in the SCP-fermenter under three different H_2S concentrations. When the H_2S content in the biogas increased from 1000 (S-1) to 5000 ppm (S-2) with the inflow rate of 50 mL·d^{-1}, the growth of MOB was not significantly influenced, with the maximum OD_{600} of 1.17 ± 0.08 (S-1) and 1.14 ± 0.08 (S-2) respectively. The final SCP concentration was 458.46 ± 17.22

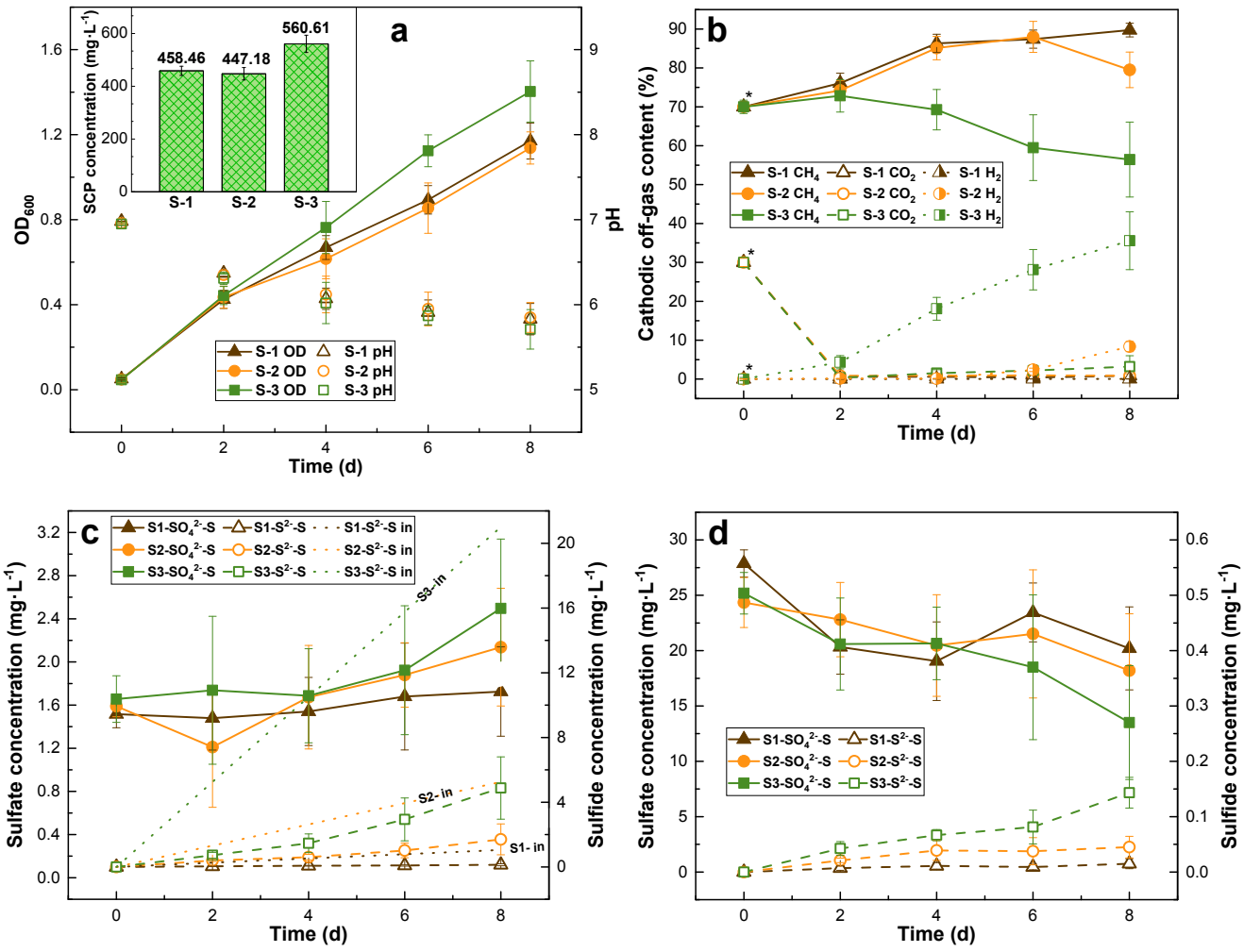

Fig. 3. . Effect of H_2S in synthetic biogas on BIES system performance in one batch, including 1000 ppm at the inflow rate of 50 mL·d^{-1} (Group S-1), 5000 ppm (Group S-2) at 50 mL·d^{-1}, and 5000 ppm at 200 mL·d^{-1} (Group S-3): a) final SCP concentration and the change of MOB's growth and medium pH over time; b) change of cathodic off-gas content over time (* the results of day 0 represent the inflow gas content); c) change of S^{2-} and SO_4^{2-} concentration in catholyte over time (the dotted line represents the theoretical H_2S dose in the catholyte, which was converted into the equivalent concentration of S^{2-}-S); d) change of S^{2-} and SO_4^{2-} concentration in the MOB medium over time.

(S-1) and 447.18 ± 23.55 mg·L^{-1} (S-2), both of which were slightly lower but close to the control group without H_2S (472.04 ± 22.25 mg·L^{-1}, BG-$3_{30\%CO2/70\%CH4}$). Thus, the biogas with the H_2S content lower than 5000 ppm is considered safe for BIES to produce SCP. However, it should be noted that the CH_4 content in the cathodic off-gas in Group S-2 decreased from 87.98 ± 3.98% on the 6th day to 79.50 ± 4.57% on the 8th day (Fig. 3b), which indicated that the bioconversion of "CO_2-to-CH_4" was slightly affected due to the accumulation of sulfide at the cathode.

Therefore, to further investigate the sulfide inhibition on system performance, the inflow rate of the biogas with 5000 ppm H_2S was lifted to 200 mL·d^{-1} (S-3). Obviously, CH_4 content in the cathodic off-gas in Group S-3 declined after the 4th day and dropped to 56.44 ± 9.63% at the end of the batch (Fig. 3b). Correspondingly, H_2 content gradually increased and reached 35.58 ± 7.45% on the 8th day, which proved the inhibition and toxicity of H_2S on biogas upgrading in the cathode. As reported, significant inhibition on SCP production was found if fed a methanotroph with raw biogas containing the H_2S content over 1000 ppm [21]. Unanticipatedly, the final SCP concentration in Group S-3 was not decreased but even higher than the other groups and achieved 560.61 ± 32.84 mg·L^{-1} (Fig. 3a). A maximum OD_{600} of 1.40 ± 0.14 was found at the end of the batch without any influence from the sulfide toxicity. It could be due to that the cathode was well buffered, and thus, intercepted the H_2S inhibition. H_2S content was found lower than the detection limit (<10 ppm, data not shown) in the cathodic off-gas. Thus, the SCP production was not influenced by the H_2S inflow rush. Considering that the biogas inflow rate of 200 mL·d^{-1} also contributed to four times higher accessibility of CH_4 toward MOB, the enhanced SCP production in Group S-3 was reasonable.

According to Fig. 3c, the sulfate concentration in the catholyte was not significantly changed during the batch in the three groups, except for a slight increase in Group S-3 at the end of the batch. The sulfide concentration in the catholyte showed an increasing trend in Group S-2 and S-3 but was far less than the theoretical H_2S dose amount. In Fig. 3d, the sulfate concentration in the MOB medium decreased during the batch, which could be related to the synthesis of protein [41,42]. Only a tiny amount of sulfide (<0.15 mg·L^{-1}) was detected in the SCP-fermenter, which was apparently lower than the sulfide input. Therefore, the two-stage BIES system could be a feasible solution toward the possible sulfide inhibition on SCP production observed in the conventional methanotrophic process using raw biogas. Sulfur balance analysis on the batch was summarized in Table S2. Around 55 ~ 72% of H_2S-derived sulfur was not in the forms of S^{2-} or SO_4^{2-} in the catholyte. The missing sulfide in the catholyte was speculated to be oxidized into sulfur and left in the cathode chamber, which could be the reason for the worse electromethanogensis performance in Group S-3. It was reported that some

chemolithoautotrophic sulfide-oxidizing bacteria (SOB, e.g., *Thiobacillus denitrificans* and *Candidatus Arcobacter sulfidicus*) could anaerobically oxidize sulfide to elemental sulfur together with CO_2 fixation via Reductive Tricarboxylic Acid Cycle [43–45]. Considering nitrate was not included in the catholyte, autotrophic-CO_2-assimilation-driven sulfide oxidation could be the possible sulfur metabolic pathway by SOB [46]. Afterward, the elemental sulfur was probably accumulated intracellularly in the AnGS, given that the sulfate concentration was not significantly altered during the batch [45].

3.3. Scaling-up of fermenter for higher SCP productivity

In the proof-of-concept experiment, though a comparatively high conversion efficiency of "CO_2-to-CH_4" was obtained in system Stage I, the SCP synthesis efficiency from CH_4 and O_2 in Stage II was still<25%, leaving the massive waste of unutilized CH_4 and O_2 in the gas effluent. In this case, a scaled-up SCP-fermenter with a working volume of 1 L was employed in the system for higher SCP productivity. As expected, the final SCP yield of 144.82 ± 9.47 mg (dry weight) was achieved at the end of the batch (shown in Fig. 4), which was three times higher than the yield from Group BG-$3_{30\%CO2/70\%CH4}$. Besides, the accumulative amount of CH_4 and O_2 in the effluent gas bag was 108.75 ± 5.32 and 110.14 ± 6.17 mL, respectively, with a 62.2% and 71.3% reduction compared with that from Group BG-3. Meanwhile, the "CH_4-to-SCP" efficiency in the scaled-up system was calculated as 70.67 ± 2.37% (shown in Table 2), which was significantly higher than Group BG-3. The better SCP yield could be attributed to less competition and higher accessibility of CH_4 per cell in the scaled-up fermenter with a longer CH_4 retention time in the medium for MOB assimilation.

3.4. Amino acid profile and microbiome composition analysis

Fig. 5 shows the amino acid profile of the SCP produced from the BIES system with the scaled-up fermenter (1 L). In general, the total amino acid content in the dry biomass (DW) was over 62.8%, which is comparable with conventional MOB studies [21,22]. The amino acid composition was balanced, covering most essential amino acids (EAAs, marked in red). The dominant amino acids (with a portion over 10%) were glutamine/glutamic acid, asparagine/aspartic acid, lysine, and alanine. The amount of EAAs was 217.11 $mg{\cdot}g^{-1}_{DW}$ on average. Two EAAs found at the highest relative content were lysine (10.51%) and histidine (6.46%). Lysine is responsible for producing protein, hormones, enzymes, and the absorption of calcium, which possesses the function of strengthening the immune system and the production of collagen and elastin [47–49]. Lysine has been utilized as an essential diet supplement in fishmeal and animal feed (e.g., swine and broiler) to optimize their growth performance and muscle development [50–52]. Histidine has unique roles in scavenging reactive oxygen and nitrogen species, erythropoiesis, and the histaminergic system [53]. The dietary supplementation of histidine has been demonstrated to be beneficial in clinical conditions because of its association with improved glucoregulatory outcomes and its potential for cancer therapy enhancement (e.g., pediatric blood cancers) [54,55].

Fig. 6 exhibits the community composition of the MOB consortium from the scaled-up SCP-fermenter. Results showed that over 90% of bacteria were represented by ten OTUs presenting with relative ambulance higher than 1%. The most abundant bacteria were characterized as *Methylomonas methanica,* represented by OTU1 (99.3% similarity) and OTU7 (99.6% similarity). *Methylomonas methanica* accounted for over 30% of the entire community. The previous study indicated that *Methylomonas methanica* is a type I methanotroph that mediates methane oxidation through soluble methane monooxygenase (sMMO) [56]. *Methylophilus* sp. was the second abundant bacteria (15.6%), with only one OTU representative. The observation was consistent with the previous MOB study [22], which suggested that *Methylomonas methanica* oxidizes methane, while *Methylophilus* sp. assimilates the accumulated metabolites (e.g., methanol). The dynamicity between *Methylomonas* sp. and *Methylophilus* sp. also contributes to the acclimation of the MOB consortium to the change of CH_4 source and cultivation media [57]. Besides the bacteria known for methane oxidation, *Terrimonas* sp. and *Rurimicrobium* sp. were observed with a high relative abundance. Although their functional roles have not been fully unveiled, their contribution to CH_4 oxidation can be suggested for their ubiquitous presence in CH_4 oxidation environments [58,59].

3.5. Implications and perspectives

The two-stage BIES system proposed in this study successfully realized upstream biogas upgrading (Stage I) and downstream biogas valorization to SCP (Stage II). Among other technologies, this BIES system has the following advantages. (1) Biogas upgrading enables the further application and valorization of biomethane into high-value

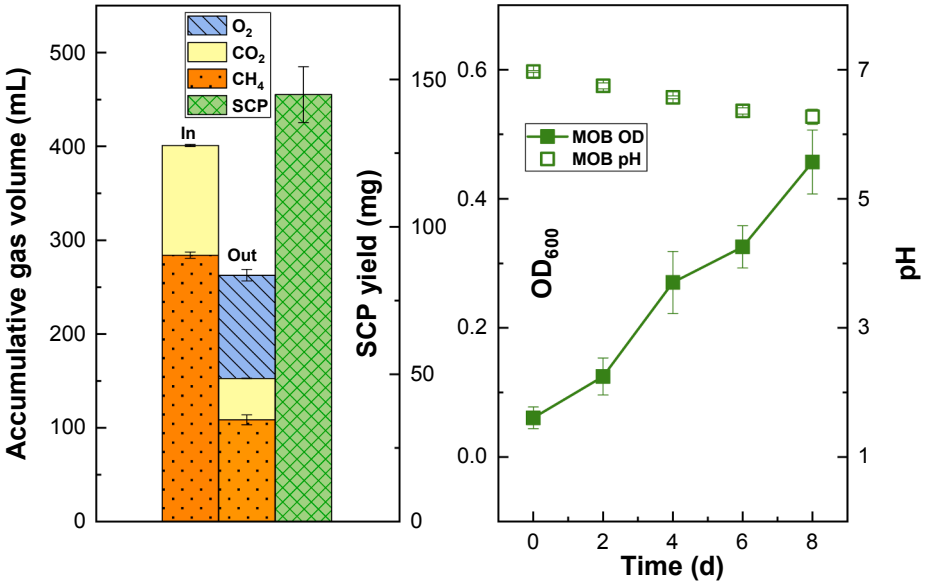

Fig. 4. . Final SCP yield and accumulative effluent gas amount from the upscaled fermenter (1 L) at the end of the batch, as well as the change of MOB's growth and medium pH during the batch.

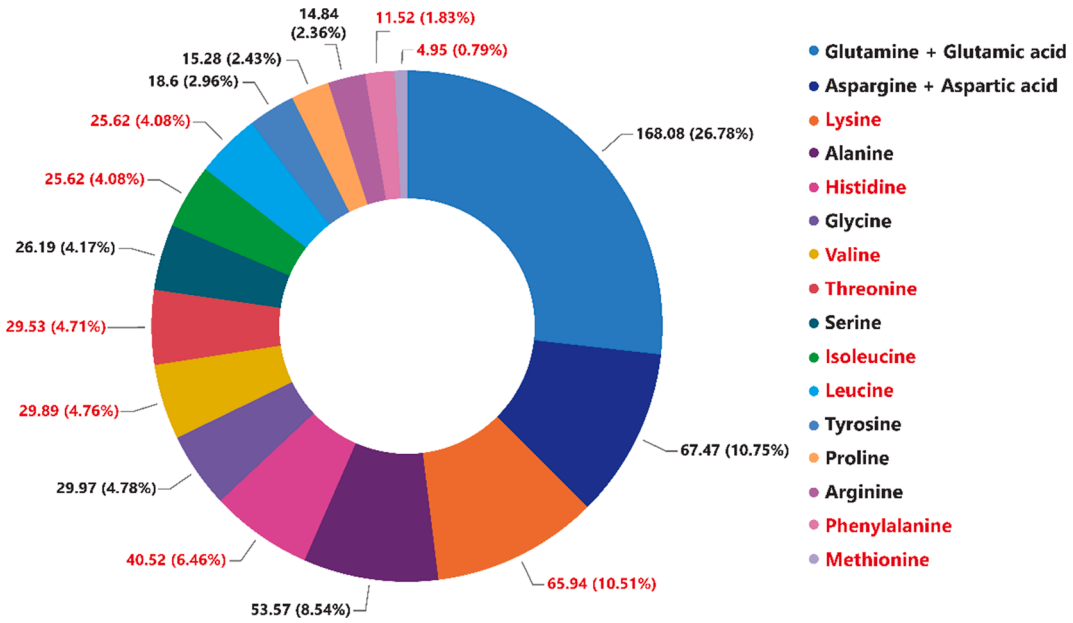

Fig. 5. . Amino acid profile of the SCP produced from the upscaled fermenter at the end of the batch (Unit: $mg \cdot g_{DW}^{-1}$).

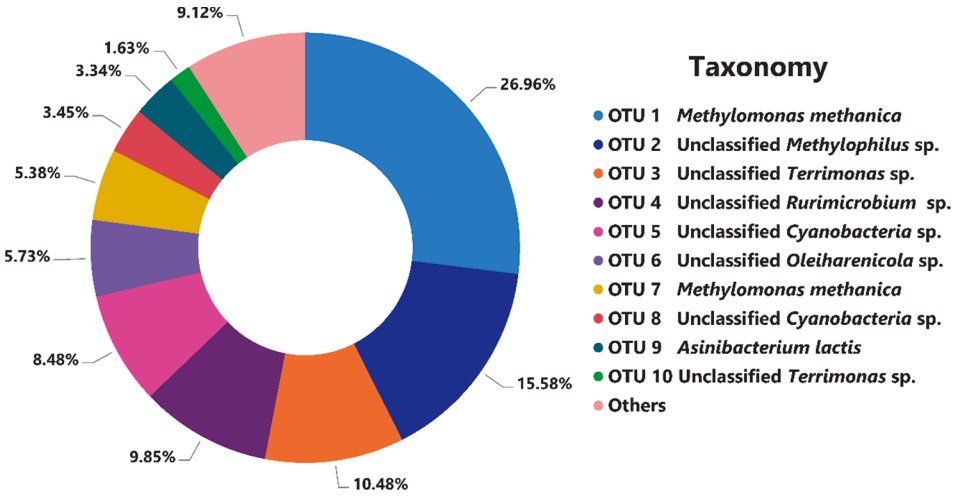

Fig. 6. . The community composition of MOB consortium.

products (e.g., SCP) instead of being used at the generation site. Compared with the conventional biogas upgrading technologies, the BIES can further convert the upgraded biogas into higher-value products (e.g., protein). Besides, the system is proved resilient to the toxic H_2S in biogas up to 5000 ppm, which meets the challenge of sulfide inhibition on the growth of MOBs when converting raw biogas into SCP [21]. (2) Compared with traditional agricultural proteins, using biogas for SCP production through BIES could offer a more sustainable and climate-friendly solution. For example, 7 kg N feedstock and 0.39 g pesticides are required to harvest 1 kg soybean protein, and meanwhile, 6.1 kg CO_2 will be emitted into the atmosphere during the cultivation [60,61]. In comparison, the proposed BIES can theoretically produce 1 kg SCP with 1.91 kg raw biogas (70%CH_4/30%CO_2) and 0.11 kg N input. (3) The electrochemical approaches for carbon capture and utilization and proteinaceous biomass production (up to 15.91 $mg_{protein} \cdot kJ^{-1}$) could be less energy-intensive than the CBB cycle (~6.89 $mg_{protein}$ kJ^{-1}) or Wood-Ljungdahl pathway (~10.98 $mg_{protein}$ kJ^{-1}) [62]. Besides, compared with other "power-to-protein" systems (e.g., the biosynthesis route of electrochemical "CO_2-acetate-yeast"), the proposed BIES system does not involve organic matters, preventing potential contamination by other heterotrophic bacteria, and therefore ensuring high purity of SCP product [63]. (4) Feeding BIES with biogas (e.g., 70%CH_4/30%CO_2) instead of pure CO_2 as the carbon source may need much less energy due to less H_2 requirement. Besides, around 3.5 times higher SCP concentration could be received because of higher CH_4 content in the cathode off-gas and more stable biomethane supply to the SCP fermenter.

Though promising, this technology is still in its infancy. Several challenges were found in this proof-of-concept study. Firstly, the final SCP concentration is still too low for practical uses. Essential optimizations on the SCP fermentation stage are necessary. It should be noted that the gas effluent from this process (after Stage II) contained 30 ~ 50% CH_4 and 45 ~ 60% O_2, which is not suitable for energy purposes (like biomethane) but an ideal gas feedstock for MOB's growth. Thus, two strategies are considered to simultaneously improve SCP production, enhance carbon conversion efficiency, and avoid the waste of gases: 1) optimization on SCP fermentation to achieve the carbon captured by the system ending in SCP to the maximum extent; 2) process control on gas generation (Stage I) and supply (Stage II) to meet the gas requirement for SCP production. Feasible solutions toward the first strategy include recirculating the effluent gas backward to feed MOBs,

operating the system at continuous mode, upscaling SCP fermenter size, and optimizing gas-liquid mass transfer efficiency of CH_4 (e.g., better stirring condition, employment of bubble-free membrane) [64]. For the second strategy, considering CH_4 (from the cathode) and O_2 (from the anode) were produced via two separated processes in Stage I and collected by two individual gas effluent tubes, it is possible to control the gas supply for Stage II and collect the excessive biomethane or O_2 as an independent byproduct before they are mixed and fed into the SCP fermenter. Besides, gas uplift velocity should be well controlled in future system optimizations.

Second, though the impact of H_2S on SCP yield was not notable in this study, high H_2S content in biogas did negatively affect the bioconversion of "CO_2-to-CH_4" in the BIES system. The sulfide accumulation at the cathode could still possibly challenge the system performance when running at continuous mode. Thus, an appropriate hydraulic retention time of the catholyte depending on the biogas inflow rate and H_2S content should be investigated further. Besides, the mechanism of H_2S inhibition on electromethanogensis performance and microbial sulfur metabolism in the cathode was still unclear. For example, the possible interspecies electron transfer (direct or indirect) between hydrogenotrophic methanogens and SOB could be another reason for the decrease of sulfide concentration. Thus, systematic research on the underlying bacterial interactions in the BIES cathode will be beneficial in solving the sulfide inhibition problem in the future.

4. Conclusion

In this proof-of-concept study, the combination of bio-electrochemical biogas upgrading and the following SCP production was achieved in a two-stage BIES system. The underlying dynamics and mechanisms of the two synergistic bioprocesses involved in the system were investigated. Higher CH_4 content in the biogas contributed to better "CO_2-to-CH_4" efficiency and SCP yield. The H_2S content within 5000 ppm in the biogas would not affect the SCP production. A scaled-up fermenter was proved to be beneficial for higher SCP yield and synthesis efficiency. This study offers insight into the development of viable and profitable biogas upgrading technology and an impact on the sustainable production of edible protein for a green transition and circular economy.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2021.131837.

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