Performance of cocultivation of Chlorella vulgaris and four different fungi in biogas slurry purification and biogas upgrading by induction of strigolactone (GR24) and endophytic bacteria

Yuan Ji
Yantai Academe of China Agricultural University
Luanbei Huang
Jiaxing Nanhu University
Zhengfang Wang
Suzhou Institute of Trade & Commerce
Jie Xu
Jiaxing University
Jing Wei
Jiaxing Nanhu University
Yongjun Zhao (zyjun2007@126.com)
Jiaxing University

Research Article

Keywords: Pelletization, Plant hormone, Symbiotic system, Photosynthetic performance, Removal efficiency

Posted Date: March 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2673020/v1

Abstract

This study aimed to determine the best fungi to form the algal-bacterial-fungi symbiotic system and identify the optimal conditions for the synchronous processing of biogas slurry and biogas. *Chlorella vulgaris* (*C. vulgaris*) and endophytic bacteria (S395-2) isolated from it and four different fungi (*Ganoderma lucidum, Pleurotus ostreatus, Pleurotus geesteranus, and Pleurotus corucopiae*) were used to form different symbiotic systems. Four different concentrations of GR24 were added to systems to examine the growth characteristics, the content of chlorophyll a (CHL-a), the activity of carbonic anhydrase (CA), the photosynthetic performance, the removal of nutrients, and the biogas purification performance. The results suggested that the growth rate, CA, CHL-a content, and photosynthetic performance of the *C. vulgaris*-endophytic bacteria-*Ganoderma lucidum* symbiosis were higher than the other three symbiotic systems when 10^{-9} M GR24 was added. The highest nutrients/CO_2 removal efficiency 78.36 ± 6.98% for chemical oxygen demand (COD), 81.63 ± 7.35% for total nitrogen (TN), 84.05 ± 7.16% for total phosphorus (TP) and 65.18 ± 6.12% for CO_2 was obtained under the above optimal conditions. This approach will provide a theoretical basis for the selection and optimization of an algal-bacterial-fungi symbiotic system for biogas slurry and biogas purification.

Introduction

Strigolactones (SLs), a class of plant secondary metabolites, were initially identified and isolated from the root secretions of plants such as cotton, sorghum, maize, and rice (Siame et al. 1993). Since natural SLs are rarely produced, they have been synthesized for plant signal transduction studies. Moreover, the synthetic SLs are primarily a series of witchweed alcohol analogs (germination releaser, GR), with GR24 having the highest biological activity effect. GR24 is often used in plant signal transduction of related research (Al-Babili &Bouwmeester 2015). Among the various biological functions of SLs, it promotes arbuscular mycelial branching and nutrient absorption (Al-Babili &Bouwmeester 2015), affecting plant morphology and symbiosis performance, which has drawn the attention of many researchers.

Many breakthrough achievements have been achieved recently in the research of algae treatment technology for biogas purification and slurry (Cao et al. 2017, Jiang et al. 2019, Wilson et al. 2014). However, technical bottlenecks in difficult extraction and low purification efficiency of single microalgae have prevented this technique from being widely used. How to remove this problem quickly is an urgent task. The biogas system of algal-fungal symbionts synchronized with biogas slurry can effectively overcome the above-mentioned problems associated with the treatment of single microalgae so that the microalgal and fungi symbiosis system can be widely promoted and applied. Although the algal-bacterial-fungi symbiosis system has broad application prospects, microalgae grow faster than fungi, and microalgae and fungi are not easy to form particles (Luo et al. 2019, Zamalloa et al. 2017). Consequently, spheres formed by microalgae and fungi are not stable and have unequal sizes. In general, the factors affecting the granulation of algae and fungi include stirring speed, the synchronous formation of algal cells and fungal mycelia, and charge on the surface of a sphere (Luo et al. 2019, Zamalloa et al. 2017). It is important but difficult to build algal symbionts with stable growth and long growth cycles. As

a highly potent phytohormone, GR24 can promote fungal mycelial branching and shorten the formation time of mycelial pellets. Moreover, the formed algal pellets have good homogeneity and strong stability. The algal-fungi symbiosis system induced with GR24 can be beneficial for biogas slurry and biogas treatment. Previous studies have shown that exogenous GR24 in the appropriate concentration range can significantly improve the photosynthetic efficiency of algal cells and effectively accelerate the formation of microalgal-fungal spheres.

The organic matter produced by algae through photosynthesis is utilized by fungi, while algae benefit from the winding and protection of fungal filaments, absorbing various nutrients needed for growth from the environment (Jiang et al. 2019). Studies have shown that in the process of symbiosis between microalgae and fungi, the sugar alcohols (ribitol, sorbitol, and erythritol) produced by several algal cells of the class Eucoccus of *Chlorophyta phylum* can be absorbed by fungi (A.Mukhin et al. 2018). Prior to absorption, the fungi produce specific signaling substances to promote the secretion of nutrients that can be absorbed (Dong et al. 2022). The symbiosis of microalgae and fungi achieved a survival advantage that could not be achieved individually, while SLs and analogists enhanced the symbiosis of algal cells and fungal hyphae. Studies have revealed that the biogas treatment effects of the symbiotic system formed by different algae and bacteria are significantly different. According to a previous study, the low content of GR24 stimulates the symbiotic system composed of *C. vulgaris*, *Ganoderma lucidum* (*G. lucidum*), and endophytic bacteria (Dong et al. 2022). The processing of biogas slurry and biogas was better than the symbiosis system composed of *Strigostrea-oyster mushroom*-endophytic bacteria. Cao et al. (Cao et al. 2017) used three different fungi associated with *C. vulgaris* to form a symbiotic system and simultaneously treated biogas slurry. The results revealed that the symbiotic system formed by *G. lucidum* assisted *C. vulgaris* had a higher removal rate of nutrients and CO_2 when simultaneously treated with biogas slurry and biogas. This might be because *G. lucidum* is more likely to form algal spheres with *C. vulgaris* and its specific surface area is larger when it has the same biomass as the other two symbionts, resulting in a better removal rate of nutrients and CO_2.

Furthermore, GR24 has been shown to significantly improve the number and length of fungal mycelia, enhance the production of lipids in fungi, and strengthen the life metabolic activities of fungi (Song et al. 2019). Hence, GR24 promotes the increase in the number and length of fungal mycelia, increasing the probability of mycelia wrapping around the algal cells. GR24 is also beneficial to pellet forming and plays a crucial and positive role. The improvement of pellet-forming performance and rapid growth of algal symbionts are key to efficiently removing the nutrients such as N, P, and so on. It is still unclear about the way for improving biogas and biogas slurry biological processing after adding GR24.

The present work selected *C. vulgaris*, endophytic bacteria, and four fungi namely *G. lucidum, Pleurotus ostreatus* (*P. ostreatus*), *Pleurotus geesteranus* (*P. geesteranus*) and *Pleurotus corucopiae* (*P. corucopiae*) to form different algal-fungual pellets as the research samples. Exogenous GR24 with different concentrations was utilized to examine the change characteristics of CHL-a content, growth, photosynthetic characteristics, and biogas upgrading effect of the algal symbionts. The change in the growth of algae-fungi symbionts, photosynthetic characteristics, CA activity, and biogas upgrading were

analyzed after adding different content of GR24. Finally, the optimal fungal species and GR24 concentration were determined. The development of this study may offer novel ideas and methodologies to the application of SLs in the biogas purification project of algae and bacteria symbionts, which has significant theoretical and application value.

Methods And Materials

Microalgae, endophytic bacteria, and fungi

This work obtained C. vulgaris (FACHB-8) in Freshwater Algae Culture Collection at the Institute of Hydrobiology (Wuhan, China) and cultured it within BG-11 medium under constant cool-white LED light and 200 μmol m^{-2} s^{-1} photosynthetic photon flux density conditions. The light-dark ratio is 12 h:12 h, and the flask is shaken manually 3 times a day (Zhao et al. 2015). The four fungi, G. lucidum (5.765), P. ostreatus (5.705), P. corucopiae (NAECC0457) and P. geesteranus (Px-02) were used in this experiment.

This work obtained P. ostreatus and G. lucidum from China General Microbiological Culture Collection Center, while P. geesteranus and P. corucopiae were cultivated by our research team. The preparation method of culture medium was based on previous literature (Wang et al. 2017).

This study cultured inoculum within the shaking flask for seven days with the cultivated temperature set at 25 ± 1 °C and stirring at a rate of 150 rpm. The cultures were washed and then dispersed using sterile distilled water (100 mL), with those resulting fungal strains being used in subsequent symbiosis with microalgae.

Using the previous method, the endophytic bacterial strain (S395–2) was isolated in C. vulgaris (Xu et al. 2021). Subsequently, using Luria Bertani medium, the bacteria produced by algae-fungi pellets was co-culture, and the cultivated temperature was set to 37 ±1 °C.

Formation and domestication of algae-fungi microspheres

Firstly, the C. vulgaris cells at the logarithmic growth period and four fungi pellets (G. lucidum, P. ostreatus, P. geesteranus, and P. corucopiae) were washed using BG11 medium thrice. Then, C. vulgaris pellets were co-cultured with four fungi cells in the BG11 medium (150 mL) to form algae-fungi pellets, respectively.

The co-culture system of C. vulgaris, endophytic bacteria and G. lucidum was labeled as Treatment 1. Symbiotic system obtained by the symbiosis of C. vulgaris pellets and P. ostreatus cells with endophytic bacteria was labeled as Treatment 2. Treatment 3 refers to the symbiotic system of C. vulgaris pellets and P. geesteranus cells with endophytic bacteria. Furthermore, the symbiotic system of C. vulgaris pellets and P. corucopiae cells with endophytic bacteria was considered as Treatment 4. A total of 2.0×10^7 cells mL^{-1} endophytic bacteria were used in the symbiotic system. The concentration of GR24 added to the treatment systems was 0, 10^{-11}, 10^{-9}, and 10^{-7} M, separately.

Initial dry weight biomass of four symbionts was 107.68 ± 8.59 mg L^{-1}. The four symbiotic systems were grown under 25 ±1 °C, and the stirring speed was 160 r/min. Firstly, 100 mL C. vulgaris suspension containing 88.26 ± 5.32 mg L^{-1} dry weight was prepared. Secondly, the C. vulgaris suspension and four prepared fungal pellet suspension (G. lucidum, P. ostreatus, P. geesteranus, and P. corucopiae) were mixed separately to form four algae-fungi symbiotic systems. The dry weights of the four fungal suspensions used were 49.36 ± 2.85, 52.37 ± 4.28, 50.19 ± 3.77, 53.08 ± 4.32 mg L^{-1}. The initial content of C. vulgaris used for co-culture with fungal cells was 118.95 ± 8.53 mg L^{-1}. The biogas slurry was first diluted to 10%, and then the algae cells were placed in it for gradual domestication. After the fungal microalgae cells tolerated the biogas slurry and grew rapidly, the obtained high biomass yield was compared with the original strain. This work kept those cultured strains to co-culture with microalgae cells.

Photobioreactor

This reactor included 2 glass bottles, each of 16.8 L in volume (Cao et al. 2017). Before processing, biogas slurry (2.8 L) was added to one tank and biogas (14 L) to another. At the same time, four strains of symbiotic treatment and a certain amount of GR24 were added to the glass tank on the left side of the reactor respectively, and the simultaneous purification treatment experiments were carried out for biogas and biogas slurry using these four algae technologies. The purified biogas slurry was collected from the outlet of the left glass tank; the biogas after quality improvement was collected from the biogas outlet of the right glass tank of the reactor. All inlet and sampling ports were sealed with rubber plugs during the experimental treatment. Both blue and red lights were used in processing biogas slurry and biogas with the 225 μmol m^{-2} s^{-1} intensity. During this whole treatment cycle, the intensity ratio remained 5:5.

Biogas and biogas slurry

This study obtained biogas slurry in the anaerobic digestion reactor from one pig farm in Jiaxing, Zhejiang (China), and put under 4 °C for standby immediately after taking it back. Physicochemical features of the filtrate after sterilization were shown below: pH, 6.78 ± 0.46; chemical oxygen demand (COD), 1256.38 ± 22.14 mg L^{-1}; total phosphorus (TP), 15.43 ± 2.09 mg L^{-1}; total organic carbon (TOC), 1006.27 ± 19.54 mg L^{-1}; total nitrogen (TN), 118.95 ± 6.27 mg L^{-1}; total suspended solids (SS), 1065.02 ± 41.25 mg L^{-1}.

Meanwhile, we obtained biogas in one biogas plant from JiaYuan green meadow. Before treatment, this work passed biogas through an absorption reactor for desulfurizing and ensuring that the content of H_2S did not exceed 100 mL L^{-1}. Four component contents (v/v) in biogas comprised CH_4, CO_2, O_2, and H_2O at 62.37 ± 4.58%, 34.28 ± 1.76%, 0.34 ± 0.03%, and 3.01 ± 0.24%, separately.

Treatment

Four different GR24 contents (0, 10^{-11}, 10^{-9}, 10^{-7} M) were added to the four algal-bacterial-fungi symbiotic systems. Furthermore, the biogas purification of biogas slurry was performed to explore the

growth performance and photosynthetic performance of the four algal-bacterial-fungi spheres. The intensity of red light and blue light was maintained at 225 μmol m^{-2} s^{-1} with the 12-h/12-h L/D cycle for a 10-day period under 25 ± 2 °C. The entire processing time was 10 days.

Analytical methods and definitions

Estimation of four symbiont growth

In these four treatment systems, the mean daily yield (P, g L^{-1} d^{-1}) and growth rate (d^{-1}) of symbionts was obtained according to Formula (1) (Zhao et al. 2019):

$$P = (DW_k - DW_0)/(t_k - t_0) \qquad (1)$$

Where, DW_k and DW_0 are biomass concentrations under t_k (g L^{-1}) and t_0 (g L^{-1}), separately.

The concentration analysis of Chlorophyll a (CHL-a)

The CHL-a contents within four treatment systems were analyzed and determined based on published literature (Shen et al. 2020). Firstly, C. vulgaris suspension (10 mL) was absorbed, followed by 10-min centrifugation at 10000 g. Secondly, the algae was washed thrice using distilled water. Then, this slurry was dispersed into 10 mL aqueous acetone solution for extraction of photosynthetic pigment of CHL-a. With aqueous acetone solution being blank control, absorbance of supernatant was determined with spectrophotometry at 4 wavelengths (630, 645, 663, and 750 nm), respectively. The content of CHL-a was obtained using Formula (2):

$$A_{CHL\text{-}a} = [11.64 \times (A_{663} - A_{750}) - 2.16 \times (A_{645} - A_{750}) + 0.10 \times (A_{630} - A_{750})]\, V \qquad (2)$$

In the formula, $A_{CHL\text{-}a}$ indicates chlorophyll a content (μg L^{-1}); A_{663}, A_{750}, A_{645}, and A_{630} were the absorbance of the corresponding wavelength; V was the sample volume (L) (Ji et al. 2018a, Ji et al. 2018b).

Activity analysis of intracellular carbonic anhydrase (CA)

CA is often used to assess the ability of microalgae to absorb CO_2 through photosynthesis. The activity of CA was analyzed using the electricity consumption method (Chinnasamy et al. 2009). Frist, this work pipetted algae treatment solution (10 mL) and centrifuged it for 15 min (10,000 × g). Then, the lower layer of centrifuged sediment was washed 3-4 times using distilled water. Finally, it was dispersed into 4 mL of barbituric acid buffer (2×10^{-2} M, pH=8.3). The diluted dispersion (1.0 mL) was mixed with pre-chilled barbituric acid buffer (8 mL). Then, 4.0 mL of precooled saturated CO_2 solution was supplemented with this solution.

Finally, the time required for changing the pH = 8.3 to pH = 6.3 at 0-4 °C was determined using Formula (3) to calculate the value of enzyme activity (EU).

$EU = 10\times[(t_0/t)-1]$ (3)

Where, t_0 refers to the time spent by algae without bacteria; t is the time required by each algae treatment group.

Determination of photosynthetic performance parameters

To analyze how GR24 content affected C. vulgaris photosynthesis and assess C. vulgaris' photosynthetic efficiency of four diverse symbiotic systems, the co-culture liquid of algae-fungi on the 10th day following biogas and biogas slurry processing via four symbiotic systems was analyzed. Firstly, 2 mL of algal-fungi solution on the 10th day of the experiment of different algal treatment systems was pipetted out and kept away from light for a 25-min period under 25 °C. Next, Aquapen handheld chlorophyll fluorescence meter (OJIP Open-JIP Test) was used for testing change in chlorophyll fluorescence. Moreover, the OJIP analysis was used to acquire several photosynthetic performance variables (PI_{ABS}, F_V/F_M, Ψ_O, Φ_{Do}, Φ_{EO}).

Nutrient removal rate analysis and biogas upgrade

The COD, TN, and TP concentrations in biogas slurry were analyzed according to the methods described in the previous work (APHA 1995). CO_2, CH_4, O_2 and H_2O concentrations within the treated biogas were set by gas chromatography (TSQ 9000). Then, COD, CO_2, TN and TP removal rates were calculated according to Equation (4):

$RE = (1-C_i/C_0) \times 100$ (4)

In the formula, RE was removal rate, %; C_0 represented the initial content of pollutants or CO_2 (mg L^{-1}); C_i was the final content of pollutants or CO_2 (mg L^{-1}).

Statistical analyses

Each experimental treatment group was repeated thrice in parallel. All the experimental data in this experiment were subject to statistical analysis with SPSS19.0. This study employed Duncan's multiple comparison method to test the significant differences in the effects of GR24 addition amount on algal growth, photosynthetic parameters, biogas slurry, and biogas purification effect among the three algal technologies. $p<0.05$ stood for statistical significance.

Results And Discussion

The growth of four algae-fungi pellets at different content of GR24

The key indicators of fungi-assisted algal particle growth primarily include average daily output, growth rate, and CHL-a levels. Average daily outputs and growth rates of four symbiotic systems domesticated for ten days were examined by adding different GR24 concentrations to the symbiotic systems. The experimental results are presented in Table 1. Furthermore, the average daily output and growth rate

induced with GR24 remarkably increased in comparison with control ($P<0.05$; Table 1). Among each GR24 concentration treatments, Treatment 1 exhibited the greatest average daily output and growth rate. Mean daily output and growth rates of all four treatments peaked after adding GR24 (10^{-9} M). The above conclusion showed that SL (GR24) had a positive effect on microalgal growth (Zhang et al. 2021a, Zhang et al. 2021b, Zhao et al. 2019). Previous studies have shown that GR24 could upregulate the cell division-related genes due to the interference of auxin and other hormones, hence, promoting tooth root elongation (Arite et al. 2011, Hu et al. 2018). Moreover, Song et al. found a low concentration of GR24 was beneficial for the growth of Monoraphidium sp. QLY-1 (Song et al. 2019). We found that, GR24 promoted growth of algae-fungi symbiosis and provided maximal biomass as well as growth rate, conforming to the previous report (Shen et al. 2020). The results suggested remarkably elevated C. vulgaris growth with the increase of the content of GR24 in the system between 10^{-9} and 10^{-7} M. Nonetheless, our results were different from prior works (Song et al. 2019), which showed that GR24 did not significantly improve the growth rate of Monoraphidium sp. Also, the conclusion of the present study was different from other research (Song et al. 2019), which showed that the biomass content of Monoraphidium sp. did not alter when GR24 was induced.

[Table 1]

In general, the microalgae biomass increased as CHL-a content elevated. In the study, CHL-a isolated in four symbiotic systems was analyzed in a 10-day period after simultaneous purification and upgrading of biogas slurry and biogas. Figure 1 displays how GR24 affects CHL-a in four different systems. The CHL-a contents of four symbiotic systems increased in comparison with blank control (Figure 1). When GR24 concentrations were 0, 10^{-11}, 10^{-9}, and 10^{-7} M, CHL-a levels in four symbiotic systems showed the same change trend, increasing first and then decreasing. Furthermore, CHL-a contents among four treatment systems could reach the peak if induced with GR24 (10^{-9} M). After adding GR24 (10^{-9} M), CHL-a level reached 261.72 ± 22.05, 186.67 ± 16.74, 243.16 ± 21.39, and 172.09 ± 14.28 μg L^{-1} under four treatments on day 7, respectively. Treatment 1 had the greatest content of CHL-a, indicating that the symbiotic system composed of C. vulgaris, endophytic bacteria and G. lucidum (Treatment 1) had highest generation and CHL-a level. Hence, the appropriate GR24 level can increase CHL-a content. Previous studies demonstrated the highest CHL-a level within a symbiotic system after adding GR24 (10^{-9}–10^{-7} M) (Shen et al. 2020, Xu et al. 2021, Zhang et al. 2021a).

[Figure 1]

According to the above analysis and conclusions, the growth of algal-fungal symbionts depends not only on the growth environment but also on the type of algae and fungi selected after adding GR24 (Ling et al. 2020, Min et al. 2019, Sharifi &Bidabadi 2020). By analyzing and comparing the data of CHL-a on four symbiotic systems, Treatment 1 growth (C. vulgaris-G. lucidum-endophytic bacteria symbionts) was superior to other three treatment systems. According to the results, microalgae growth rate and average daily output of symbionts will vary depending on the fungi added.

The intracellular carbonic anhydrase activity of the four treatments induced with different content of GR24

Carbonic anhydrase (CA) represents the vital enzyme function to enrich carbon in microalgae. CA rapidly converts CO_2 to HCO_3^- with the energy generated from photosynthesis at a speed of up to 10^6 s^{-1} (Jun et al. 2020). Increased CA activity indicates that algae fixation efficiency for CO_2 is further enhanced (García Lobo et al. 2019, Teodoro et al. 2019). In experimental process of synchronous biogas slurry and biogas treatment, the CA activity data of the four treatments are depicted in Figure 2.

[Figure 2]

According to the bar charts of the four systems in Figure 2, the CA activities of the four symbiotic systems showed the same trend. The activity increased when added to GR24 (0 M-10^{-9} M) and then decreased after adding GR24 (10^{-9} M-10^{-7} M) concentration. On day 7 of the experiment, the CA activity of the four symbiosis systems reached the peak after adding 10^{-9} M of GR24 in the following order: Treatment 1 >Treatment 3 >Treatment 2 >Treatment 4. CA activity under Treatment 1 posed the maximum added 10^{-9} M GR24. CA activity was optimized under Treatment 1 at 10^{-9} M GR24, conforming to previous studies (Dong et al. 2022). This may be because GR24 (10^{-9} to 10^{-7} M) enhanced the growth of algal symbionts by improving the photosynthesis of C. vulgaris, which could thus increase the fixation efficiency of CO_2.

The photosynthetic characteristics of the four algae-fungi symbioses under different GR24 content

Open-JIP (OJIP) test was adopted for detecting the photosynthetic characteristics of C. vulgaris in four treatment systems (Bates et al. 2019). PI_{ABS} is a performance measure of energy conservation from the absorption of photons by PSII to the reduction of electron acceptors between systems.

F_V is the maximum fluorescence variable, and F_M is the maximum fluorescence intensity recorded (Strasser &Tsimilli-Michael 2004). In the four treatment systems, the values of PI_{ABS} and F_V/F_M elevated as GR24 content elevated (Table 2), followed by a decreasing trend. These findings suggested that increasing the GR24 content in the algal-fungi symbiosis system could promote the photo-promoting effect of PSII. However, the results conformed to the other work (Sun et al. 2020), which proved that atrazine prevented the PSII in microalgae instead of promoting it. The reason for these two different conclusions may be that the suitable content of GR24 was beneficial for the C. vulgaris growth, and it also stimulated photosynthetic ability, whereas microalgae photosynthetic characteristics decreased in toxicological study. Q_A is an electron acceptor of primary quinone and PSII. Ψ_O represents the probability of further travel distance of electron than Q_A, and Φ_{EO} indicates electron transport quantum yield (Strasser &Tsimilli-Michael 2004). These two parameters (Ψ_O and Φ_{EO}) were used to explore how GR24 affected C. vulgaris photosynthetic ability (Table 2). As the content of GR24 increased, the Ψ_O and Φ_{EO} variation trends conformed to F_V/F_M and PI_{ABS}, indicating that light absorption of C. vulgaris was

enhanced by GR24. Moreover, the electron transfer rate and maximum electron transport yield increased accordingly. The photosynthetic performance parameters of the four treatment systems could reach the highest value if induced by 10^{-9} M of GR24. The order of photosynthetic C. vulgaris parameters (Table 2) was as follows: Treatment 1 >Treatment 3 >Treatment 2 >Treatment 4. Among the four systems, there existed obvious differences among photosynthetic performance parameters ($p < 0.05$).

[Table 2]

The above analysis and conclusion showed that Treatment 1 was an ideal algal treatment system. Therefore, adding an appropriate amount of GR24 can stimulate the photosynthesis of microalgae. In the symbiotic system, the photosynthetic performance parameters Ψ_O, Φ_{EO}, F_V/F_M, and PI_{ABS} showed the same changing trend with the increasing concentration of GR24 because GR24 promoted the light absorption of microalgae and subsequently increased the electron transfer productivity and the maximum electron transfer output. From the results of the OJIP test, it can be observed that the variation trend of photosynthetic performance parameters was consistent with the change of CHL-a content (Figure 2), as CHL-a is an essential substance for algae to absorb light in the photosynthesis process (Ramanna et al. 2017).

Removal efficiencies of nutrient-induced different content of GR24 for the four algae-fungi symbiosis system

Generally, the purification impact of biogas and biogas slurry was analyzed by removal rate of COD (RE-COD), TP (RE-TP) and TN (RE-TN). The four treatment systems simultaneously treated biogas and biogas slurry according to induction of four different concentrations of GR24. The relevant data for RE-COD, RE-TP and RE-TN are shown in Table 3. Following is the order of the effects of the four symbiosis systems: Treatment 1 >Treatment 3 >Treatment 2 >Treatment 4, therefore, Treatment 1 had optimal nutrient removal efficiency. Adding GR24 into algal-fungal symbiosis system for stimulating its biogas and biogas slurry purification had been found to have positive effects on removing nutrients such as N and P and COD of algal cells.

[Table 3]

Figure 2 represents nutrients and CO_2 removal rates under Treatment 1 at 3d, 7d, and 10d under different concentrations of GR24. By adding GR24 (10^{-9} M), average RE-COD under Treatment 1 was the highest (78.36 ± 6.98%). From the average RE-COD in Table 3, it was clear that the removal effect of Treatment 1 was the best among the four treatment systems (Table 3). RE-COD under Treatment 1 increased from 3d to 7d and then decreased (Figure 3a). The changing trend of RE-COD of biogas and biogas slurry was consistent with one published article (Xu et al. 2021). Yang et al. confirmed that algae-fungi co-culture system showed a superior RE-COD than the monoculture system (algae or fungi), and the RE-COD of molasses wastewater reached 70.68% (Yang et al. 2019). Compared with the monoculture system, the biogas slurry treatment co-culture system based on microalgae showed a significant removal effect on ammonia nitrogen and organic nitrogen (Leng et al. 2021).

[Figure 3]

According to the experimental data (Table 3), the average RE-TN of Treatment 1 was the highest among the four treatment systems. The changing trend of average RE-TN in Treatment 1 was the same as the average RE-COD (Figure 3a). On day 7 during experimental processing, at GR24 content of 10^{-9} M, the RE-TN removal rate was the maximum (81.63 ± 7.35%) (Figure 3b).

TP removal rate is a key index for measuring the removal rate of nutrient algal-fungi symbiosis. Studies have shown that phosphorus in biogas slurry is a crucial element to synthesize phospholipids, nucleic acids, proteins, as well as lipids, which can be absorbed, then converted to organic matter by Chlorella within aerobic surroundings (Cai et al. 2013, Zhu et al. 2019). The surroundings in the purification system were shown to be more conducive to phosphorus absorption and removal at 10^{-9} M exogenous GR24 (Shen et al. 2020). RE-TP under Treatment 1 was highest among the four treatment systems (Table 3). The average RE-TP of Treatment 1 was 84.05 ±7.16% when added with 10^{-9} M GR24, which was slightly higher than that of Treatment 3 (82.13 ± 7.25%), Treatment 2 (80.52 ± 7.19%), and Treatment 4 (77.39 ± 7.25%). The trend of RE-TP first increased and then decreased with time, which was the same as that of RE-COD and RE-TN (Figure 3c). On day 7 during experiment, when supplemented with 10^{-9} M of GR24, RE-TP was the highest (85.24 ± 8.15%).

Treatment 1 was the optimal co-culture system in this study because it exerted the best removal impact on several traditional nutrients in biogas slurry. Induced with 10^{-9} M exogenous GR24, it had a positive impact on the algal-fungi system metabolism, enhancing microalgae photosynthesis in symbiont system and improving the metabolism of algal symbionts and microalgae photosynthesis. Therefore, algal-fungal symbionts growth rate was accelerated, and the purification of effect was improved.

In Treatment 1, due to endophytic bacterial metabolism as well as combined influence of microalgae with endophytic bacteria on pollutant removal, the algal-bacterial-fungi symbiosis culture system had increased metabolic activity compared with microalgae mono-culture system and fungi-microalgae culture system (to some extent), making the nutrient removal efficiency of the biogas slurry more remarkable. Maybe, endophytic bacteria complementarity and co-metabolism are most evident in providing CO_2 as well as growth-promoting materials to host microalgae and synthesizing photosynthetic enzymes in host microalgae (Wang et al. 2015).

Biogas upgrading under different content of GR24 for the four different algae-fungi symbiotic system

CH_4 content in biogas is a vital indicator for evaluating the quality of biogas (Mesbah et al. 2019). Biogas microalgae purification technology employs microalgae to convert excess CO_2 in biogas into solid organic matter containing carbon and remove it, which indirectly increases the concentration of CH_4 (Zhao et al. 2019). Hence, the carbon fixation efficiency of microalgae is the key to microalgae purification technology (Kim et al. 2013). The RE-CO_2 of crude biogas was adopted for measuring the capability of biogas purification using algal technology. The four treatment systems were induced with

different concentrations of GR24, and the RE-CO_2 (Table 3) of biogas was in the following order: Treatment 1 >Treatment 3 >Treatment 2 >Treatment 4. RE-CO_2 values for four treatment systems were different ($p < 0.05$). The experimental data analysis conformed to other nutrient removal effects. Figure 3d illustrates influence of Treatment 1 on the CO_2 removal rate at diverse GR24 doses. The RE-CO_2 changing trend was the same as that of other nutrients discussed above (Figure 3d). Furthermore, the maximum value could be found on the 7th day of the treatment, which was the same as the change in growth performance and photosynthetic performance of the system. The RE-CO_2 in Treatment 1 was the highest (66.57 ± 6.34%) when added with 10^{-9} M of GR24, with the average removal efficiency being 65.18 ± 6.12% at the end of the treatment (Table 3).

Experimental results of biogas purification in Treatment 1 induced by different GR24 concentrations are presented in Figure 4. The biogas upgrading efficiency of CH_4 in Treatment 1 (Figure 4a), the growth of the algal-bacterial-fungi symbiosis system (Table 1), the photosynthetic capacity (Figure 1, Table 2), and the changes in microalgal CA activity (Figure 2) were consistent with the changes in nutrient removal efficiency of biogas slurry (Figures 4a-4c). In other words, the faster the microalgae grow, the higher the CHL-a content in the system. The RE-CO_2 is proportional to the CA activity in biogas. This is because about 50% of the dry weight of microalgae comes from CO_2 consumed for photosynthesis. On the other hand, the concentration of impurities such as CH_4 in biogas is also a key index for evaluating biogas (Mesbah et al. 2019). On days 3, 7 and 10 during treatment experiment (Treatment 1), CH_4, CO_2, H_2O, and O_2 concentrations in biogas changed according to the change in concentration GR24 (Figure 4). The results confirmed Treatment 1 with highest improvement in biogas quality triggered using 10^{-9} M of GR24. On day 7 during treatment, when Treatment 1 was triggered using 10^{-9} M of GR24, the concentrations of CH_4 (85.19 ± 8.23%) and O_2 (0.40 ± 0.04%) reached the maximum. Moreover, the H_2O and CO_2 concentrations reached the minimum values of 2.95 ± 0.17% and 10.94 ± 9.95%, respectively. Also, biogas was saturated with biogas slurry. Besides CO_2, H_2O is also an indispensable component during microalgae synthesis (Yan &Zheng 2014). As a result, H_2O in biogas has little effect on the formation of algae-fungi symbiotic particles, the biogas upgrading and nutrient removal (Zhao et al. 2019). Previous research showed that the combustion performance of biogas could be improved by appropriately increasing the O_2 content in biogas (Papadias et al. 2012). With consideration of the absolute content, O_2 in the biogas before and after purification is relatively low, along with the possibility of CH_4 and O_2 forming explosive mixtures within this concentration (Ryckebosch et al. 2011), so this technology offers high security and operability.

[Figure 4]

Conclusions

In this study, the synchronous nutrients and CO_2 removal within biogas slurry, and biogas upgrading by four different algal-bacterial-fungi symbiosis co-culture systems was studied by changing the

concentration of GR24. The addition of GR24 significantly increased the symbiont growth and biomass within 4 co-culture systems, along with CO_2 and nutrient removal rates. Adding GR24 was found to enhance the metabolism and photosynthesis of microalgae in the four symbiotic systems, and achieved rapid pelletization of algal-bacterial-fungi symbiosis, and improved biogas upgrading and biogas slurry purification of each system. *C. vulgaris*-*G. lucidum*-endophytic bacteria symbionts system produced optimal results in all the experimental groups. Moreover, the present study may offer novel ideas and methodologies for the application of SLs using algal symbionts. In addition, this study can also provide novel perspectives and methods for the engineering of the biogas upgrading and biogas slurry purification via an algal-fungal symbiosis system.

Declarations

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Yuan Ji: Study, Data curation, Writing-original draft, Writing-review & Editing. **Luanbei Huang:** Methodology & Conceptualization. **Zhengfang Wang**: Formal analysis. **Jie Xu:** Methodology, Data curation, **Jing Wei:** Conceptualization. **Yongjun Zhao**: Supervision, Funding acquisition & Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or

personal relationships that could have appeared to influence the work reported in this

paper.

Data availability

Data will be made available on request.

Acknowledgments

This research was was supported by“National Natural Science Foundation of China” (Grant numbers 31971514, 31670511) and the “Project of Jiaxing Science and Technology Bureau” (Grant number 2020AY10007).

References

1. A.Mukhin V, Neustroeva NV, Patova EN et al (2018). Lichen-like Symbiotic Associations of Wood-decaying Fungi and Algae. I. Biodiversity and Ecology of Photobionts. The fourth International Scientific Conference on Ecology and Geography of Plants and Plant Communities, KnE Life Sciences, 9
2. Al-Babili S, Bouwmeester HJ (2015). Strigolactones, a Novel Carotenoid-Derived Plant Hormone. Annual Review of Plant Biology 66, 161-186
3. APHA (1995). Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.
4. Arite T, Kameoka H, Kyozuka J (2011). Strigolactone Positively Controls Crown Root Elongation in Rice. Journal of Plant Growth Regulation 31, 165-172
5. Bates H, Zavafer A, Szabó M et al (2019). A guide to Open-JIP, a low-cost open-source chlorophyll fluorometer. Photosynthesis Research 142, 361-368
6. Cai T, Park SY, Li Y (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renewable and Sustainable Energy Reviews 19, 360-369
7. Cao W, Wang X, Sun S et al (2017). Simultaneously upgrading biogas and purifying biogas slurry using cocultivation of Chlorella vulgaris and three different fungi under various mixed light wavelength and photoperiods. Bioresource technology 241, 701-709
8. Chinnasamy S, Ramakrishnan B, Bhatnagar A et al (2009). Biomass Production Potential of a Wastewater Alga Chlorella vulgaris ARC 1 under Elevated Levels of CO_2 and Temperature. International Journal of Molecular Sciences 10, 518-532
9. Dong X, Wei J, Huang J et al (2022). Performance of different microalgae-fungi-bacteria co-culture technologies in photosynthetic and removal performance in response to various GR24 concentrations. Bioresource technology 347, 126428
10. García Lobo JM, Ortiz Y, Gonzalez-Riancho C et al (2019). Polymorphisms in Brucella Carbonic Anhydrase II Mediate CO_2 Dependence and Fitness in vivo. Frontiers in Microbiology 10, 2751
11. Hu Q, Zhang S, Huang B (2018). Strigolactones and interaction with auxin regulating root elongation in tall fescue under different temperature regimes. Plant Science 271, 34-39
12. Ji X, Cheng J, Gong D et al (2018a). The effect of NaCl stress on photosynthetic efficiency and lipid production in freshwater microalga-Scenedesmus obliquus XJ002. The Science of the total environment 633, 593-599
13. Ji X, Jiang M, Zhang J et al (2018b). The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater. Bioresource technology 247, 44-50
14. Jiang M, Li H, Zhou Y et al(2019). The interactions of an algae–fungi symbiotic system influence nutrient removal from synthetic wastewater. Journal of Chemical Technology & Biotechnology 94, 3993-3999
15. Jun S-H, Yang J, Jeon H et al (2020). Stabilized and Immobilized Carbonic Anhydrase on Electrospun Nanofibers for Enzymatic CO_2 Conversion and Utilization in Expedited Microalgal Growth.

Environmental Science & Technology 54, 1223-1231

16. Kim T-H, Lee Y, Han S-H et al (2013). The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using Scenedesmus sp. for wastewater treatment. Bioresource technology 130, 75-80
17. Leng L, Li W, Chen J et al (2021). Co-culture of fungi-microalgae consortium for wastewater treatment: A review. Bioresource technology 330, 125008
18. Ling F, Su Q, Jiang H et al (2020). Effects of strigolactone on photosynthetic and physiological characteristics in salt-stressed rice seedlings. Scientific reports 10(1), 6183
19. Luo S, Wu X, Jiang H et al (2019). Edible fungi-assisted harvesting system for efficient microalgae bio-flocculation. Bioresource technology 282, 325-330
20. Mesbah M, Momeni M, Soroush E et al (2019). Theoretical study of CO_2 separation from CO_2/CH_4 gaseous mixture using 2-methylpiperazine -promoted potassium carbonate through hollow fiber membrane contactor. Journal of Environmental Chemical Engineering 7, 102781
21. Min Z, Li R, Chen L et al (2019). Alleviation of drought stress in grapevine by foliar-applied strigolactones. Plant Physiology and Biochemistry 135, 99-110
22. Papadias DD, Ahmed S, Kumar R (2012). Fuel quality issues with biogas energy – An economic analysis for a stationary fuel cell system. Energy 44, 257-277
23. Ramanna L, Rawat I, Bux F (2017). Light enhancement strategies improve microalgal biomass productivity. Renewable and Sustainable Energy Reviews 80, 765-773
24. Ryckebosch E, Drouillon M, Vervaeren H (2011). Techniques for transformation of biogas to biomethane. Biomass and Bioenergy 35, 1633-1645
25. Sharifi P, Bidabadi SS (2020). Strigolactone could enhances gas-exchange through augmented antioxidant defense system in Salvia nemorosa L. plants subjected to saline conditions stress. Industrial Crops and Products 151, 112460
26. Shen X, Xue Z, Sun L et al (2020). Effect of GR24 concentrations on biogas upgrade and nutrient removal by microalgae-based technology. Bioresource technology 312, 123563
27. Siame BA, Weerasuriya Y, Wood K et al (1993). Isolation of strigol, a germination stimulant for Striga asiatica, from host plants. Journal of Agricultural and Food Chemistry, **41**(9), 1486-1491.
28. Song X, Zhao Y, Li T et al (2019). Enhancement of lipid accumulation in Monoraphidium sp. QLY-1 by induction of strigolactone. Bioresource technology 288, 121607
29. Strasser RJ, Tsimilli-Michael M, Srivastava A (2004). Analysis of the Chlorophyll a Fluorescence Transient. in: Chlorophyll a Fluorescence: A Signature of Photosynthesis, (Eds.) G.C. Papageorgiou, Govindjee, Springer Netherlands. Dordrecht, 321-362.
30. Sun C, Xu Y, Hu N et al (2020). To evaluate the toxicity of atrazine on the freshwater microalgae Chlorella sp. using sensitive indices indicated by photosynthetic parameters. Chemosphere 244, 125514

31. Teodoro KBR, Migliorini FL, Facure MHM et al (2019). Conductive electrospun nanofibers containing cellulose nanowhiskers and reduced graphene oxide for the electrochemical detection of mercury(II). Carbohydrate Polymers 207, 747-754
32. Wang R, Xue S, Zhang D et al (2015). Construction and characteristics of artificial consortia of Scenedesmus obliquus-bacteria for S. obliquus growth and lipid production. Algal Research 12, 436-445
33. Wang X, Gao S, Zhang Y et al (2017). Performance of different microalgae-based technologies in biogas slurry nutrient removal and biogas upgrading in response to various initial CO_2 concentration and mixed light-emitting diode light wavelength treatments. Journal of Cleaner Production 166, 408-416
34. Wilson RA, Wrede D, Taha M et al (2014). Co-Cultivation of Fungal and Microalgal Cells as an Efficient System for Harvesting Microalgal Cells, Lipid Production and Wastewater Treatment. PLoS ONE 9, e113497
35. Xu M, Ou D, Xue Z et al (2021). Enhancement of the photosynthetic and removal performance for microalgae-based technologies by co-culture strategy and strigolactone induction. Bioresource technology 339, 125579
36. Yan C, Zheng Z (2014). Performance of mixed LED light wavelengths on biogas upgrade and biogas fluid removal by microalga Chlorella sp. Applied Energy 113, 1008-1014
37. Yang L, Li H, Wang Q (2019). A novel one-step method for oil-rich biomass production and harvesting by co-cultivating microalgae with filamentous fungi in molasses wastewater. Bioresource technology 275, 35-43
38. Zamalloa C, Gultom SO, Rajendran A et al (2017). Ionic effects on microalgae harvest via microalgae-fungi co-pelletization. Biocatalysis and Agricultural Biotechnology 9, 145-155
39. Zhang J, Zhao C, Sun S et al (2021a). Performance of different microalgae-based technologies in nutrient removal and biogas upgrading in response to various GR24 concentrations. International Biodeterioration & Biodegradation 158, 105166
40. Zhang W, Zhao C, Liu J et al (2021b). Effects of exogenous GR24 on biogas upgrading and nutrient removal by co-culturing microalgae with fungi under mixed LED light wavelengths. Chemosphere 281, 130791
41. Zhao Y, Sun S, Hu C et al (2015). Performance of three microalgal strains in biogas slurry purification and biogas upgrade in response to various mixed light-emitting diode light wavelengths. Bioresource technology 187, 338-345
42. Zhao Y, Guo G, Sun S et al (2019). Co-pelletization of microalgae and fungi for efficient nutrient purification and biogas upgrading. Bioresource technology 289, 121656
43. Zhu S, Qin L, Feng P et al (2019). Treatment of low C/N ratio wastewater and biomass production using co-culture of Chlorella vulgaris and activated sludge in a batch photobioreactor. Bioresource technology 274, 313-320

Tables

Table 1

Growth rates and average daily output of the four algae-fungi pellet induced with different contents of GR24.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Parameter	Growth rate d^{-1}			
0M	0.277^{c}±0.02	0.236^{c}±0.02	0.258^{d}±0.02	0.209^{c}±0.02
10^{-7} M	0.332^{b}±0.03	0.275^{b}±0.03	0.312^{b}±0.03	0.244^{b}±0.02
10^{-9} M	0.369^{a}±0.04	0.323^{a}±0.03	0.341^{a}±0.03	0.305^{a}±0.03
10^{-11} M	0.319^{b}±0.03	0.269^{b}±0.03	0.284^{c}±0.03	0.231^{b}±0.02
	Mean daily productivity(gL^{-1} d^{-1})			
0M	0.089^{c}±0.009	0.065^{c}±0.006	0.078^{c}±0.008	0.058^{c}±0.006
10^{-7} M	0.149^{b}±0.12	0.119^{b}±0.010	0.136^{b}±0.012	0.105^{b}±0.009
10^{-9} M	0.171^{a}±0.015	0.137^{a}±0.012	0.159^{a}±0.014	0.127^{a}±0.011
10^{-11} M	0.138^{b}±0.011	0.122^{b}±0.011	0.124^{b}±0.011	0.094^{b}±0.009

Note: In table1,the data were recorded as mean ± SD (n = 3).

Different superscript letters of data indicate significant difference ($p < 0.05$)

The superscript letters of the data are the same, indicating that the difference is not significant.

Table 2

The photosynthetic performance parameters measured by the OJIP test on the 10th day of the four selected fungi-assisted microalgae pellet.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Parameter	**FV/FM**			
0M	0.51 ^{c}±0.05	0.42 ^{c}±0.04	0.48 ^{c}±0.05	0.34 ^{c}±0.03
10^{-7} M	0.68^{b}±0.07	0.57^{b}±0.06	0.62^{b}±0.06	0.52^{b}±0.05
10^{-9} M	0.89^{a}±0.09	0.77^{a}±0.08	0.81^{a}±0.08	0.69^{a}±0.07
10^{-11} M	0.64 ^{b}±0.06	0.53 ^{b}±0.05	0.58 ^{b}±0.06	0.47 ^{b}±0.05
	PIABS			
0M	4.19^{d}±0.39	3.75^{c}±0.32	4.02^{c}±0.37	3.39^{c}±0.31
10^{-7} M	5.16^{b}±0.44	4.82^{b}±0.43	5.04^{b}±0.42	4.67^{b}±0.41
10^{-9} M	6.35^{a}±0.61	5.74^{a}±0.51	6.18^{a}±0.53	5.38^{a}±0.49
10^{-11} M	4.42^{c}±0.41	4.76^{b}±0.45	4.92^{b}±0.48	4.46^{b}±0.43
	ΨO			
0M	0.74^{b}±0.07	0.63^{b}±0.06	0.69^{b}±0.07	0.55^{b}±0.06
10^{-7} M	0.93^{a}±0.09	0.78^{a}±0.08	0.85^{a}±0.09	0.74^{a}±0.07
10^{-9} M	0.98^{a}±0.09	0.87^{a}±0.09	0.91^{a}±0.09	0.85^{a}±0.08
10^{-11} M	0.77^{b}±0.08	0.66^{b}±0.07	0.73^{b}±0.07	0.59^{b}±0.06
	ΦEO			
0M	0.41^{b}±0.04	0.31^{b}±0.03	0.36^{b}±0.04	0.27^{b}±0.03
10^{-7} M	0.59^{a}±0.06	0.44^{a}±0.04	0.52^{a}±0.05	0.41^{a}±0.04
10^{-9} M	0.64^{a}±0.06	0.48^{a}±0.05	0.58^{a}±0.06	0.43^{a}±0.04
10^{-11} M	0.43^{b}±0.04	0.35^{b}±0.03	0.39^{b}±0.04	0.31^{b}±0.03

Note: In table2, the data were recorded as mean ± SD (n = 3).

Different superscript letters of data indicate significant difference ($p < 0.05$)

The superscript letters of the data are the same, indicating that the difference is not significant.

Table 3

Mean values ±SD of the removal efficiency of COD, TN and TP and CO_2 of the four selected fungi-assisted microalgae pellet under different concentrations of GR24.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Parameter	COD Removal efficiency (%)			
0M	66.84^{c}±6.23	62.09^{c}±6.03	64.12^{c}±6.11	60.14^{c}±5.76
10^{-7} M	73.58^{b}±6.61	70.18^{b}±6.16	71.35^{b}±6.36	68.07^{b}±6.32
10^{-9} M	78.36^{a}±6.98	74.31^{a}±6.35	76.27^{a}±6.64	71.49^{a}±6.68
10^{-11} M	68.15^{c}±6.39	64.77^{c}±6.12	66.23^{c}±6.19	62.56^{c}±5.84
	TN Removal efficiency (%)			
0M	67.16^{c}±6.58	63.27^{c}±6.26	65.16^{c}±6.58	61.48^{c}±5.81
10^{-7} M	74.19^{b}±7.14	72.43^{b}±7.11	73.13^{b}±7.02	69.39^{b}±6.77
10^{-9} M	81.63^{a}±7.35	77.36^{a}±7.48	79.55^{a}±7.21	74.24^{a}±7.12
10^{-11} M	72.54^{b}±6.86	70.36^{b}±6.75	71.27^{b}±6.92	67.22^{b}±6.43
	TP Removal efficiency (%)			
0M	73.51^{b}±6.83	69.15^{b}±6.55	71.06^{b}±6.32	66.21^{b}±6.01
10^{-7} M	77.42^{b}±7.28	73.27^{b}±6.83	75.09^{b}±7.01	70.38^{b}±6.22
10^{-9} M	84.05^{a}±7.16	80.52^{a}±7.19	82.13^{a}±7.25	77.39^{a}±7.25
10^{-11} M	74.18^{b}±6.92	70.46^{b}±6.54	72.55^{b}±6.54	68.75^{b}±6.09
	CO_2 Removal efficiency (%)			
0M	53.26^{c}±4.71	47.61^{c}±4.37	50.77^{c}±4.58	46.35^{c}±4.07
10^{-7} M	60.05^{b}±5.51	54.65^{b}±5.14	56.34^{b}±5.24	53.16^{b}±4.72
10^{-9} M	65.18^{a}±6.12	60.24^{a}±5.82	62.57^{a}±6.03	58.79^{a}±5.21
10^{-11} M	58.44^{b}±5.57	52.32^{b}±5.05	55.08^{b}±5.26	51.09^{b}±4.35

Note: In table 3, the data were recorded as mean ± SD (n = 3).

Different superscript letters of data indicate significant difference ($p < 0.05$)

The superscript letters of the data are the same, indicating that the difference is not

Figures

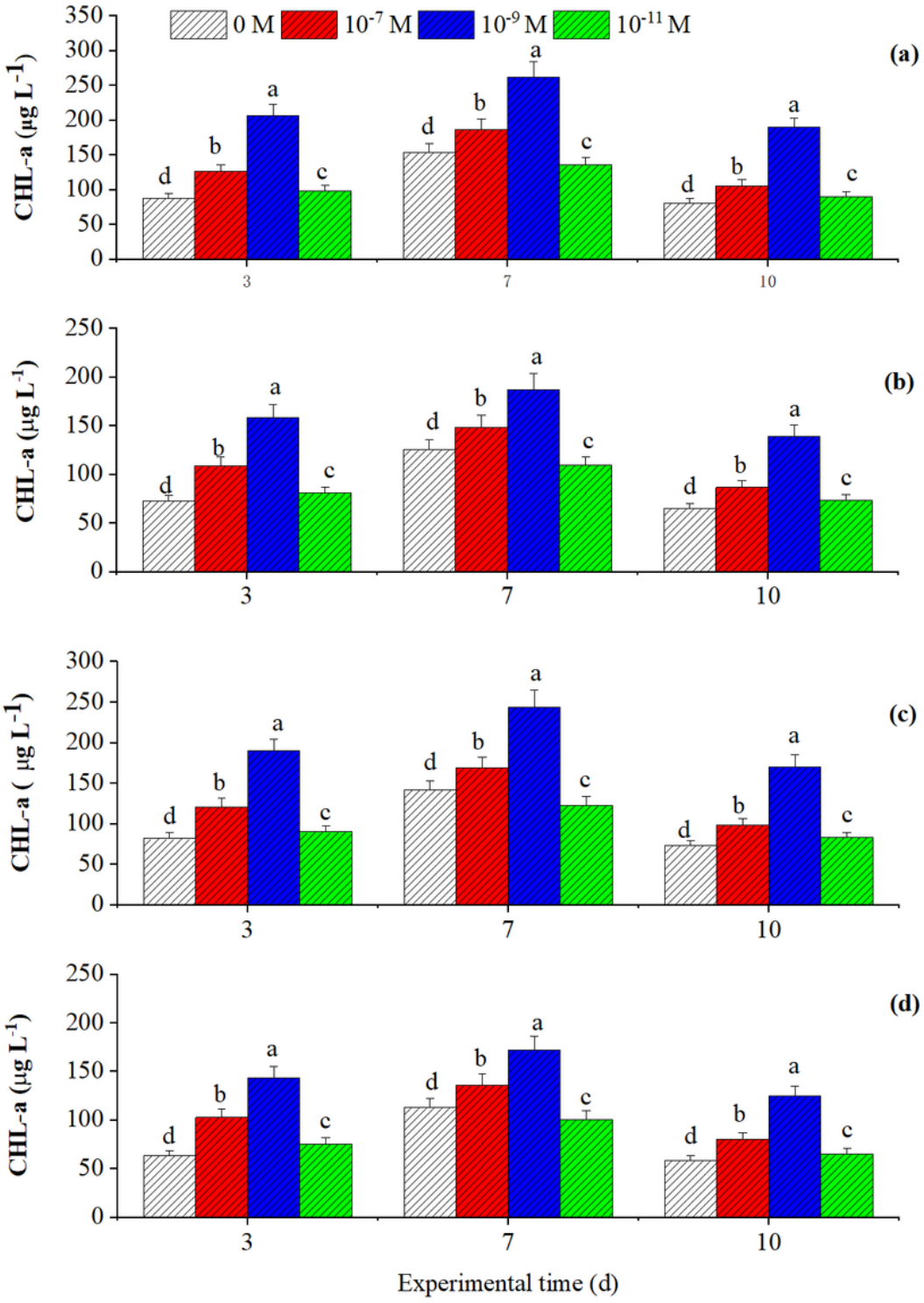

Figure 1

The CHL-a content of the four algae-bacteria-fungi pellets for 3d, 7d and 10 treatments.

(a) Treatment 1, (b) Treatment 2, (c) Treatment 3, (d)Treatment 4

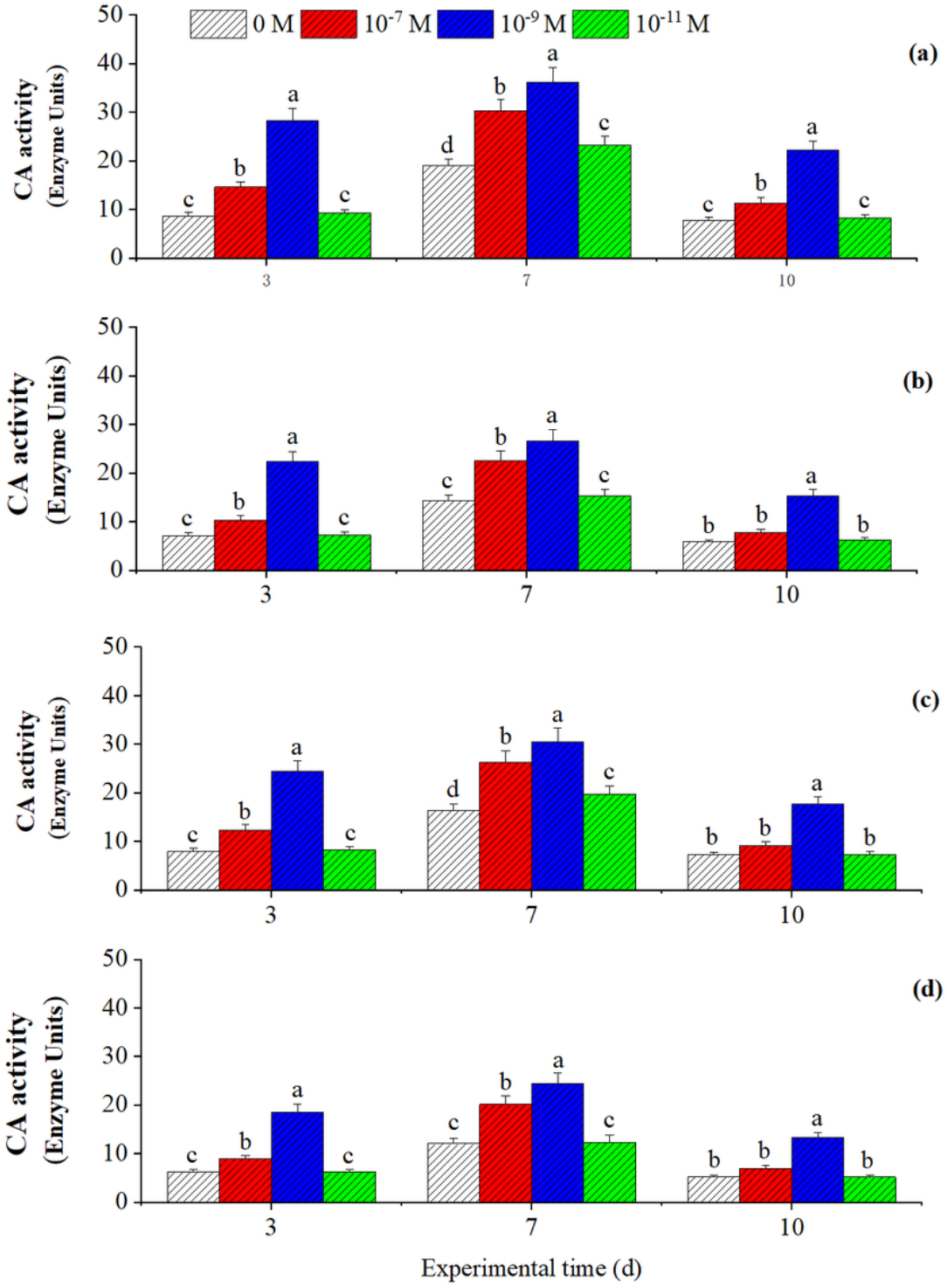

Figure 2

The extracellular CA activity of the four algae-bacteria-fungi pellets for 3d, 7d and 10 treatments.

(a) Treatment 1, (b) Treatment 2, (c) Treatment 3, (d)Treatment 4

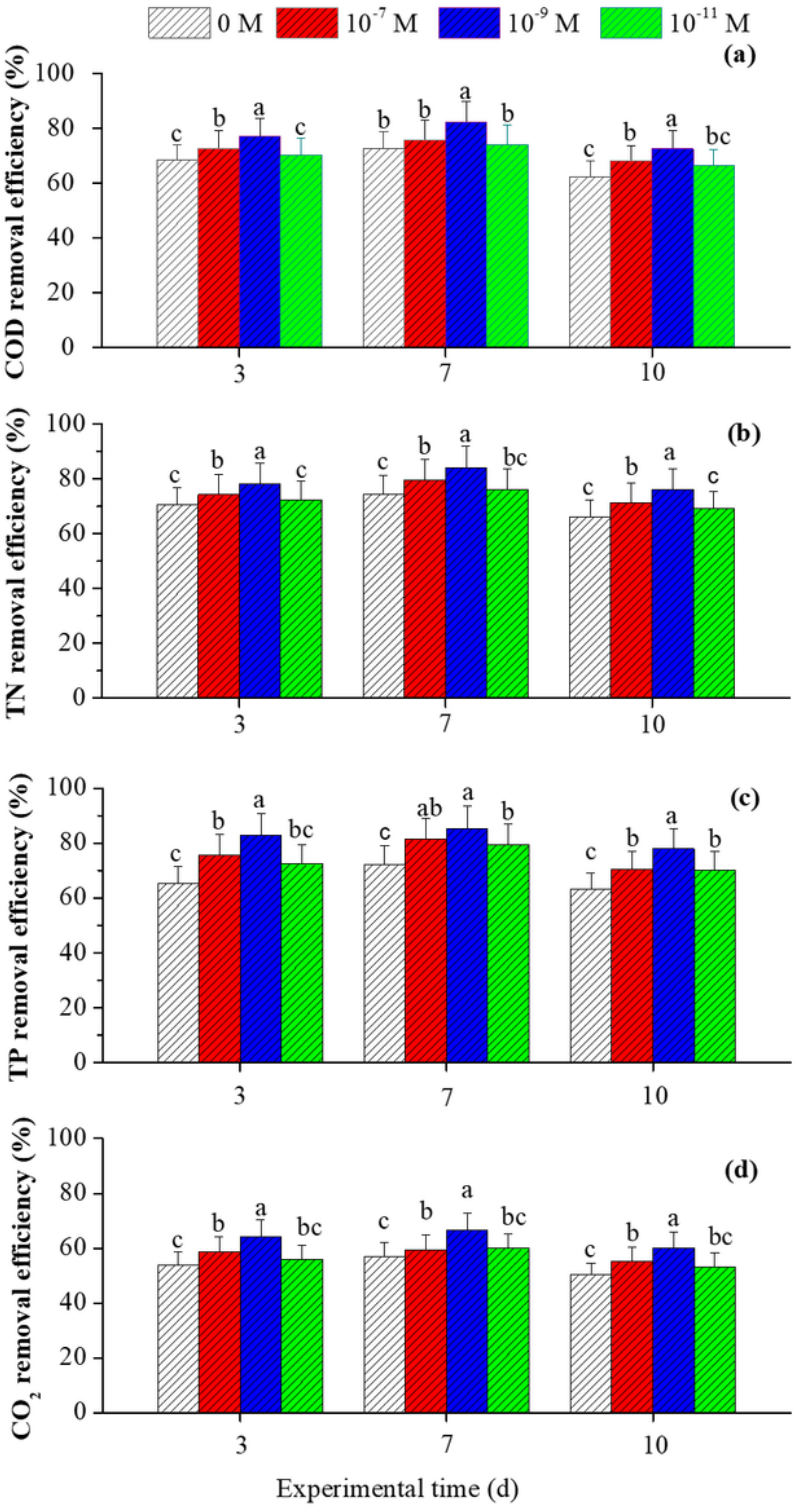

Figure 3

The removal efficiencies of the nutrients and CO_2 induced with different content GR24 for Treatment 1 at 3d, 7d and 10d treatments .

(a) RE-COD, (b) RE-TN,

(c) RE-TP and (d) RE-CO_2 .

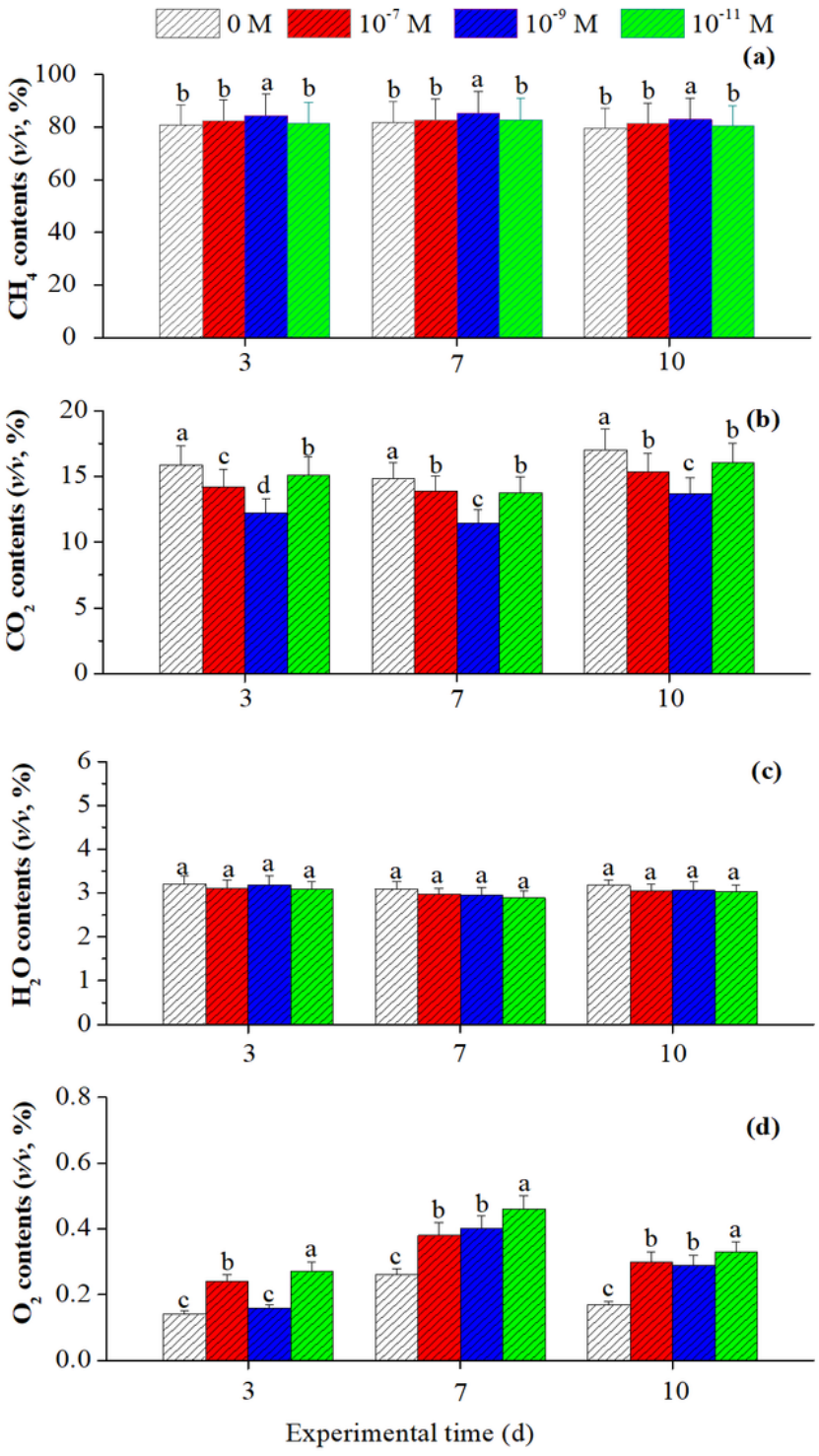

Figure 4

The contents of CH_4, CO_2, H_2O and O_2 in the biogas induced with different content of GR24 for Treatment 1 at 3d, 7d and 10 treatments.

(a) The contents of CH_4, (b) The contents of CO_2,

(c) The contents of H_2O and (d) The contents of O_2