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A Comparative Evaluation of Simultaneous Nutrient Removal and Biomass Production for Treatment Vinasse Biogas Slurry by Microalgae

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Abstract: The substantial discharge of vinasse biogas slurry (VBS) from anaerobic digestion poses significant environmental risks, while microalgae could recover nutrient for biomass production from VBS treatment. This study comprehensively investigated the bio-remediation potential of microalgae via rotating biofilm reactor, focusing on microalgae species and inoculation ratios, as well as VBS dilution ratios. The results revealed that *Desmodesmus* sp. had a better performance in biomass productivity (0.62 g/L), and total phosphorus (TP) removal efficiency (77.03%) than the other two microalgae ($p < 0.05$). The low VBS proportion (≤30%) obtained a higher biomass accumulation of 0.87–0.89 g/L than groups of high VBS proportion of 50%. These growth inhibition in high VBS proportion groups were attributed to high ammonium nitrogen (NH_4^+-N) toxicity and elevated turbidity of biogas slurry. Both the total nitrogen (TN) removal of 34.11–36.48% and NH_4^+-N removal of 83.63–97.38% revealed ammonium assimilation during the treatment of VBS via microalgae. Inoculum optimization demonstrated that reduced initial inoculation ratio (10% *v*/*v*) obtained highest TN removal efficiency of 46.52% and biomass growth rate of 0.04 g/(L·d), likely through alleviated light limitation. In the rotating biofilm reactor, treatment with 50-time diluted VBS achieved the highest biomass growth rate of 1.60 g/(m^2·d), and the removal of TN, NH_4^+-N, and TP was 74.93%, 93.94% and 98.01%, respectively. Moreover, coarse ribbed fiber fabric (CRFF) exhibited higher attached biomass (48.76 g/m^2) and NH_4^+-N removal efficiency (76.30%) than other attached materials. The research offered insights into trade-offs between the nutrient recovery and accumulating microalgae biomass during biogas slurry treatment.

Keywords: microalgae culture; biogas slurry treatment; rotating biofilm reactor; vinasse; nutrient recovery

1. Introduction

Vinasse is a by-product of liquor distillation, which reached 57 million tonnes production in 2021 according China national statistics [1]. Anaerobic digestion has emerged as a predominant strategy for vinasse resource recovery and eco-friendly treatment, yielding biogas slurry. The vinasse biogas slurry (VBS) featured high levels of ammonia nitrogen (NH_4^+-N, 500–3000 mg/L) and chemical oxygen demand (COD, 35,000–50,000 mg/L) [2]. Direct discharge of untreated VBS can lead to eutrophication of water bodies, depletion of dissolved oxygen, and groundwater contamination [3]. It constitutes primary constraints to sustainable industrial development and environmental strategy. Numerous physical and chemical methods have been used to treat VBS, including the

ammonia stripping, membrane filtration, and advanced oxidation [4,5]. However, these methods have limited comprehensive removal efficiency and high chemical costs, which can also lead to resource waste and secondary pollution [6,7]. Therefore, it is necessary to explore alternative technologies to achieve thorough treatment of VBS pollutants and resource recovery.

As photosynthetic autotrophs, microalgae exhibit dual environmental remediation capabilities through atmospheric CO_2 assimilation and aqueous nutrient fixation. Microalgae demonstrate distinct advantages including superior photosynthetic efficiency, rapid growth cycles, non-arable land requirements, and high biomass productivity. The autotrophic biosynthesis of 1 kg microalgae biomass requires approximately 1.83 kg of CO_2 [8], establishing microalgae as promising renewable feedstocks for fossil fuel substitution. Various wastewater types including municipal, livestock, and biogas slurry can serve as viable nutrient matrices for microalgae growth [9–11]. The waste water generally contains sufficient nitrogen and phosphorus to support microalgae growth [12]. Significantly, freshwater consumption constitutes 6–20% of total production costs of microalgae culture, making wastewater-based cultivation economically advantageous [13]. This approach simultaneously conserves freshwater resources for microalgae biomass production.

Currently, many studies reported the microalgae culture from biogas slurry for biomass production [9,14,15]. There are two restrictive problems need to take consideration. On the one hand, the characteristic brown pigmentation, high ammonia nitrogen content, and elevated turbidity of biogas slurry could reduce microalgae photosynthetic efficiency and biomass productivity. On the other hand, conventional suspended cultivation systems made harvesting costs account for 21–30% of total production expenditure due to energy-intensive dewatering processes [16]. The use of immobilized attached microalgae can effectively address the aforementioned disadvantages.

The immobilized attached microalgae system alternately drives the contact between microalgae on the attached material, culture medium, and air, thereby facilitating material exchange among them. For instance, Christenson and Sims (2012) conducted laboratory and pilot experiments using the rotating biofilm reactor with solid woven cotton thread as the attached material to treat municipal secondary effluent [17]. Their results indicated that the biomass productivity of the reactor ranged from 20.00 to 31.00 g/(m^2·d), while the lipid productivity was between 2.20 and 2.50 g/(m^2·d), both biomass and lipid significantly higher than those achieved through suspension culture methods. Gross et al. (2013) and Zhao et al. (2018) designed rotating biofilm culture systems for microalgae with “triangular structures” and “vertical conveyor belt structures” for laboratory and pilot tests [18,19]. The results showed that the system with the “vertical conveyor belt structure” achieved the highest algal biomass productivity of 11.36 g/(m^2·d), which was 302% higher than that of suspension culture. Additionally, the corresponding total phosphorus (TP) and total nitrogen (TN) removal efficiencies was 80% and 87%, respectively. Despite numerous studies demonstrating the suitability of the rotating biofilm reactors for microalgae processing wastewater, but the reactor design, attached material, as well as the operating parameters and microalgae species dependence remains need to be further study.

Thus, this study using microalgae for treatment of the vinasse biogas slurry (VBS) with high ammonia content. The objective was to explore the effects of different microalgae species, inoculum ration, and biogas slurry proportions on the microalgae growth and the nitrogen and phosphorus removal efficiencies in batch tests. Furthermore, constructed a semi-immersion rotating biofilm reactor for microalgae cultivation in VBS, and the microalgae biomass production and nutrient removal within the rotating biofilm reactors also were evaluated. This research can supply a insight for the sustainable microalgal-based biogas slurry treatment coupled with valuable bio-products production.

2. Materials and Methods

2.1. Microalgae Strains and Pre-Cultivation

Microalgae species of *Chlorella vulgaris* FACHB-31, *Desmodesmus* sp. FACHB-2097, and *Scenedesmus quadricauda* FACHB-44 used in this study belong to the genera *Chlorella*, *Desmodesmus*, and *Scenedesmus* in the phylum Chlorophyta, respectively. These microalgae are not only common, but also have been proven to have good wastewater treatment effects in many studies [20]. All of these were purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. All microalgae were cultured in Erlenmeyer flasks containing sterilized BG11 medium.

2.2. Collection and Characterization of Vinasse Biogas Slurry (VBS)

The biogas slurry used in this study was collected from the biogas project in Zunyi, Guizhou Province. It was the liquid product obtained after solid-liquid separation from the mesophilic anaerobic fermentation of vinasse in a CSTR (continuously stirred tank reactor). The biogas slurry was stored in a cold storage at 4 °C, centrifuged at 8000 r/min for 15 min to remove suspended solids, and then sterilized for subsequent use. The concentration of ammonia nitrogen (NH_4^+-N), total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) in vinasse biogas slurry (VBS) was 5196.45 ± 133.65 mg/L, 5859.67 ± 195.26 mg/L, 532.59 ± 15.51 mg/L, 29,118.52 ± 52.38 mg/L, respectively. The values of pH was 8.36, and the concentration of total solids and volatile solids was 2.51% and 1.69%, respectively. Considering elements required for the growth of the microalgae and bacteria, to each liter of the synthetic wastewater, was added 1 mL of the trace element solution (2.86 mg/L H_3BO_3, 1.81 mg/L $MnSO_4 \cdot 4H_2O$, 0.079 mg/L $CuSO_4 \cdot 5H_2O$, 0.222 mg/L $ZnSO_4 \cdot 7H_2O$, 0.21 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.05 mg/L $CoCl_2 \cdot 6H_2O$).

2.3. Batch Cultivation of Microalgae Using VBS

The batch tests were performed in the 2 L Erlenmeyer flask with an effective volume of 1.9 L. After preparation, they were placed in a temperature-controlled photo-bioreactor at 25 °C, with a light intensity of 4500 lx and a light/dark cycle of 15:9. To ensure adequate contact between microalgae, VBS and air, the Erlenmeyer flasks were manually shaken three times a day for 2 min each time. Samples were taken every 2 d to measure the biomass of microalgae, the pH value, and the concentrations of TN, TP, COD, and NH_4^+-N in VBS.

2.3.1. Different Microalgae Species Test

Three microalgae species, *Chlorella vulgaris* FACHB-31, *Desmodesmus* sp. FACHB-2097, and *Scenedesmus quadricauda* FACHB-44, were cultured until they reached the logarithmic growth phase. They were then inoculated into a mixed culture medium at a 10% VBS (with a ratio of VBS to BG11 of 1:9) with the TN, NH_4^+-N, and TP concentrations of 239.50 mg/L, 17.54 mg/L, and 9.78 mg/L, respectively. The microalgae were inoculated at a 10% inoculum volume with the biomass concentration of 0.12 g/L. Each experimental group was set up in triplicate, and a control check group (CK) without inoculated microalgae was also prepared. The cultivation period was 32 d.

2.3.2. Different VBS Proportions Test

The VBS was diluted to 10%, 30%, and 50% (*v*/*v*) using BG11 medium. *Desmodesmus* sp. was cultured until they reached the logarithmic growth phase, and then inoculated into the mixed culture medium at a 10% inoculum volume (with biomass concentration of 0.12 g/L). Each experimental group was set up in triplicate. The cultivation period was 36 d.

2.3.3. Different Inoculation Ratios of Microalgae

Desmodesmus sp. were cultured until they reached the logarithmic growth phase, and then inoculated into a 10% mixed culture medium (with a ratio of VBS to BG11 of 1:9). The microalgae inoculum volumes were 10%, 20%, 40%, and 60% with biomass concentration of 0.12 g/L, 0.24 g/L, 0.48 g/L, and 0.72 g/L, respectively. Each experimental group was set up in triplicate. In order to observe the growth situations of the microalgae, the cultivation period was 54 d.

2.4. The Rotating Biofilm Reactor for VBS Treatment by Desmodesmus sp.

2.4.1. The Rotating Biofilm Reactor Setup

The rotating biofilm reactor comprised a rotating control system, a single-sided toothed synchronous belt, synchronous belt pulleys, attached materials, a biogas slurry tank, and a harvesting scraper. The synchronous belt featured a 30° inclined structure supported by a stainless-steel frame consisting of six profiles, employing top-down illumination to maintain optimal light exposure for the attached materials. In this reactor, the middle and lower sections of the attached materials remained immersed in the liquid medium while upper portions were aerially exposed. The surface area of the attached material was 0.1152 m^2. The rotating biofilm reactor enables simultaneous microalgae biofilm enrichment, separation, and resource recovery through wastewater treatment.

The rotating biofilm reactor used in this experiment has a total volume of 15 L, with an effective working volume of 12 L. The reactor rotating speed was set at 20 r/h. Light intensity was maintained at 5000–6000 lx, and

given throughout the day. The temperature was stable at 25 ± 2 °C. To compensate for the evaporation loss, distilled water was added at a fixed time every day. Samples were conducted every 2 d to determine the suspended microalgae biomass, the pH value, and the concentrations of TN, TP, and NH_4^+-N in the culture medium. Attachment microalgae biomass was harvested on the last day.

2.4.2. Different VBS Dilution Ratios Tests

The VBS was diluted to three gradients of 25, 50, and 100 times as the culture medium, named VBS25, VBS50, and VBS100, respectively. The attached material used was coarse ribbed fiber fabric (CRFF), and the inoculum biomass of *Desmodesmus* sp. was 0.8 g/L. The cultivation period was 12 d.

2.4.3. Different Attached Materials Tests

The VBS was diluted by 50 times to be used as the culture medium. Considering the high surface roughness, non-toxicity, and low cost, the attached materials of twill, CRFF, cotton and linen coarse cloth (CLCC) were selected. The inoculum biomass of *Desmodesmus* sp. was 0.8 g/L. A semi-continuous cultivation mode was adopted, with a hydraulic retention time (HRT) set at 20 days. The culture medium was partially renewed every 10 d, with 6 L of the culture medium being replaced each time.

2.5. Analytical Methods

The microalgae biomass was measured and calculated using the dry weight method based on the study [21]. The biomass growth rate of microalgae was calculated by the following Equation (1):

$$M_T = (M_t - M_0)/t \quad (1)$$

where M_T was the biomass growth rate (g/(L·d) or g/(m^2·d)), t was culture days (d), M_t was microalgae dry weight in time t (g/L or g/m^2), M_0 was initial microalgae dry weight (g/L or g/m^2).

The pH value was measured by the glass electrode method. The concentrations of TN, NH_4^+-N, TP, COD were tested according to standard methods [22]. The pollutant removal efficiency was calculated by the following Equation (2):

$$\eta = (C_t - C_0)/C_0 \times 100\% \quad (2)$$

where η was the removal efficiency (%), C_t was the pollutant concentration in time t (mg/L), C_0 was the initial pollutant concentration (mg/L).

2.6. Data Analysis

SPSS 26.0 software was used for one-way analysis of variance (one-way ANOVA) with a p value of 0.05 as the significance level. Microsoft Office Excel 2021 was used for statistical calculation and Origin 2022 was used for mapping.

3. Results and Discussion

3.1. Effect of Different Microalgae Species

The dynamic of biomass among three microalgaes *Chlorella vulgaris*, *Desmodesmus* sp., and *Scenedesmus quadricauda* are shown in Figure 1a. These three microalgae grew slowly within 10 d, and then they gradually obtained a logarithmic phase of growth with rapid biomass accumulation. By the end of the cultivation, the biomass of *Chlorella vulgaris*, *Desmodesmus* sp., and *Scenedesmus quadricauda* were 0.62 ± 0.01 g/L, 0.56 ± 0.06 g/L, and 0.68 ± 0.05 g/L, respectively. There was no significant difference (p > 0.05) between *Chlorella vulgaris* and *Scenedesmus quadricauda*, but *Desmodesmus* sp. was slightly higher. The pH value was basically maintained between 8.0 and 9.5 with slight fluctuations. The phylum Chlorophyta features wide tolerance pH range of 6.5–9.5 [23], while *Chlorella vulgaris*, *Scenedesmus quadricauda*, and *Desmodesmus* sp. selected in this study was shown to grow well in an alkaline (7.0–9.5) environment. Microalgae can control the pH value through their own metabolism to keep it within a range suitable for their own growth [24]. Moreover, because the preponderant pH range of ammonia stripping is between 10 and 12 [6], the NH_4^+-N loss caused by ammonia stripping in this study was negligible.

The growth of microalgae requires the synthesis of proteins, nucleotides and other organic substances to maintain life activities, of which nitrogen is an important component of these organic substances. Microalgae

mainly removed N in the system by directly assimilating NH_4^+-N, reducing NO_3^--N and decompose organic nitrogen with bacteria [25]. Studies have shown that nitrogen makes up 1% to 11% of the dry weight of microalgae [26–28]. In addition, in VBS, nitrogen existed mainly in the form of NH_4^+-N. Therefore, this study focused on the changes of NH_4^+-N and TN in the systems. The TN concentration decreased from 238.90 mg/L to 170.67 mg/L, 175.80 mg/L, and 163.74 mg/L for *Chlorella vulgaris*, *Desmodesmus* sp., and *Scenedesmus quadricauda* after 32 d, with the removal efficiency of 28.56%, 26.41%, and 31.46%, respectively (Figure 1b). The continuous increase in biomass and slow utilization of TN during the cultivation of microalgae indicated that the nitrogen source was not the main limiting factor for microalgae growth in this experiment. The concentrations of NH_4^+-N decreased rapidly and were largely removed around 16 d (Figure 1c). The utilization of NH_4^+-N by all three microalgae was more than 94.50% and was not significantly different ($p > 0.05$). It suggests that NH_4^+-N can be directly utilized by microalgae and is the preferred nitrogen source for microalgae growth, which is consistent with the findings of many studies [26,27,29].

Microalgae achieve P removal by adsorption, transport, and assimilation [25]. The changes in P removal from the VBS by three microalgae are shown in Figure 1d. In the first 10 d, there was little difference in P concentrations of three microalgae cultivation. From the 10th day, the TP removal efficiency of *Desmodesmus* sp. was higher than the other two microalgae, *Chlorella vulgaris* and *Scenedesmus quadricauda*. After 32 d of cultivation, the TP removal efficiency were 54.97%, 77.03%, and 51.41% for *Chlorella vulgaris*, *Desmodesmus* sp., and *Scenedesmus quadricauda*, respectively. Among them, *Desmodesmus* sp. showed better phosphorus removal among the three microalgae, and it showed strong resistance in other studies [30]. It has been reported that *Desmodesmus* sp. surface has a high density of functional groups, which can quickly adsorb phosphate ions in water by electrostatic interaction [31]. Compared to *Chlorella vulgaris* and *Scenedesmus quadricauda*, *Desmodesmus* sp. can more efficiently use the phosphorus transporter protein system to actively uptake and utilization phosphorus [32], which makes it have the higher phosphorus removal efficiency.

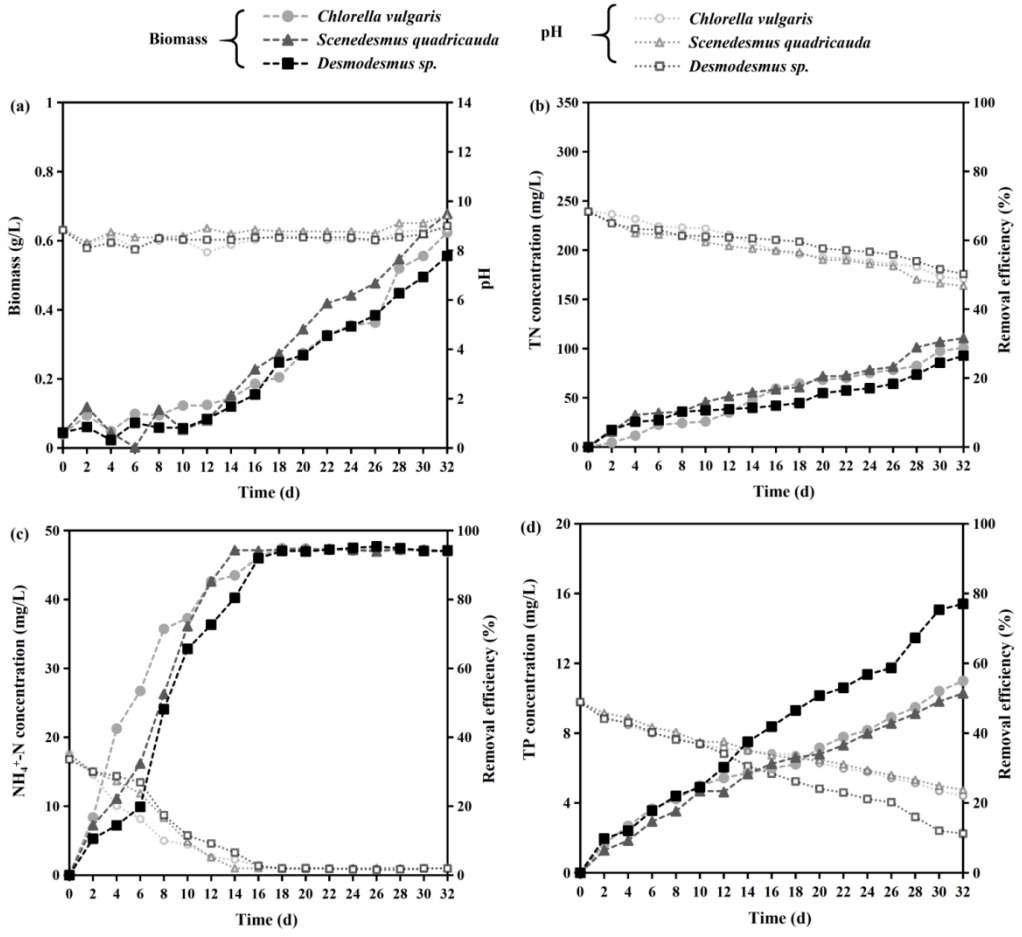


Figure 1. Effects of different microalgae species (*Chlorella vulgaris*, *Scenedesmus quadricauda*, *Desmodesmus* sp.) on the biomass and pH values changes (**a**), concentrations and removal efficiency of TN (**b**), NH_4^+-N (**c**), TP (**d**) in vinasse biogas slurry (VBS).

3.2. Effect of Different Proportions of VBS

Figure 2a shows the growth of *Desmodesmus* sp. in different VBS proportions cultivation. The microalgae biomass in the 50% VBS group grew slowly as the experiment progressed, with the biomass of only 0.33 g/L obtained at 36 d. In contrast, the microalgae in the groups with 10% and 30% of VBS grew relatively fast. The biomass appeared to increase and then decrease during the cultivation process, and the maximum obtained was 0.89 and 0.87 g/L, respectively. It indicated that a suitable proportion of VBS can promote microalgae growth, while too high a proportion of VBS can inhibit microalgae growth. In addition, certain substances such as phytohormones that promote the growth of microalgae may be present in VBS, resulting in a culture solution with a suitable proportion of VBS being more effective than a pure BG11 medium. The result of the present study also illustrated that a nitrogen source containing multiple types of nitrogen promotes the growth of microalgae more than a single nitrogen source, which was the same as the findings of Liu et al. (2022) [33].

The variation of TN during the cultivation of *Desmodesmus* sp. in different VBS proportions is shown in Figure 2b. The TN concentration of four groups gradually decreased with the growth of microalgae in the culture solution, and the trend of change was basically the same. After 32 d of cultivation, the removal efficiency of TN in the groups with 10%, 30%, and 50% VBS proportion were 35.87%, 34.11%, and 36.48%, respectively (Figure 2f).

As shown in Figure 2c, the NH_4^+-N concentration continued to decrease until the absorption was complete. The NH_4^+-N in the group with 10% VBS was consumed up on the 10th day of cultivation, and the NH_4^+-N in the 30% VBS group was completely removed on the 24th day. Furthermore, the removal efficiency of NH_4^+-N in these two groups reached 97.38% and 96.07%, respectively, while it was 83.63% in the 50% VBS group (Figure 2f). It suggested that *Desmodesmus* sp. had a good effect on the removal of NH_4^+-N during the cultivation process. Earlier study showed that a suitable concentration for microalgae growth was 60 mg/L NH_4^+-N, it was observed stunted growth and even death when the NH_4^+-N concentration greater than 100 mg/L of *Desmodesmus* sp. [34].

In this study, the NH_4^+-N concentration was in the range that promoted microalgae growth in groups with 10% and 30% of VBS. However, the concentration of NH_4^+-N in the group with 50% VBS was 85.94 mg/L, which exceeded the condition suitable for the growth of microalgae, and thus produced an inhibitory effect. When the NH_4^+-N concentration in the 50% VBS group was lower than 60 mg/L, the biomass accumulation of microalgae did not increase significantly. This suggested that the high concentration of NH_4^+-N was not the only reason to inhibit the growth of microalgae, and the high concentration of some components of the digestate may also be an inhibiting factor.

The TP concentration in different groups showed a decreasing trend with the extension of cultivation time (Figure 2d). At the 24th day of cultivation, the TP in the 10% VBS group were almost completely absorbed, and the removal efficiency was 96.64% (Figure 2f). The group with 30% VBS was also completely absorbed at the 34th day. The concentration of TP in the group with 50% VBS decreased from 13.44 mg/L to 3.40 mg/L, and the removal efficiency was 74.74%.

Microalgae can actively uptake small molecular organic matter by transport proteins on the cell membrane, or remove COD by degrading large molecular substances by extracellular enzymes [35]. The variation of COD concentration is shown in Figure 2e, which shows an overall trend of decreasing, then slightly increasing and leveling off. The COD removal efficiency in the 10% VBS group was −22.60% (Figure 2f). The small increase in COD concentration may be attributed to the production of extracellular polymers (EPS) by the microalgae under the stress of VBS [36]. The highest COD removal efficiency of 28.37%, 21.44% and 36.24% was obtained at the groups with 10%, 30% and 50% VBS, respectively. The above results showed that *Desmodesmus* sp. had the ability to reduce the COD concentration of VBS.

Table 1 lists the concentrations of elements C, H, N and S in microalgae harvested after 36 d of cultivation under different proportions of VBS. Comparison of the results of this experiment with those of Harman-Ware et al. (2013) and Li Gang (2014) showed that in this study, the N concentration of microalgae rose with the increase of the VBS proportions [37,38]. The highest C concentration was obtained from the group with 30% VBS, and the lowest C concentration was obtained from *Desmodesmus* sp. with pure BG11. Compared with the pure BG11, VBS contains more organic carbon, humus species and trace elements, which promote the accumulation of N in the microalgae composition [39]. There was no significant difference in the concentration of H and S in microalgae of different groups. In addition, the concentration of elemental C and N in *Desmodesmus* sp. (FACHB-2097) produced in this study was significantly higher than that of *Desmodesmus* sp. (EJ8-10) reported by Li Gang (2014) [38]. The possible reason was that VBS in this study was in a more suitable range for microalgae growth after BG11 dilution. However, the anaerobic fermentation liquid was not diluted in the study of Li Gang (2014) [38], which may cause the low C and N concentration of *Desmodesmus* sp. (EJ8-10).

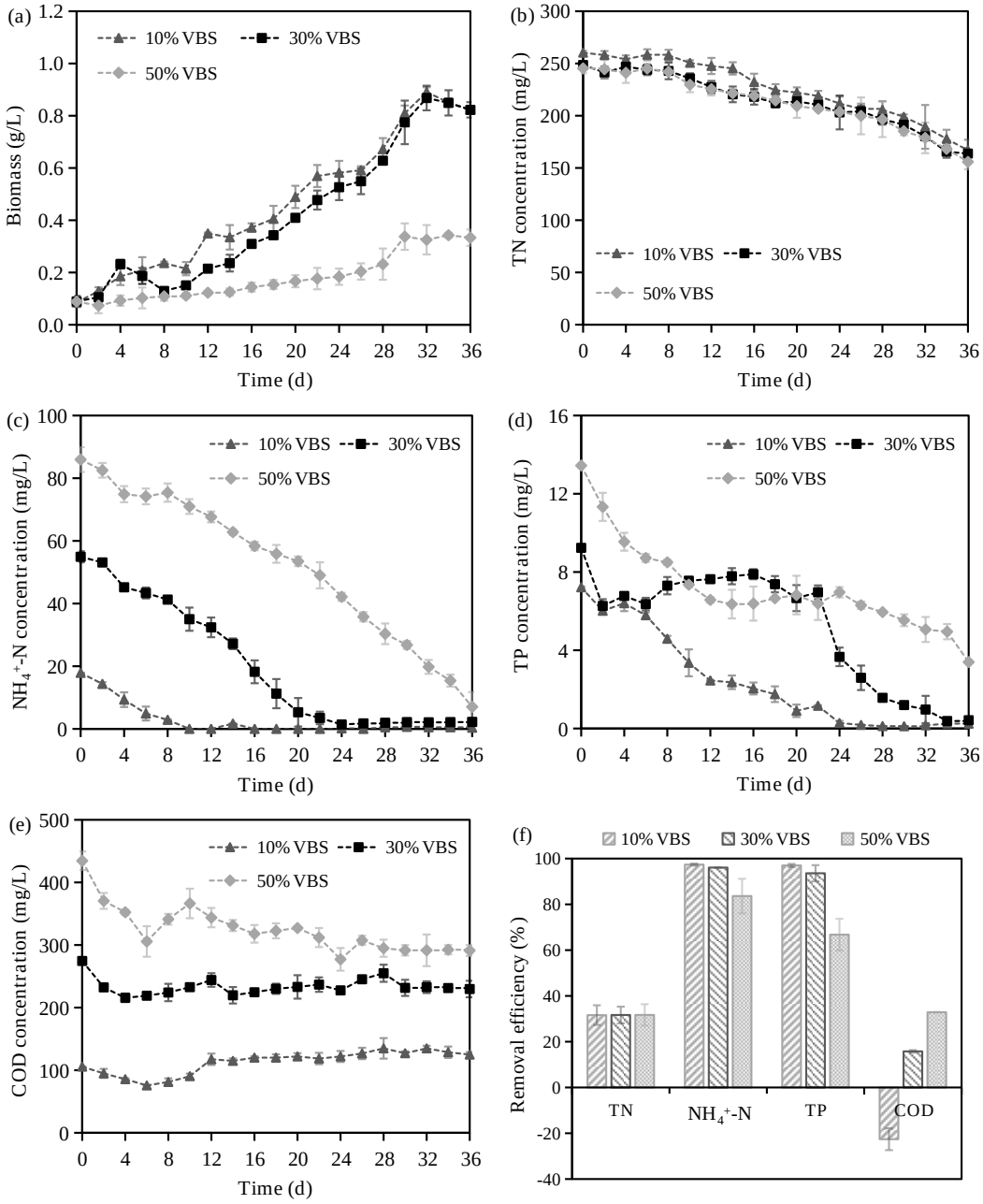


Figure 2. Effects of different proportions of vinasse biogas slurry (VBS) on the biomass of *Desmodesmus* sp. (**a**), concentrations of TN (**b**), NH_4^+-N (**c**), TP (**d**), and COD (**e**), and average removal efficiency of pollutants after day 32 (**f**).

Table 1. Elemental analysis of the microalgae under different proportions of biogas slurry.

Microalgae Species	Culture Medium	Elemental Analysis (%)				Data Sources
		N	C	H	S	
Desmodesmus sp. FACHB-2097	100% BG11	7.91	47.56	6.25	0.45	This research
	10% VBS + 90% BG11	8.40	49.61	6.42	0.49	
	30% VBS + 70% BG11	8.64	50.30	6.50	0.48	
	50% VBS + 50% BG11	8.78	49.20	6.45	0.59	
Desmodesmus sp. EJ8-10	BG11	7.67	38.05	6.30	0.56	[38]
	Anaerobic fermentation liquid	6.20	25.80	5.37	0.57	
Scenedesmus quadricauda	Pond water	5.30	32.10	5.30	0.50	[37]

3.3. Effect of Different Microalgae Inoculation Ratios

The biomass of *Desmodesmus* sp. cultivation with different inoculum ratios is shown in Figure 3a. All of four groups had similar growth tendency during the whole period. After cultivation of 54 days, the microalgae biomass of the 10%, 20%, 40% and 60% inoculum ratios groups was 2.29, 2.08, 2.40, and 2.53 g/L, respectively. The 10% inoculum group had highest biomass growth rate of 0.04 g/(L·d) (Figure 3b). However, the growth rate of microalgae biomass in the higher inoculum groups slowed down with increasing cultivation time and biomass. Similar results were found by Han Hao (2018) and Ferreira et al. (2021) [30,40]. Higher microalgae inoculum ratios resulted in increased turbidity of the culture solution, which affected the light transmission of the culture solution, thus limiting the photosynthetic efficiency of microalgae and reducing the amount of biomass accumulation. The pH value in the groups with different inoculum ratios showed a small increase and gradually stabilized. There was no significant difference in the pH values of the different experimental groups with the pH keeping in the range 9.2 to 10.2.

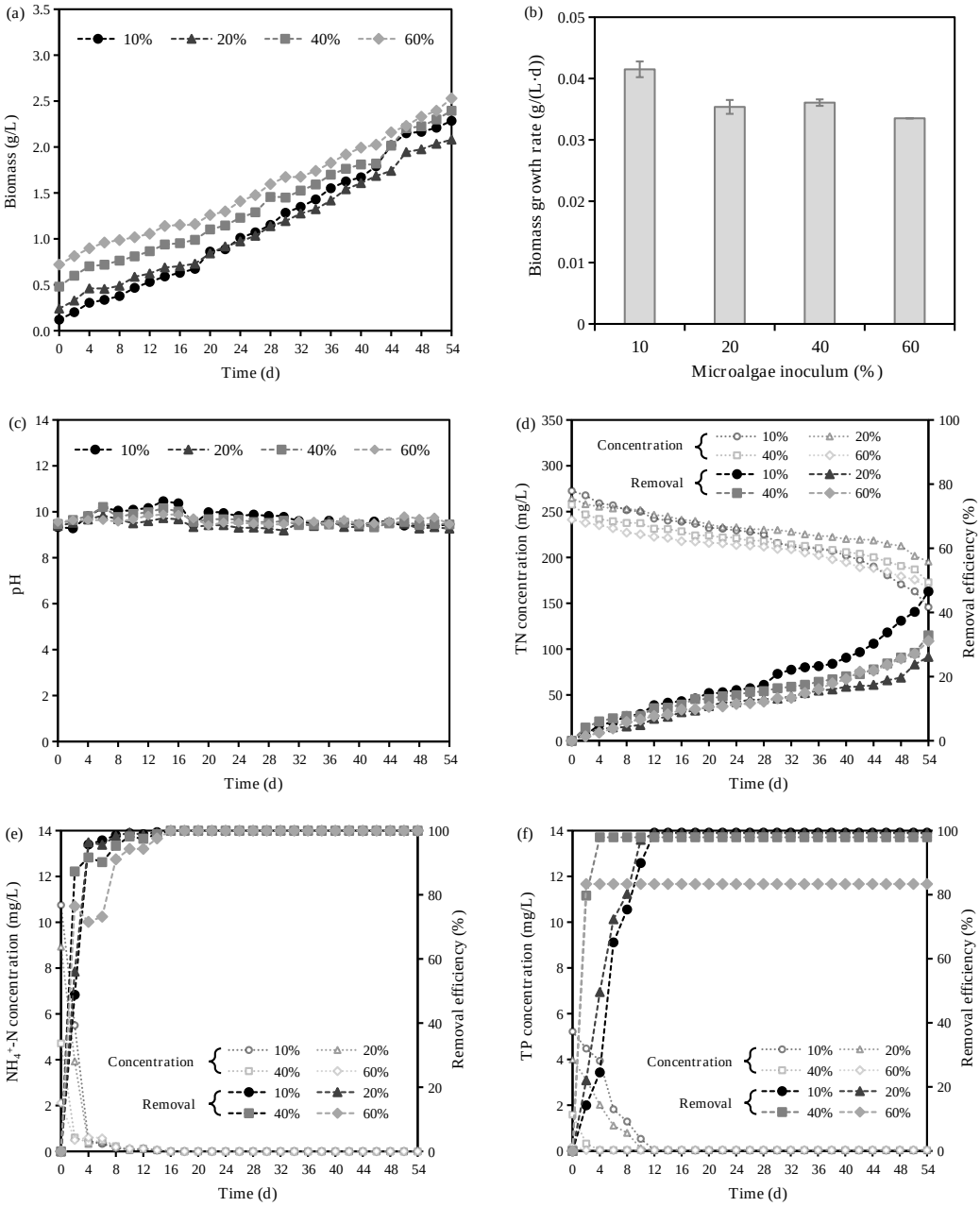


Figure 3. Effects of different microalgae inoculation amounts on the biomass (**a**) and growth rate (**b**) of *Desmodesmus* sp., pH values (**c**), and concentrations and removal efficiency of TN (**d**), NH_4^+-N (**e**), and TP (**f**) in vinasse biogas slurry (VBS).

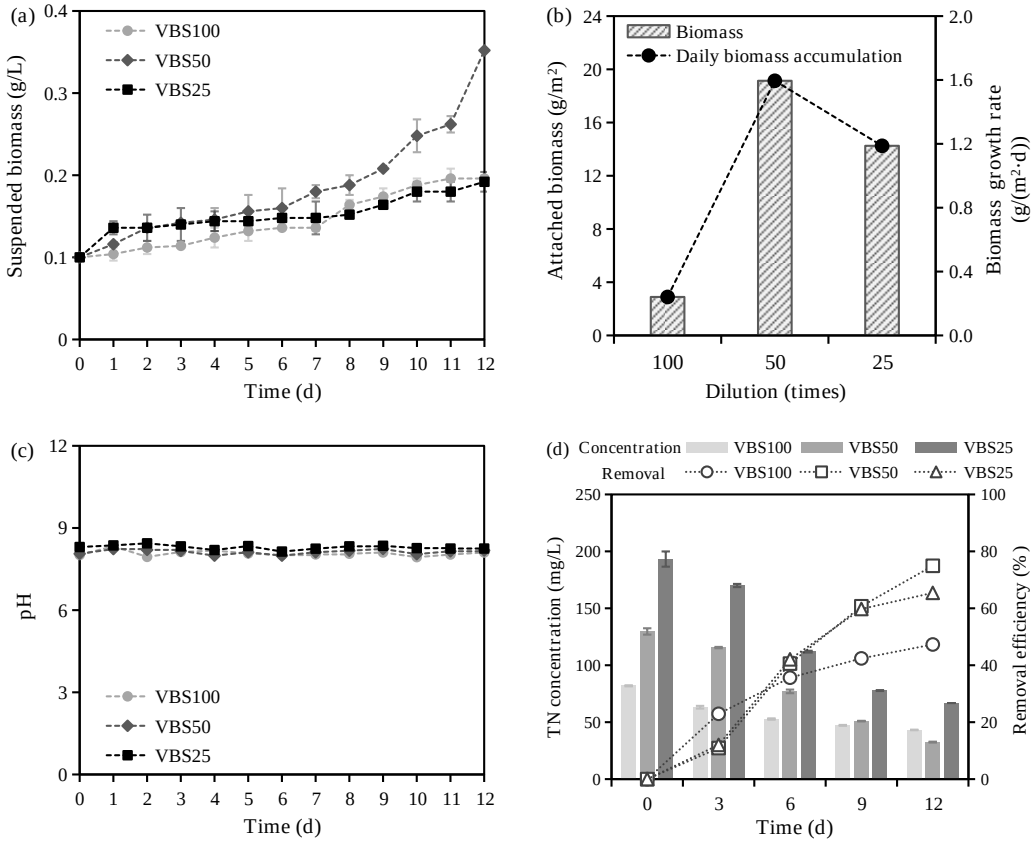
After 54 days of cultivation, the removal efficiency of TN in the groups under the inoculum ratios of 10%, 20%, 40%, and 60% was 46.52%, 26.14%, 32.85%, and 31.00%, respectively (Figure 3b). For NH_4^+-N, it was almost completely removed within 6 days of cultivation (Figure 3c). The removal efficiency of NH_4^+-N was 98.56%, 98.27%, 95.27% and 91.06% with microalgae inoculation of 10%, 20%, 40% and 60%, respectively. The group with the highest removal efficiency of TN and NH_4^+-N was 10% inoculum ratio, and this result was consistent with the results of microalgae under the same conditions to obtain the best biomass accumulation.

As shown in Figure 3d, TP was absorbed in the groups with 10% and 20% inoculum ratios, and the removal efficiency was 99.37% and 99.17% on the 12th day, respectively. However, in the groups with inoculum ratios of 40% and 60%, TP concentration was drastically reduced on the 4th day, and the removal efficiency was 97.92% and 83.33%, respectively.

The appropriate microalgae specie, inoculum ratio and VBS proportion were determined by batch tests. These results indicated that *Desmodesmus* sp. showed the strong resistance causing high biomass accumulation and TP removal efficiency of 0.62 g/L and 77.03%, respectively. Microalgae worked better in low VBS proportion (10–30%) groups than high VBS proportion (50%) because of limited ammonia inhibition. Compared high inoculum, the 10% microalgae inoculum guaranteed the light transmission, which had best biomass growth rate of 0.04 g/(L·d) and TN removal of 46.52%.

3.4. Effect of Different VBS Concentrations in the Rotating Biofilm Reactor

The biomass of *Desmodesmus* sp. cultured in the rotating biofilm reactor under three different VBS dilution group is shown in Figure 4a. After 6 days, the biomass of the group VBS50 increased rapidly, and the biomass growth rate was significantly higher than that of group VBS25 and VBS100. After 12 days of cultivation, the suspended microalgae biomass in the VBS25, VBS50, and VBS100 groups was 0.20, 0.35 and 0.19 g/L, respectively. The biomass on the attached material of the rotating biofilm reactor in the VBS25, VBS50, and VBS100 groups was 2.89, 19.14 and 14.25 g/m^2 as shown in Figure 4b. The biomass growth rate in the VBS50 group was the highest 1.60 g/(m^2·d). The pH of the culture solution was maintained stably between 7.9 and 8.5 after 12 days of cultivation in diluted VBS (Figure 4c).



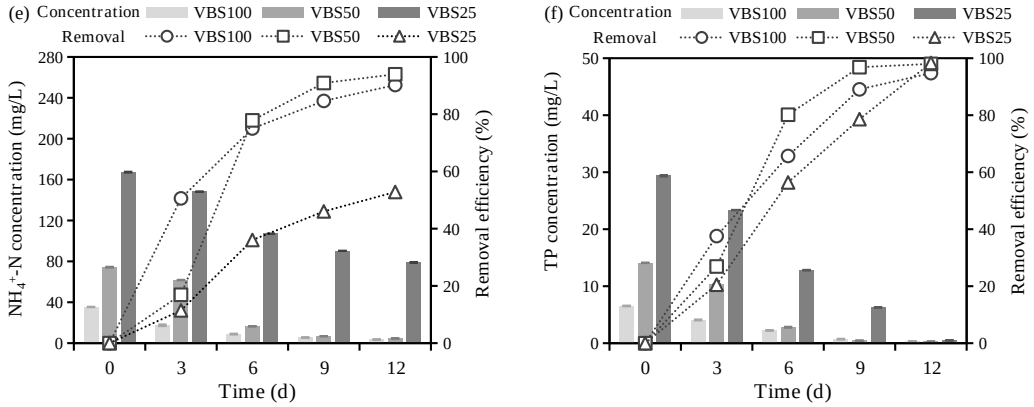


Figure 4. Effects of different concentrations of vinasse biogas slurry (VBS) on suspended microalgae biomass (**a**) and attached microalgae biomass changes (**b**), pH changes (**c**), and concentrations and removal efficiency of TN (**d**), NH_4^+-N (**e**), and TP (**f**) in VBS; Note: VBS100, VBS50, and VBS25 designate VBS diluted at 100 times, 50 times and 25 times, respectively.

Using the rotating biofilm reactor to cultivate *Desmodesmus* sp. for 12 days, the TN concentration gradually decreased, and the TN removal efficiency of the VBS25, VBS50, and VBS100 groups was 47.29%, 74.93% and 65.43%, respectively (Figure 4d). The maximum TN removal was observed in the VBS50 group. Figure 4e shows the NH_4^+-N removal in groups at different dilution levels. The NH_4^+-N concentrations decreased rapidly during the first 5 days, but the decrease slowed down after 5 days. At the 8th day, the NH_4^+-N concentrations in the VBS50 and VBS100 groups were depleted. The subsequent growth of the microalgae required the use of other forms of nitrogen sources, such as nitrate nitrogen and nitrite nitrogen, to maintain cell growth and metabolism [41]. After 12 days, NH_4^+-N removal was 52.81%, 93.94% and 90.19% in the VBS25, VBS50, and VBS100 groups, respectively (Figure 4f). Within 12 days, the TP removal efficiency in the VBS25, VBS50, and VBS100 groups was 94.76%, 98.01%, and 98.34%, respectively. In conclusion, the VBS50 group was effective for microalgae culture and treatment.

3.5. Different Attached Materials in the Rotating Biofilm Reactor

The 50-time diluted VBS was used as the culture medium in this test. The microalgae biomass in the rotating biofilm reactor with different attached materials of twill, cotton and linen coarse cloth (CLCC), and coarse ribbed fiber fabric (CRFF) is shown in Figure 5a. The initial microalgae biomass concentration was 0.48 g/L, which increased to 0.61, 0.54, and 0.54 g/L by 10 days, respectively. On the 10th day, the microalgae concentration was halved, but thereafter the microalgae biomass concentration continued to rise. At 20 days of cultivation, the biomass concentrations reached 0.39, 0.33, and 0.34 g/L, respectively. During the cultivation process, the motor failure of the CLCC reactor caused the test of this group to terminate on the 22nd day, and the suspended biomass concentration in the reactor was grown to reach 0.18 g/L. The concentrations of suspended biomass in the twill and CRFF reactors was grown to the 30th day to reach 0.24 and 0.25 g/L, respectively, with little difference ($p > 0.05$).

The growth situation and biomass accumulation of microalgae on attached materials are important indicators for evaluating attachment materials. Microalgae biomass on three attached materials harvested on the final day of culture are shown in Figure 5b. The attached microalgae biomass in the three groups with twill, CRFF, and CLCC was 22.40, 48.76, and 7.20 g/m^2, and the daily biomass accumulation was 0.75, 1.63, and 0.24 g/(m^2·d), respectively. The surface situation of the three attached materials after cultivation is shown in Figure 5c. The material, CRFF, showed good microalgae attached performance, but it showed obvious decay when cultivation to 30 days, indicating its poor durability. The attached capacity of CLCC to algae was not inferior to that of twill, and the algae was evenly distributed. Taken together, CRFF was optimal for the growth of microalgae attached biomass.

Figure 6a shows that the NH_4^+-N concentration decreased in a straight line during the cultivation process. To the 10th day, the NH_4^+-N removal efficiency in the three reactors of twill, CRFF, and CLCC was 60.90%, 76.30% and 65.90%, respectively (Figure 6b). After the renewal of the culture medium, the NH_4^+-N still showed a intensively decrease trend. After 20 days of cultivation, the concentration of NH_4^+-N in the culture medium was decreased to 16.05, 6.79, and 20.06 mg/L, respectively. Among them, CRFF attached materials had the best effect on removing NH_4^+-N. The TP concentration showed a linear decrease during the culture (Figure 6c). By 10 days, TP removal was above 98% with no significant difference ($p > 0.05$) (Figure 6d). After updating part of the culture

medium on the 10th day and the 20th day, the TP concentration was first increased to 9 mg/L and gradually utilized during the subsequent 10 days cultivation.

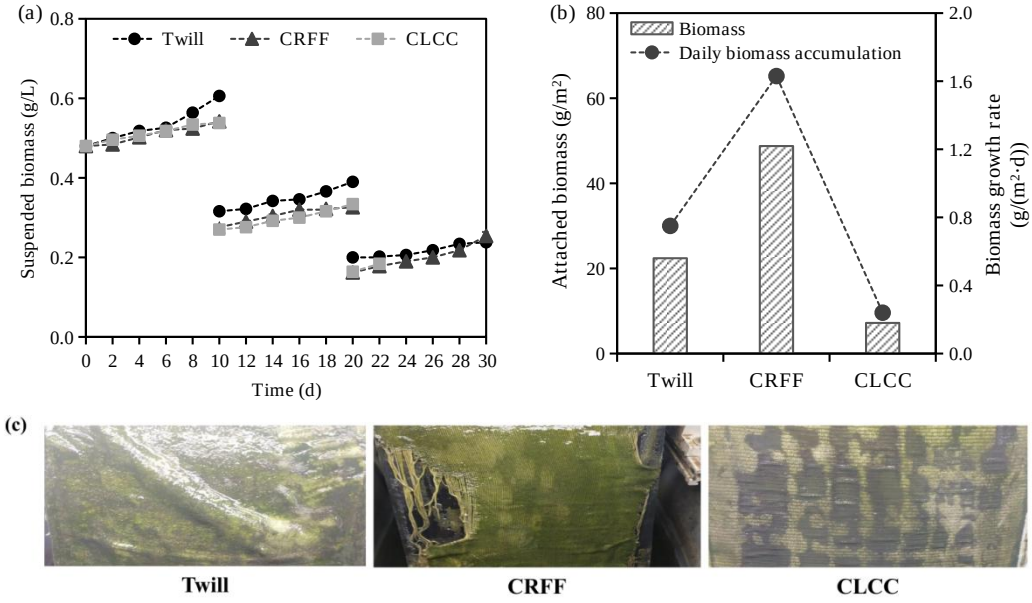


Figure 5. Effects of different attachment materials (twill; CRFF, coarse ribbed fiber fabric; CLCC, cotton and linen coarse cloth) on suspended microalgae biomass (**a**) and attached microalgae biomass changes (**b**), the surface condition after 30 days of cultivation (**c**) in vinasse biogas slurry (VBS).

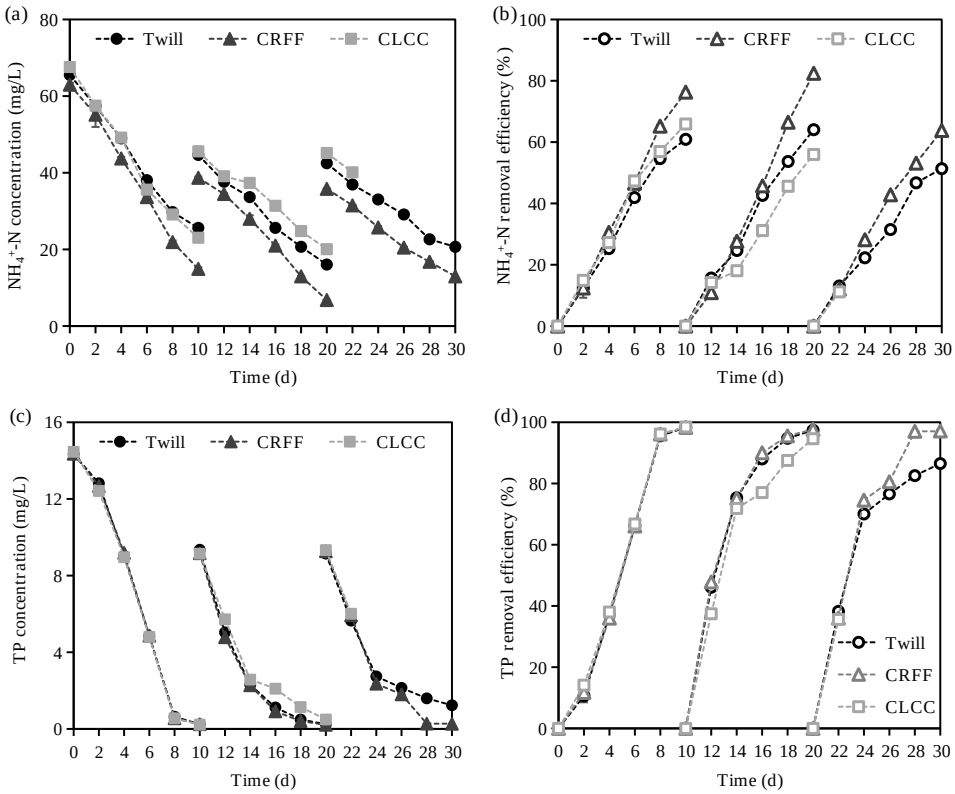


Figure 6. Effects of different attachment materials (twill; CRFF, coarse ribbed fiber fabric; CLCC, cotton and linen coarse cloth) on concentrations of NH_4^+-N (**a**), TP (**b**), and removal efficiency of NH_4^+-N (**c**), TP (**d**).

The effects of different VBS dilutions and attached materials on VBS treatment by microalgae were investigated in the rotating biofilm reactor. Experimental data revealed neither high (100 times) nor low (25 times) dilution supported optimal biomass cultivation. The 50-time dilution group demonstrated peak productivity with 0.35 g/L suspended biomass and 19.14 g/m^2 attached biomass, concurrently achieving maximum nutrient removal

efficiencies. Among tested materials, the coarse ribbed fiber fabric (CRFF) exhibited superior biofilm formation capacity, attaining 76.30% NH_4^+-N removal. These findings establish CRFF as the optimal carrier material for enhanced nutrient recovery in the photo-bioreactor system.

4. Conclusions

This study demonstrated that *Desmodesmus* sp. exhibited better performance than *Chlorella vulgaris* and *Scenedesmus quadricauda* for vinasse biogas slurry (VBS) treatment, achieving the highest biomass and phosphorus removal efficiency. The low VBS proportion (10–30%) groups exhibited better microalgae growth and nutrient removal than the 50% VBS group. The microalgae inoculum ratio of 10% obtained the highest TN removal and biomass accumulation. The rotating biofilm reactor with 50-time diluted VBS achieved 19.14 g/m^2 attached biomass and 93.94% NH_4^+-N removal, and coarse ribbed fiber fabric (CRFF) obtained higher attached biomass than twill and cotton and linen coarse cloth (CLCC). These results further confirmed the potential of integrating microalgae culture and nutrient removal for wastewater treatment using rotating biofilm reactor. In the future, the coupling of the rotating biofilm reactor system with the traditional wastewater treatment process can be further explored, so as to realize the biomass energy recovery while improving the efficiency of N and P removal. The further study may focus on the treatment stability under different water quality characteristics, cost optimization and life cycle assessment. It lays the engineering foundation for the construction of low-cost and high-load microalgae-biofilm collaborative treatment process, and promotes the substantial leap of this technology from laboratory research to industrial application.

Author Contributions

S.L. (Shupeng Lin): Conceptualization, Investigation, Data curation, Writing—original draft. Z.C.: Conceptualization, Data curation, Writing—Review & Editing. H.W.: Conceptualization, supervision, Writing—Review & Editing. R.D.: Conceptualization, Methodology. S.L. (Shan Liu): Supervision, Conceptualization, Writing—review & editing, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflict of interest.

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