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Effects of C/N ratio on pollution removal efficiency and cell proliferation during the bioconversion of wastewater by photosynthetic bacteria

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ABSTRACT

Photosynthetic bacteria (PSB) bioconversion is a new technology for wastewater treatment and resource recovery. The C/N ratio is an important factor in biological wastewater treatment. For the first time, the efficient C/N ratio range for PSB bioconversion, and the effects of the C/N ratio on wastewater treatment efficiency and cell proliferation, were studied. The results of this study showed that PSB bioconversion was efficient when the wastewater C/N ratio was within the range of 400–0.1. Chemical oxygen demand (COD) removal was higher than 60% in this C/N range. The change in the NH_4^+-N concentration was fitted by the logistic model for each C/N ratio. The V_{max} of nitrogen removal was similar to that of other technologies and it decreased as the C/N ratio decreased. This shows that this technology exhibits reliable pollutant removal in the C/N ratio range of 400–0.1. The nitrogen transformation study indicated that the PSB might use a specific mechanism under a low C/N ratio. PSB cell proliferation was fitted by the logistic model in the C/N ratio range of 400–2, and μ_{max} was found to be not correlated with the C/N ratio. The protein content in the cells was 40–60% in the C/N ratio range of 400–0.1. The results showed that PSB bioconversion technology has a very high resource value and the value of the C/N ratio places little restriction on it.

Keywords: Photosynthetic bacteria; C/N ratio; Logistic model; Nitrogen removal

1. Introduction

Photosynthetic bacteria (PSB) bioconversion technology has attracted extensive attention since the 1960s [1]. Firstly, this technology can effectively remove pollutants in wastewater [2]. For example, chemical oxygen demand (COD), total nitrogen, and total phosphorus removal reached 99%, 98%, and 97%, respectively [3,4]. Secondly, this technology can realize resource recovery. The reason is that PSB cells contain high-value products such as coenzyme Q10 and 5-aminolevulinic acid, which are widely used as additives for breeding in livestock and aquaculture [5]. The market price of PSB suspensions is 0.3–2 USD/L. Furthermore, the resource recovery of PSB cells has solved the problem of residual sludge caused by conventional technologies [6,7]. Thus, PSB bioconversion of wastewater is a novel technology of great potential.

The C/N ratio is the ratio of COD to total nitrogen (TN) or biochemical oxygen demand (BOD) to TN [8,9]. It is not only a key factor for microorganism growth, but it is also an important wastewater quality index [10–12]. Thus, the C/N ratio is an important factor in biological wastewater treatment. Generally, when the C/N ratio is too high, the lack of nitrogen inhibits microorganism growth; when the C/N ratio is too low, the removal of nitrogen and the final nitrogen concentration of the effluent cannot meet the amount required. For different technologies, the effects of the C/N ratio are different. For the activated sludge method, the appropriate C/N ratio is 10–20 [13,14]. Kim et al. [15] reported that the optimal C/N ratio was 8 in an anaerobic granular membrane bioreactor. For algae bioconversion technology, the appropriate C/N ratio was

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found to be in the range of 7–39 [16]. However, there is no research about the effect of the C/N ratio on PSB wastewater bioconversion.

Thus, this study investigated the effects of the C/N ratio on PSB wastewater bioconversion. The initial C/N ratios used in this study were 400, 100, 50, 20, 10, 5, 2, 1, 0.5, and 0.1. The C/N ratio of wastewater can reach 300, such as in brewery wastewater. Therefore, a maximum C/N ratio of 400 was chosen. It has been reported that PSB have a good efficiency in a C/N ratio range of 10–40; thus, C/N ratios of 10, 20 and 50 were selected [17]. A middle value of 100 was chosen between 400 and 50. The optimal C/N ratio for conventional aerobic treatment is 20. In recent years, low C/N ratios (<5) have been frequently studied [9–11] and so low C/N ratios of 5, 2, 1, 0.5 and 0.1 were also included. A middle value of 10 was also chosen between 20 and 5.

This study aimed to investigate the fundamental characteristics of PSB under different C/N ratios. COD removal, nitrogen removal, PSB cell proliferation and protein content under different initial C/N ratios were all investigated.

2. Materials and methods

2.1. PSB

The PSB strain used was *Rhodobacter Sphaeroides*, with a Gen Bank accession number of CP001151.1. This strain was isolated from a local pond and it has been effectively applied in wastewater bioconversion processes. The PSB strain was cultured in RCVBN medium (70/30, v/v). The thermostat shaker was 120 rpm and 26–30°C [18].

2.2. Wastewater

Sugar wastewater was chosen for this study. Artificial sugar wastewater was formulated with ammonium sulfate, malic acid, saccharose, sodium bicarbonate, and potassium dihydrogen phosphate. The specific components and characteristics of each reactor are shown in the Supplementary Materials tables S1 and S2. The C/N ratio range of 400–0.1 was determined via data from the literature and data of actual wastewater [19,20]. The initial pH was 6.8–7.2. Please note that all C/N ratios refer to the initial C/N ratio.

2.3. Experimental setup

Experiments were conducted in batch bioreactors in 1 L conical flasks at the laboratory scale. The ratio of wastewater to PSB was 80/20 (v/v). The light oxygen condition was dark-aerobic. Darkness was achieved by wrapping aluminum foil onto the reactors. The dissolved oxygen concentration of 2–4 mg/L was achieved by aeration. The temperature was maintained at 26–30°C.

2.4. Analysis methods

The samples were taken out at 0, 12, 24, 48, and 72 h. Separation of the PSB was achieved by centrifugation at 9000 rpm for 10 min. COD and NH_4^+-N were tested using the supernatant by the APHA Method [18]. PSB biomass was measured via OD_{660} [21]. The pH was measured by a pH tester (Mettler Toledo FE20 Five Easy Plus). The dissolved oxygen concentration was measured by a YSI 550A dissolved oxygen meter. Protein mass was tested using determination kits by the Lowry method [22]. The protein content of the PSB cells was calculated using Eq. (1).

$$\textit{protein content}(\%) = \frac{\textit{protein}(g)}{\textit{dry cell weight}(g)} * 100\% \quad (1)$$

2.5. Statistical analysis

All experiments were carried out three or more times. The reported values are the averages of the repeated experiments. SPSS 22.0 was used to analyze the significance.

2.6. Calculation of kinetics

The logistic model was applied for the calculation of kinetics because it is frequently used as a tool in biological growth and material utilization studies [23]. The logistic model equation can be seen in Eq. (2):

$$r = \frac{a_1 - a_2}{1 + \left(\frac{x}{x_0}\right)^{r_{\max}}} + a_2 \quad (2)$$

where r is reaction rate, r_{max} is the maximal rate, x is the substrate concentration and a_1, a_2 and x_0 are coefficients.

The Gompertz and Bertallanffy models were also tested but the fitting results were unsatisfactory and thus, the results are not reported.

3. Results and discussion

3.1. COD removal under different initial C/N ratios

COD removal was higher than 60% over the entire range of C/N ratios from 400 to 0.1 (Fig. 1). This showed that for COD removal, PSB bioconversion is very flexible and the C/N ratio is not restrictive. Other studies [24,25]

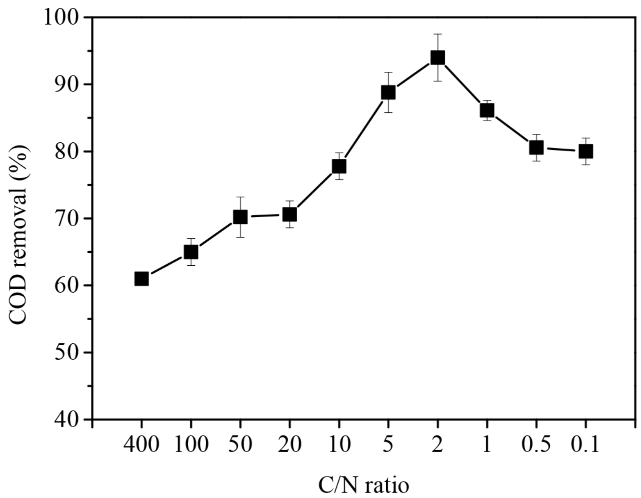

Fig. 1. COD removal under different initial C/N ratios.

have reported that PSB could remove more than 60% of COD wastewater loads under extreme C/N ratios. Zhi et al. [26] showed less than 50% COD removal with a C/N ratio of 2 in a constructed wetland. Miao et al. [27] reported about 50% COD removal with a C/N ratio of 1 using an activated sludge process. Compared with these conventional technologies, the PSB in this study achieved a higher removal efficiency (more than 85%) with a C/N ratio range of 1–2.

In the high C/N ratio range (400–50) and middle C/N ratio range (20–5), COD removal decreased as the C/N ratio increased. This was due to the fact that COD had increased relative to the biomass in the system. As the C/N ratio varied from 400 to 0.1, the COD concentration varied from 40000 to 500 mg/L. A high C/N ratio indicates high COD concentration. Under a high COD concentration, the removal efficiency was low but the absolute amount removed was still high. This phenomenon is common in traditional microorganisms. In the low C/N ratio range (2–0.1), COD removal increased as C/N ratio increased. When the C/N ratio increased to 1–0.1, the relatively high nitrogen concentration might become inhibitive and lower the bioactivity of the PSB, thus decreasing the COD removal ability of the PSB. Within this C/N ratio range, a high nitrogen concentration inhibited the pollution removal ability of the PSB.

3.2. NH_4^+-N removal under different initial C/N ratios

3.2.1. NH_4^+-N removal

The PSB could remove nitrogen over the entire range of C/N ratios from 400 to 0.1 (Fig. 2). In an activated sludge membrane bioreactor and constructed wetland, the NH_4^+-N removal was about 50% with a C/N ratio of 2 [10,26]. Compared with these technologies, the PSB achieved similar removal efficiency with a C/N ratio of 2.

On the whole, NH_4^+-N removal decreased as the C/N ratio decreased and the nitrogen concentration increased as the C/N ratio decreased. Therefore the nitrogen removal ratio decreased with a given dosage of bacteria but the absolute amount of nitrogen removal was higher at lower C/N ratios. Another study [28] reported that NH_4^+-N removal by PSB in a pilot-scale bioreactor decreased when the C/N ratio increased from 3 to 12. The change in ammonia removal as the C/N ratio varied was different between PSB bioconversion and activated sludge. This was because the nitrogen utilization pathways of PSB were different from those in conventional biological wastewater treatment technologies [18,29–31].

NH_4^+-N removal with a C/N ratio of 2 is higher than that of a C/N ratio of 5. By combining the result of the highest COD removal with a C/N ratio of 2 (3.1) and this result, it can be seen that a specific mechanism may be used by the PSB when there is a low C/N ratio (2–0.1).

To thoroughly study the nitrogen removal process of PSB, the NH_4^+-N, NO_3^--N, and NO_2^--N concentration changes in the high, middle, and low C/N ratio ranges were investigated (Fig. 3). The representative high, middle, and low C/N ratios were C/N ratios of 100, 20, and 0.5, respectively. It can be seen from Fig. 3a and c that NH_4^+-N and NO_2^--N decreased, and no NO_2^--N accumulation occurred under all C/N ratios. The concentration of NO_3^--N increased from the start and reached 30 mg/L with a C/N ratio of 100, whereas it decreased from the start and then was not observed at all when the C/N ratio was 20 and 0.5. In the activated sludge process, as the C/N ratio increased, NO_3^--N decreased, and NO_2^--N increased [10,32,33]. Sheng et al. [34] and Mannina et al. [10] reported that the bacteria was primarily ammonia oxidizing bacteria and nitrite oxidizing bacteria with a C/N ratio of more than 1, and the bacteria was primarily anammox bacteria with a C/N ratio of less than 1. By comparing the different nitrogen transformations, the nitrogen removal efficiency of PSB was close to that of anammox bacteria at a very low C/N ratio.

3.2.2. Dynamics of NH_4^+-N removal

The change of NH_4^+-N concentration with a C/N ratio of 400–0.1 was fitted by the logistic model, which has been widely used in kinetic studies of wastewater biotreatment processes [35,36]. As Fig. 4 shows, the change in the NH_4^+-N concentration can be fitted by the logistic model for each C/N ratio. The fitted line for each C/N ratio was different. This shows that the process of NH_4^+-N removal by the PSB varied.

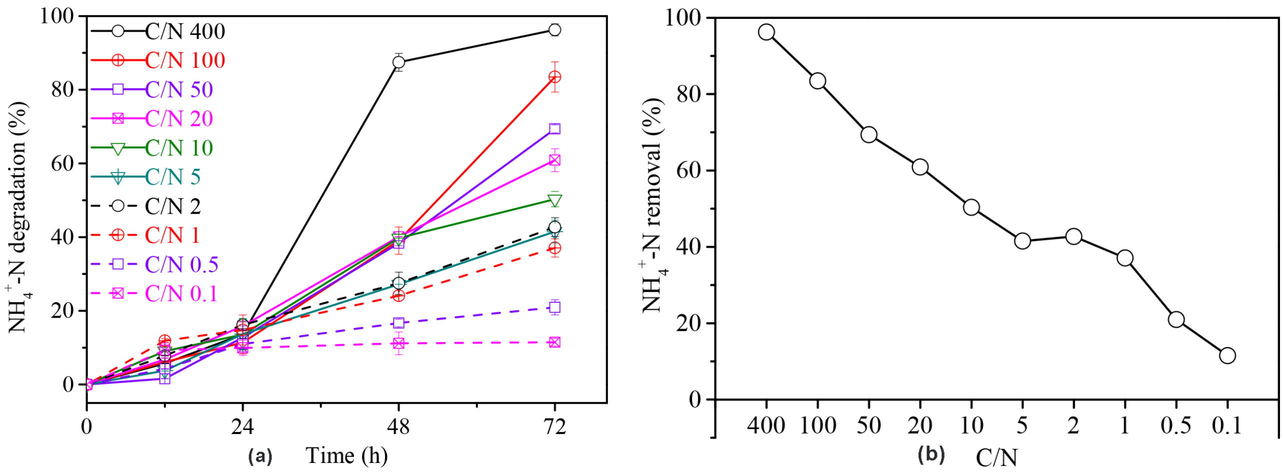

Fig. 2. NH_4^+-N removal under different C/N ratios (a) over 72 h and (b) at 72 h.

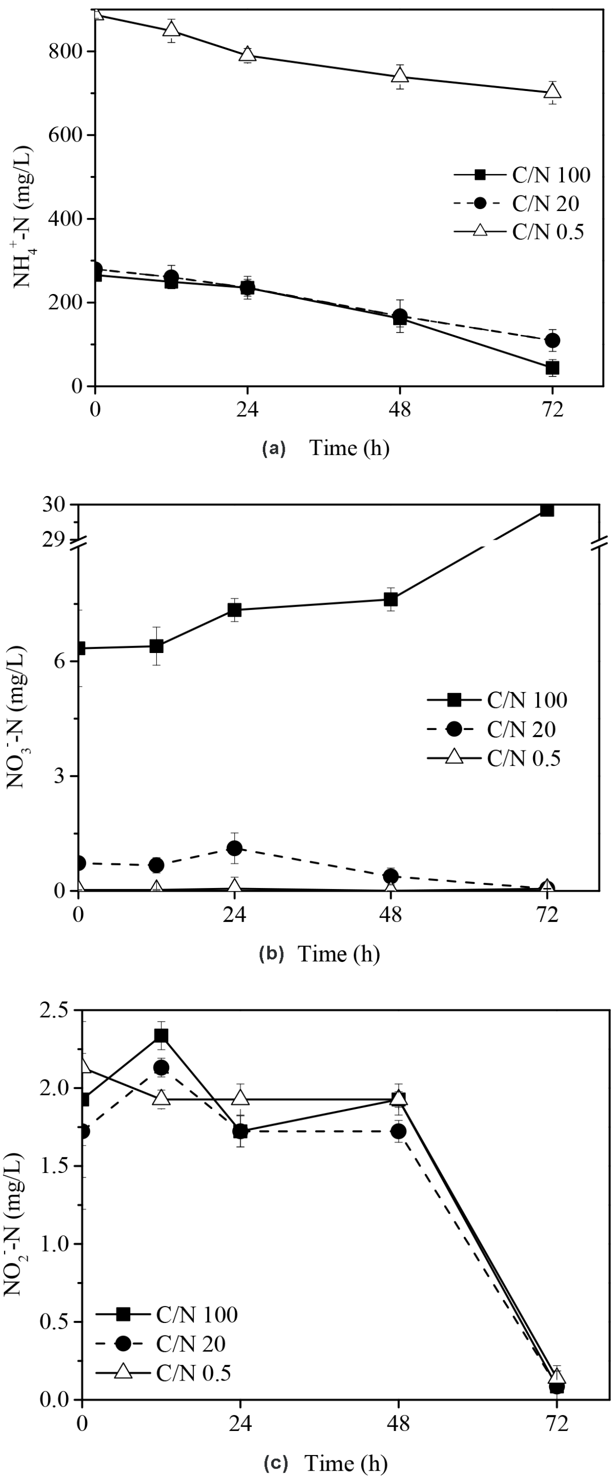

Fig. 3 (a). NH_4^+-N concentration, (b) NO_3^--N concentration, and (c) NO_2^--N concentration under different initial C/N ratios of 100, 20, and 0.5 for over 72 h

V_{max} and R^2, which were determined using the logistic model, are summarized in Table 1a. The R^2 value (>0.98) shows that the experimental data fitted the logistic model well ($P < 0.01$). Overall, V_{max} decreased as the C/N ratio decreased.

The kinetic parameters of NH_4^+-N removal from other technologies are summarized in Table 1b. The table shows that the V_{max} of PSB bioconversion was similar to that of conventional activated sludge technologies for each C/N ratio. This result showed that nitrogen removal by PSB bioconversion of wastewater is similar to nitrogen removal using conventional activated sludge technologies.

3.3. PSB cell proliferation under different C/N ratios

3.3.1. PSB cell proliferation

The PSB showed growth over the entire range of C/N ratios from 400 to 0.1 (Fig. 5). Certain PSB cell accumulation under extreme C/N ratios was also reported by previous studies [24,25]. Differing from activated sludge technology, cell proliferation is greatly advantageous because PSB cells are valuable products in PSB bioconversion technology.

In the high C/N ratio range (400–50), PSB biomass maintained a high value (more than 1800 mg/L) because the high COD concentration provided sufficient food. In the middle C/N ratio range (20–5) and low C/N ratio range (2–0.1), PSB biomass generally decreased as C/N decreased. This trend is coincident with COD removal and NH_4^+-N removal.

The information gained on COD removal, NH_4^+-N removal, and PSB cell proliferation shows that PSB bioconversion technology is efficient in the C/N ratio range of 400–0.1.

3.3.2. Kinetics of PSB cell proliferation

The change in PSB cell proliferation for each different initial wastewater C/N ratio was also fitted by the logistic model. As Fig. 6 shows, the cell proliferation of the PSB can be fitted by the logistic model with a C/N ratio range of 400–2. The data for the C/N ratios of 1–0.1 cannot be fitted by the logistic model, which might be because the mechanism of PSB growth was different under different C/N ratios.

μ_{max} and R^2, which were determined using the logistic model, are summarized in Table 2a. The R^2 value (>0.92) shows that the experimental data fit the logistic model well ($P < 0.01$). The μ_{max} value for each C/N ratio was different. There is no corresponding relationship between PSB cell proliferation and the C/N ratio.

The kinetic parameters of cell proliferation in other technologies are summarized in Table 1b. The μ_{max} values were all clearly higher. This shows that PSB cell proliferation via PSB bioconversion is notably higher than that through other biological nitrogen wastewater treatment technologies. This is a great advantage because the cells are valuable products in PSB bioconversion technology.

3.4. Protein content in PSB cells under different C/N ratios

In the PSB bioconversion process, protein-rich cells can be cultured simultaneously with nitrogen removal. The PSB cells can be used as aquatic feed and soil additives because of their high protein content and so protein content is an important index for PSB bioconversion. As Fig. 7 shows, the protein content in the PSB cells first increased and then

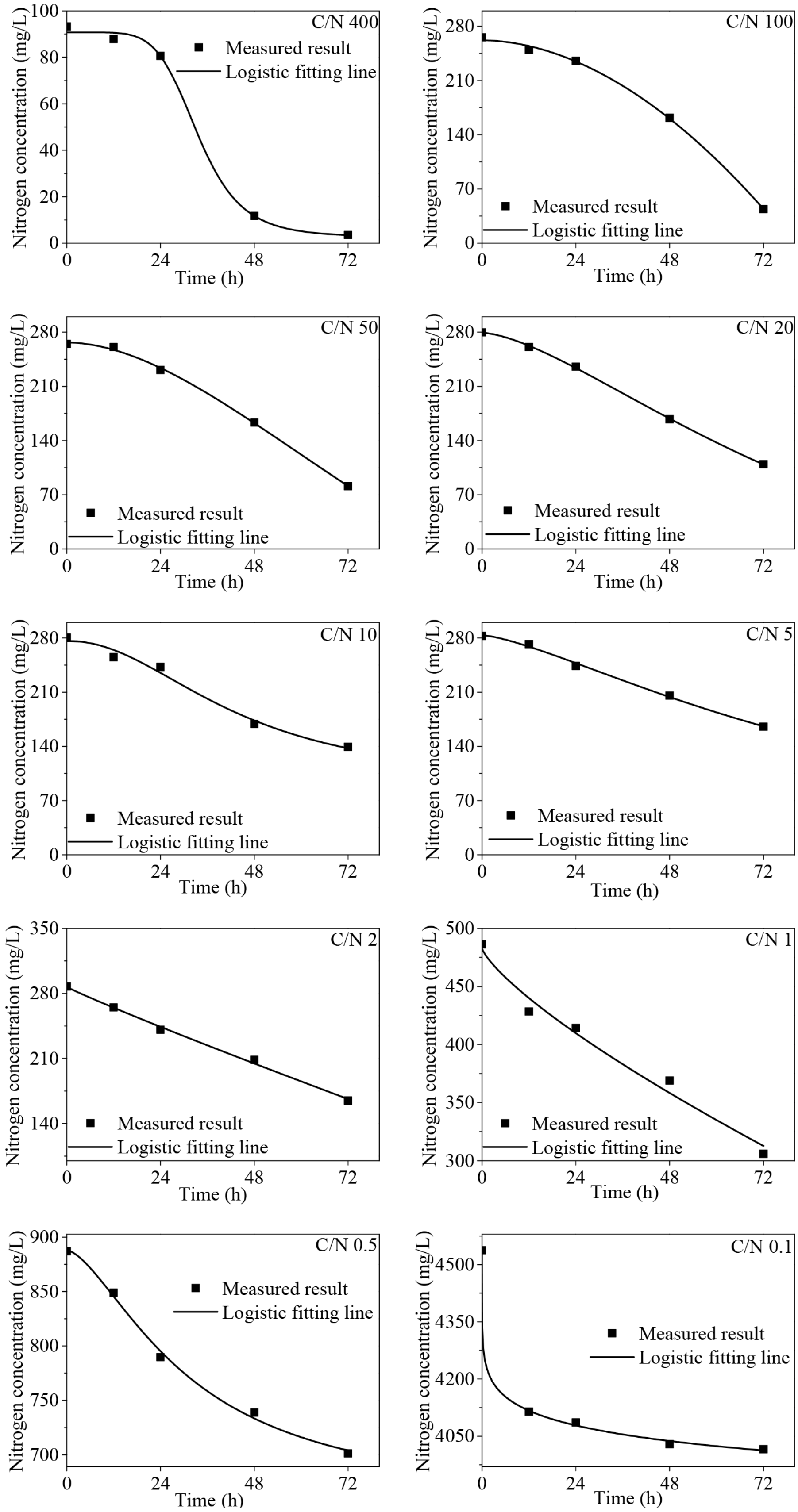

Fig. 4. Change in the NH_4^+-N concentration fitted using the logistic model.

Table 1
V_{max} of nitrogen in PSB bioconversion

a. This study

	C/N 400	C/N 100	C/N 50	C/N 20	C/N 10	C/N 5	C/N 2	C/N 1	C/N 0.5	C/N 0.1
V_{max}	6.03	1.89	1.85	1.54	2.10	1.39	0.94	0.77	1.56	0.17
R^2	0.998	0.999	0.999	0.999	0.987	0.997	0.997	0.982	0.997	0.999

b. Comparison of V_{max} of nitrogen removal by PSB bioconversion technology with those of conventional biodegradation technologies

Technology	Microorganism type	V_{max} (d^{-1})	C/N ratio	Reference
UASB	Anammox bacteria	1.3	1	[37]
Simultaneous anammox and heterotrophic denitrification process	Anammox bacteria, heterotrophic denitrifying bacteria	0.9	1.5–2.0	[38]
MBR	Ammonia oxidizing bacteria	0.18	5	[39]
A/O process	Ammonia oxidizing bacteria	2.8	5	[40]
UASB	Ammonia oxidizing bacteria	0.44	5	[41]
CAS	Ammonia oxidizing bacteria	0.18	6	[42]
SND	Paracoccusdenitrificans ISTOD1	7.6	10	[43]

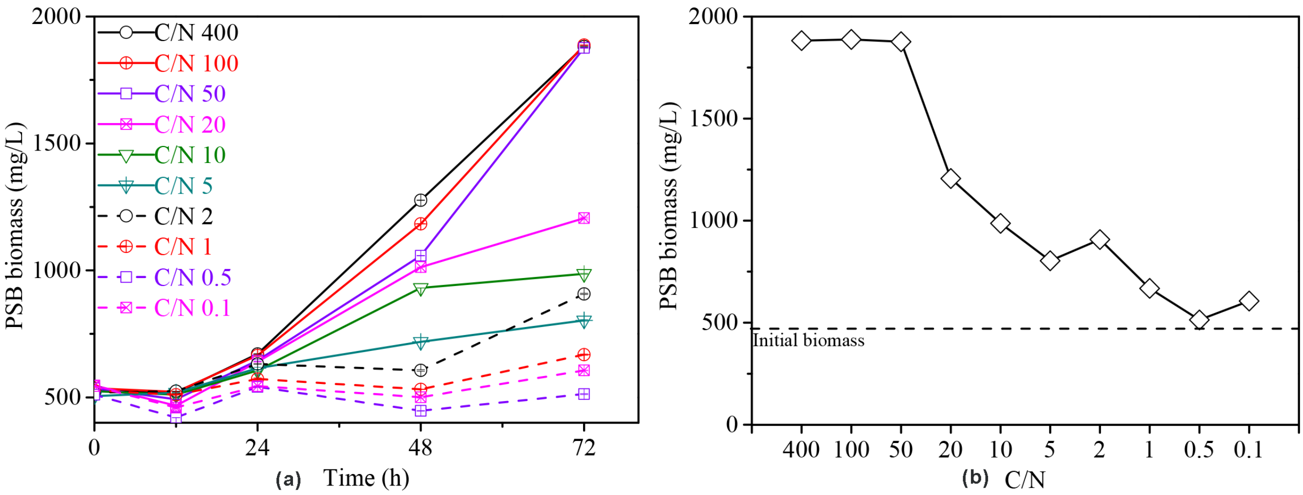

Fig. 5. PSB biomass under different C/N ratios (a) over 72 h and (b) at 72 h.

decreased with decrease in the C/N ratio. In an activated sludge process, the protein content of cells changes at different C/N ratios [47]. Dong et al. [48] also reported that protein content increased with increasing C/N ratios in an aerobic granular sludge system.

The protein content was 40–60% with a C/N ratio of 400–0.1 (Fig. 7). This protein content means the PSB cells were a high quality additive for breeding in aquaculture and livestock. The result also showed that for protein content in PSB cells, the C/N ratio is not restrictive.

Within the high C/N ratio range (400–50), protein content in PSB cells increased as the C/N ratio decreased. Within the middle C/N ratio range (20–5) and low C/N range (2–0.1), protein content decreased as the C/N ratio decreased. By looking at the results of pollution removal, PSB biomass, and protein content, it is clear that the PSB had a specific mechanism under the low C/N ratio range (2–0.1). The increase of protein content under C/N ratio of 400–20 is because that nitrogen concentration increase. The highest protein content was with a C/N ratio of 20 and this indicated that it was the best C/N ratio for PSB protein production. The decrease of protein content was also due to the nitrogen inhibition.

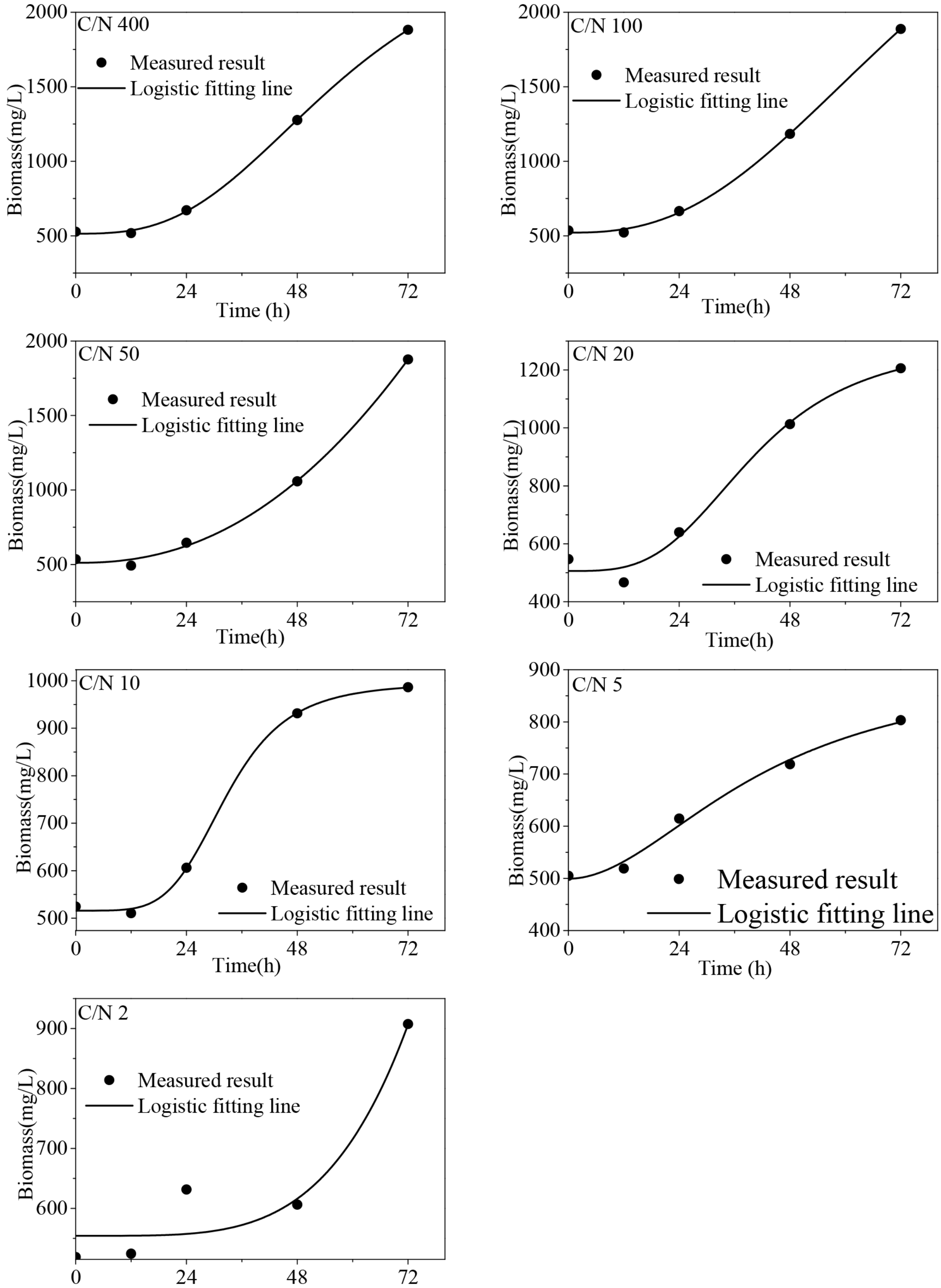

Fig. 6. PSB cell proliferation fitted by the logistic model.

4. Conclusions

Wastewater C/N ratios for PSB bioconversion were investigated for the first time in this study. PSB bioconversion was effective in the C/N ratio range of 400–0.1. COD removal was higher than 60%, and the protein content was 40–60% in the C/N ratio of 400–0.1. The change in the NH_4^+-N concentration was fitted by the logistic model for each C/N ratio, and V_{max} decreased as the C/N ratio decreased. The nitrogen transformation study indicated that PSB might use a specific mechanism under a low C/N ratio (2–0.1). PSB cell prolifer-

Table 2
μ_{max} of cell proliferation in PSB bioconversion

a. This study

	C/N 400	C/N 100	C/N 50	C/N 20	C/N 10	C/N 5	C/N 2	C/N 1	C/N 0.5	C/N 0.1
μ_{max}	2.88	2.57	2.28	3.37	4.84	1.92	4.29	–	–	–
R^2	0.999	0.999	0.998	0.988	0.999	0.993	0.923	–	–	–

b. Comparison of the μ_{max} of nitrogen removal by PSB bioconversion technology with those of conventional biodegradation technologies

Technology	Microorganism type	μ_{max} ($^{-1}$)	C/N ratio	Reference
Simultaneous anammox and heterotrophic denitrification process	Anammox bacteria, heterotrophic denitrifying bacteria	0.21	1.5–2.0	[38]
Heterotrophic denitrification process	Acinetobacter sp. strain	0.02	2	[44]
SBR	Ammonia oxidizing bacteria	0.0015	2.5	[45]
MBR	Ammonia oxidizing bacteria	0.72	3	[46]
Heterotrophic denitrification process	Acinetobacter sp. strain	0.015–0.012	4–10	[44]
MBR	Ammonia oxidizing bacteria	0.23	10	[10]

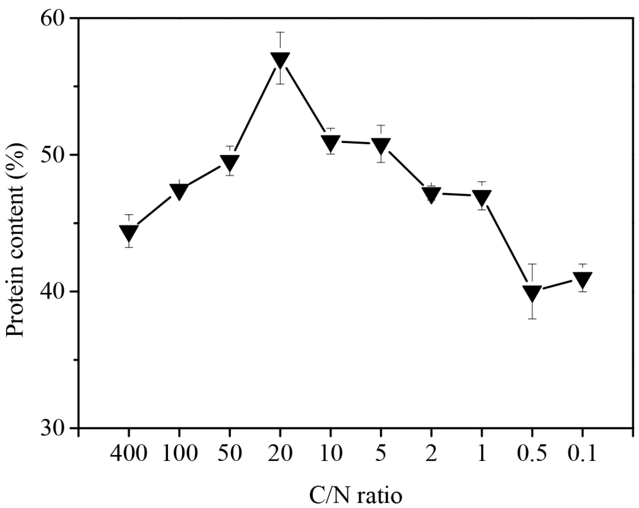

Fig. 7. Protein content in PSB cells under different initial C/N ratios.

ation was fitted by the logistic model in the C/N ratio range of 400–2. By comparing the kinetic coefficients with other technologies it has been shown that PSB bioconversion technology exhibited reliable pollutant removal and a very high resource value within the C/N ratio range of 400–0.1. The best C/N ratio was different for different purposes: for COD removal, the best C/N ratio was 2; for NH_4^+-N removal, the best C/N ratio was 400; for PSB biomass accumulation, the best C/N ratio was 100–50; and finally for protein content, the best C/N ratio was 20.

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Supplementary material

Table S1 reports the wastewater NH_4^+-N, COD, and C/N data. The NH_4^+-N range was 100–5,000 mg/L,and the COD concentration was 500–40,000 mg/L; this range was appropriate for PSB wastewater bioconversion [1,2].

Table S1
Wastewater NH_4^+-N, COD, and C/N in this study

Reactor	C/N	Initial NH_4^+-N concentration (mg/L)	Initial COD concentration (mg/L)
1	400	100	40000
2	100	300	30000
3	50	300	15000
4	20	300	6000
5	10	300	3000
6	5	300	1500
7	2	300	600
8	1	500	500
9	0.5	1000	500
10	0.1	5000	500

Table S2
The reagent and dosage (g) in each group of experiments

Reactor	1 (C/N=400)	2 (C/N=100)	3 (C/N=50)	4 (C/N=20)	5 (C/N=10)	6 (C/N=5)	7 (C/N=2)	8 (C/N=1)	9 (C/N=0.5)	10 (C/N=0.1)
Ammonium sulphate	0.11	0.34	0.34	0.34	0.34	0.34	0.34	0.57	1.13	5.65
Saccharose	7.2	6	3	1.2	0.6	0.3	0.12	0.1	0.1	0.1
Malic acid	4.32	3.6	1.8	0.72	0.36	0.18	0.072	0.06	0.06	0.06
Potassium dihydrogen phosphate	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Magnesium sulphate heptahydrate	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Sodium bicarbonate	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16

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