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The Effects of Physicochemical Factors and Cell Density on Nitrite Transformation in a Lipid-Rich *Chlorella*

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To understand the effects of physicochemical factors on nitrite transformation by microalgae, a lipid-rich *Chlorella* with high nitrite tolerance was cultured with 8 mmol/l sodium nitrite as sole nitrogen source under different conditions. The results showed that nitrite transformation was mainly dependent on the metabolic activities of algal cells rather than oxidation of nitrite by dissolved oxygen. Light intensity, temperature, pH, $NaHCO_3$ concentrations, and initial cell densities had significant effects on the rate of nitrite transformation. Single-factor experiments revealed that the optimum conditions for nitrite transformation were light intensity: 300 μmol/m^2/s; temperature: 30°C; pH: 7–8; $NaHCO_3$ concentration: 2.0 g/l; and initial cell density: 0.15 g/l; and the highest nitrite transformation rate of 1.36 mmol/l/d was achieved. There was a positive correlation between nitrite transformation rate and the growth of *Chlorella*. The relationship between nitrite transformation rate (mg/l/d) and biomass productivity (g/l/d) could be described by the regression equation y = 61.3x (R^2 = 0.9665), meaning that 61.3 mg N element was assimilated by 1.0 g dry biomass on average, which indicated that the nitrite transformation is a process of consuming nitrite as nitrogen source by *Chlorella*. The results demonstrated that the *Chlorella* suspension was able to assimilate nitrite efficiently, which implied the feasibility of using flue gas for mass production of *Chlorella* without preliminary removal of NO_X.

Keywords: *Chlorella*, NO_X, nitrite transformation, flue gas

Introduction

Microalgae are able to capture and utilize CO_2 (the main greenhouse gas) efficiently to create substantial biomass, which can be converted into biofuels, chemicals, or foods [2, 22, 28]. Owing to their high biomass productivity and lipid content, microalgae are considered as the only source of renewable biodiesel to meet the global demand for transport fuel [4].

A wide consensus has emerged that flue gas can be used as nutrition or feedstock for mass production of microalgae, which becomes an important research subject to not only decrease the cost of biodiesel and other bioproducts from microalgal biomass, but also relieve the environmental stress sustainably. Typical flue gas emitted from a boiler using low-sulfur heavy oil as fuel contains 0.01–0.05% NO_X [13, 29, 30]. In 2012, NO_X emission from industrial flue gas reached up to about 16.58 billion kilograms in China. NO_X (mainly NO and NO_2) is the major contributor of photochemical smog, acid rain, trophospheric ozone, and eutrophication [5, 11]. Traditional physicochemical treatment technologies of denitration are always related to high capital and energy cost [29]. Using microalgae to establish a new environment-friendly biodenitration method becomes an important research subject.

Nitrogen is one of the most important nutrients for the growth of microalgae. In the last few decades, extensive studies have been conducted on NO_X uptake and metabolic

pathways by microalgae [15, 18, 27], NO_X tolerance and effects on microalgae [18, 26, 35], and removal of NO_X by microalgae [7, 17, 19, 35]. Dissolution of NO_X from flue gas in aqueous phase produces the equivalent amount of nitrite under aerobic conditions [21, 29]. According to Nagase *et al*. [19], the NO removal rate achieved 50–60% in the algal suspension of *Dunaliella tertiolecta* at depth of 2 m. In our laboratory, about 85% NO was absorbed by an 80-cm-deep *Chlorella* sp. XQ-20044 suspension with pH controlled to 7.0–8.0 (unpublished data). The fact that the algal suspension was able to absorb NOx at high proportion from simulated flue gas under suitable culture conditions indicated that a large amount of nitrite would accumulated in the algal suspension when flue gas was used to cultivate algae. It was reported that a low concentration of nitrite did not affect the growth of algae, even in the case of serving as the sole nitrogen source [16, 18, 34]. However, a high concentration of nitrite may reduce the utilization or removal rate of NO_X, and inhibit microalgae growth by rising oxidative stress, damaging the cell membrane, and reducing photosynthesis [3, 34]. Obviously, accumulated nitrite will be harmful to algal growth if nitrite is not converted into harmless forms efficiently. Therefore, understanding the effects of physicochemical factors on nitrite transformation by microalgae is important for establishment of a technique of using flue gas for mass production of microalgae.

In this study, the effects of several main physicochemical factors on nitrite transformation by the lipid-rich *Chlorella* sp. XQ-20044 and the nature of nitrite transformation were investigated. The feasibility of using flue gas for mass production of microalgae without preliminary removal of NO_X was further discussed.

Materials and Methods

Microalgal Strain

The fast growing and lipid-rich microalgal strain *Chlorella* sp. XQ-20044 was provided by the microalgae culture collection in Wuhan Botanical Garden, Chinese Academy of Sciences [32]. Prior to experiment, the alga was maintained at 25°C in a 1 L flask in modified BG-11 medium under artificial light with a photoperiod of a 14:10 h light/dark cycle (60 μmol/m^2/s), and was shaken at 110 rpm on an orbital shaker for 3 days. The medium contained (per liter) $NaNO_3$ (100 mg), $K_2HPO_4{\cdot}3H_2O$ (40 mg), $MgSO_4{\cdot}7H_2O$ (75 mg), $CaCl_2{\cdot}2H_2O$ (36 mg), Fe-citrate (6 mg), citric acid (6 mg), EDTA·Na_2 (1 mg), Na_2CO_3 (20 mg), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.1104 μg), $Co(NO_3)_2{\cdot}6H_2O$ (0.0494 μg), $ZnSO_4{\cdot}7H_2O$ (0.22 μg), $MnCl_2{\cdot}4H_2O$ (1.8 μg), H_3BO_3 (2.86 μg), and $CuSO_4{\cdot}5H_2O$ (0.08 μg).

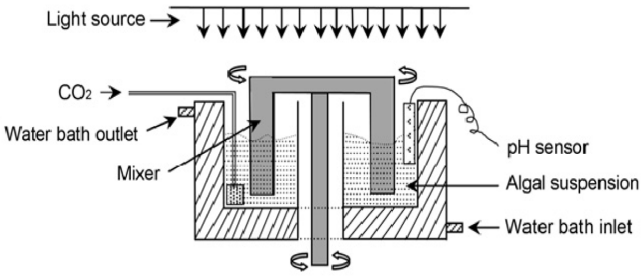

Fig. 1. Schematic diagram of the imitation system of open circular pond.

Photobioreactor

The photobioreactor was a self-designed imitation system of open circular pond with a working volume of 10 L (Fig. 1), which has been described in a previous publication in detail [36]. Light was supplied by 12 white fluorescent lamps (40 W). Light intensities were adjusted by the number of lighted tubes and the distance between light tubes and the surface of algal suspension, and measured by an irradiance meter (TES-1332A, China) at the algal suspension surface. The temperature of algal suspension was regulated by water recycling in the outer layer of the photobioreactor connected to a thermostatic circulator (error: ±0.1°C). The pH of the algal suspension was monitored by an on-line pH automatic controller (JENCO 6133, USA), and was maintained in a given range by automatic injection of pure CO_2 into the medium when pH was rising.

Culture Conditions

In this work, we investigated the effects of light intensity (0, 100, 300 μmol/m^2/s), temperature (20°C, 25°C, 30°C, 35°C), pH (7–8, 8–9, 9–10), $NaHCO_3$ (0, 2, 6, 10 g/l), and initial cell density (0, 0.01, 0.06, 0.15 g/l) on the nitrite transformation rate by *Chlorella*. All the factors and their ranges considered above were the most typical conditions for *Chlorella* growth. The basic culture conditions for temperature, light intensity, pH, and initial cell density were set at 30°C, 300 μmol/m^2/s illumination with 14:10 h light-dark cycle, pH 7–8, and initial cell density of 0.06 g (DW)/l. When one factor was being tested at different levels, the other factors were maintained as basic culture conditions.

Before culture, the culture pond of the photobioreactor was treated by 3% hydrogen peroxide solution for 24 h. Then the microalga was inoculated into sterile nitrate-deficient medium in which nitrate ($NaNO_3$) was replaced by nitrite ($NaNO_2$) and the initial nitrite concentrations were 8 mmol/l. Tests were conducted in a culture room that was kept as clear as possible to avoid contamination. The algal suspensions were stirred at 50 rpm. Sterile distilled water was added into the algal suspension every 3 h to replenish the water loss due to evaporation. In all experiments, the algae were cultured for 3 days and each experiment was repeated three times.

To investigate the roles of algal cells and dissolved oxygen in

nitrite transformation, algal cells in the exponential growth phase were collected by centrifugation and re-inoculated into 175 ml of sterile fresh modified nitrogen-free medium in a 200 ml 3-cm glass cylindrical bioreactor with an initial biomass density of 0.06 g(DW)/l, and then 8 mmol/l $NaNO_2$ was added into the algal suspension. The algal suspension was cultured for 3 days under the conditions of 200 μmol/m^2/s illumination, 14:10 h light-dark cycle at 30°C, pH 7–8, and continuous aeration. The air was filtered through a 0.45 μm filter before entering the algal suspension. The pH was controlled in the same way as that in the imitation system of open circular pond. Blank medium without cells containing 8 mmol/l $NaNO_2$ was used as the control, which was treated the same way. Each experiment was repeated three times.

Measurement of Biomass and Specific Growth Rate

Algal growth was evaluated by the change of biomass dry weight. Triplicate samples (20 ml) were withdrawn from the culture medium and filtered through pre-weighted 0.45 μm GF/C glass membrane filters. Then the cell pellet was rinsed twice with distilled water, and dried in a vacuum dryer to constant weight.

Biomass productivity (P, in units of g/l/d) and specific growth rate (μ) were calculated by Eqs. (1) and (2) as follows, respectively:

$$P = (X_1 - X_0)/t \quad (1)$$

$$\mu = \ln (X_1/X_0)/t \quad (2)$$

where X_0 is the initial biomass concentration (g/l), and X_1 is the biomass concentration after t days. The biomass concentration (g/l) was used to quantify the cell density in each experiment.

Measurement of Nitrite (NO_2^-) and Nitrate (NO_3^-) Concentrations

Microalgal cells from each test were collected by centrifugation at 7,000 ×*g* for 10 min, washed twice with nitrate-free fresh water, then lyophilized for 24 h with a vacuum freeze dryer (FD-1A-50, Beijing Changliu Scientific Instruments Co. Ltd., Beijing, China), and then stored at –20°C for further analysis. An element analyzer (Vario Microcube, Germany) was used to determine the intracellular total nitrogen. Supernatants were further filtered twice through GF/C glass membrane filters (0.45 μm), and the nitrite (NO_2^-) and nitrate (NO_3^-) concentrations in the filtered supernatants were determined.

The nitrite concentration in the medium was measured by a modified spectrophotometric method [23]. First, 1 ml of cell-free culture medium was diluted 25 times to a final volume of 25 ml, and then 0.5 ml of 1% sulfanilamide and 0.5 ml of 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride were added. The mixture was incubated in the dark for 15 min, and then the absorbance at 540 nm was measured.

Nitrite transformation rate (R, in units of mmol/l/d) was calculated by the following equation

$$R = (C_1 - C_2)/(t_2 - t_1) \quad (3)$$

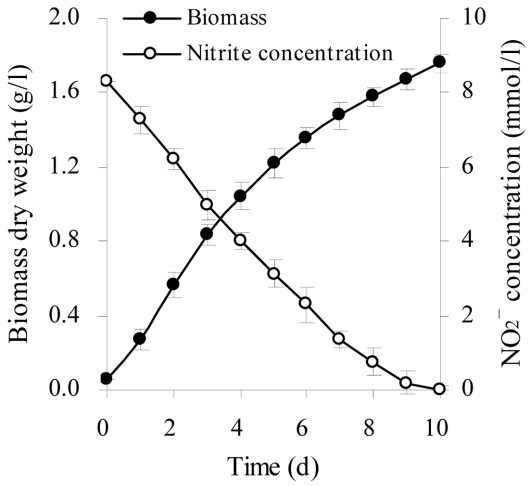

Fig. 2. Growth curve of *Chlorella* with nitrite as sole nitrogen source (●) and the changes of nitrite concentration in algal suspensions (○).

where C_1 and C_2 are the nitrite concentration in algae suspension at times t_1 and t_2, respectively.

Nitrate concentration was determined by the standard water quality analysis methods of GB7480-87 [24].

Results and Discussion

Growth of *Chlorella* with Nitrite as the Sole Nitrogen Source

To explore the effect of nitrite on the growth of microalgae and the fate of nitrite in the algal suspension, *Chlorella* cells were inoculated in 10 L of nitrogen-free modified BG-11 culture medium with an initial cell density of 0.06 g (DW)/l, and 8 mmol/l nitrite sodium was added into the medium. The culture conditions were 30°C for temperature, 300 μmol/m^2/s for illumination with 14:10 h light-dark cycle, and 7–8 for pH. The algal suspensions were stirred at 50 rpm.

As shown in Fig. 2, algal cells exhibited a rapid growth rate, which implied that nitrite could be consumed as the sole nitrogen source for algal growth. The nitrite concentration in the algal suspension decreased linearly with time, and finally could not be detected on the 10^{th} day (Fig. 2). Nitrate was never detected in the algal suspension during culture, indicating that nitrite was not transformed into nitrate. The biomass dry weight of *Chlorella* increased from 0.06 g/l to 1.64 g/l at the end of the culture and the net increase of biomass dry weight was 1.58 g/l. Element analysis showed that the harvested cells contained about 6.95% nitrogen, and 6.95% nitrogen in 1.58 g/l dry biomass

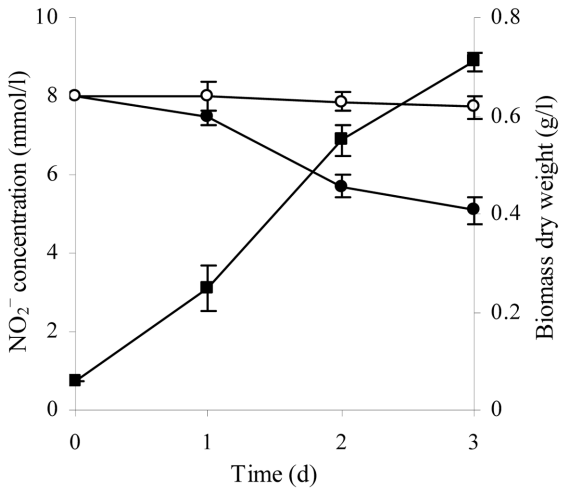

Fig. 3. Time course of nitrite concentration and microalgal biomass.

●, The nitrite concentration with algal cells; ○, the nitrite concentration without algal cells; ■, the biomass dry weight of algal cells.

of *Chlorella* just equaled to the amount of nitrogen added to the medium (8 mmol/l $NaNO_2$) at the beginning of the culture. This result indicated that the nitrite added into the suspension was completely assimilated by *Chlorella* to support its growth and was converted into organic nitrogen.

Effects of Algal Cells and Dissolved Oxygen on Nitrite Transformation

During photosynthesis, the concentration of dissolved oxygen in the algal suspension may exceed the normal air/water O_2 equilibrium concentrations. To investigate whether the dissolved oxygen contributes to nitrite transformation, the nitrite concentration in the algal suspension was compared with that in blank medium in which the concentration of dissolved oxygen was kept at about 7.0 mg/l. As shown in Fig. 3, after 3 days of culture, the nitrite concentration was reduced sharply from 8 mmol/l to 5.1 mmol/l in the algal suspension, while it only decreased slightly in the blank medium control from 8 mmol/l to 7.73 mmol/l. Based on these data, the nitrite transformation rate in the algal suspension reached up to 0.97 mmol/l/d, but was only 0.09 mmol/l/d in the blank medium. The results indicated that the nitrite transformation was mainly dependent on the metabolic activity of algal cells, and oxidation of nitrite by dissolved oxygen played little role in nitrite transformation. According to Ward [31], the conversion from nitrite to nitrate is generally mediated by bacterial activities, with very little abiotic contributions. In this study, all experiments were carried out with sterile medium, and therefore the oxidation of nitrite was not observed.

Effects of Physicochemical Factors on Nitrite Transformation and Growth of *Chlorella*

The effects of light. The effects of light intensity on nitrite transformation by *Chlorella* and algal growth are shown in Fig. 4 (A and B) and Table 1. After 3 days of culture in the dark, the nitrite concentration in the culture suspension remained about the same and the biomass of *Chlorella* decreased to 8 mg/l, which was only 13% of the initial concentration. The decrease in biomass may be due to respiration [6]. Increasing the light intensity significantly accelerated the rate of nitrite transformation and the growth of *Chlorella*. The transformation rate was increased to 0.43 mmol/l/d under the light intensity of 100 μmol/m^2/s,

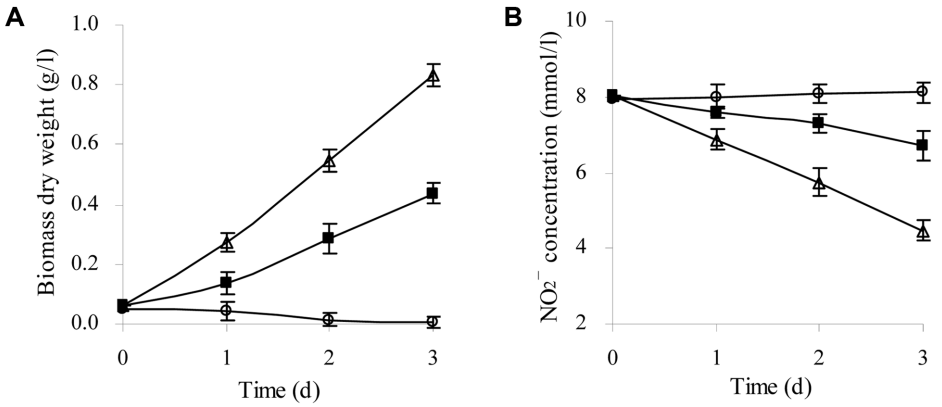

Fig. 4. The growth of *Chlorella* (**A**) and changes of nitrite concentration in algal suspension (**B**) during 3 days of culture under different light intensities.

△, 300 μmol/m^2/s; ■, 100 μmol/m^2/s; ○, 0 μmol/m^2/s.

Table 1. R, P, and μ under different light intensities.

Light intensity (μmol/m^2/s)	R (mmol/l/d)	P (g/l/d)	μ
0	–0.06 ± 0.02b	–0.02 ± 0.01c	–0.66 ± 0.17b
100	0.43 ± 0.09c	0.13 ± 0.04b	0.66 ± 0.13b
300	1.18 ± 0.23a	0.26 ± 0.09a	0.88 ± 0.15a

R, Nitrite transformation rate; P, Biomass productivity; μ, Specific growth rates. Different lowercase letters after the data in the same column mean significant difference at the 5% level by Duncan's multiple range test. The same applies to the following tables.

and further increased to 1.18 mmol/l/d under 300 μmol/m^2/s. The results indicated that nitrite transformation was associated with the activity of algal cells under light conditions when photosynthesis was active. Meanwhile, the highest biomass productivity (0.26 g/l/d) and specific growth rates (0.88) were achieved under 300 μmol/m^2/s illumination in 3 days of culture.

Light not only greatly affects algal photosynthesis and biomass productivity, as well as cell composition and metabolic pathway, but also affects the economic efficiency of the algal cultivation process [20]. Without light, removal of NO does not occur in algal culture in the absence of O_2 [19]. Consistently, this study showed that light was an essential factor for nitrite transformation by *Chlorella*. Nitrite concentration did not change in the culture in the dark, but decreased rapidly with increasing light intensity. This suggested that the nitrite transformation in the algal suspension depended on the strong photosynthesis activity of algal cells. The higher the light intensity, the more rapid photosynthesis and nitrite transformation rates can be achieved. According to Zhu *et al*. [37], the light saturation point of *Chlorella* sp. XQ-20044 was 600 μmol/m^2/s, and the alga kept active photosynthesis until 1,400 μmol/m^2/s. In the field, light intensity is much higher than 300 μmol/m^2/s. Based on the photosynthetic characteristics of *Chlorella* sp. XQ-20044, it is reasonable to expect that *Chlorella* would grow faster and give a higher nitrite transformation rate in the field.

The effects of temperature. At all temperature levels (20°C, 25°C, 30°C, and 35°C), *Chlorella* grew well, and the NO_2^- concentration was reduced essentially with time (growth curves and the curves of nitrite concentration were not shown). The average NO_2^- transformation rate (R), biomass productivity (P) and growth rate (μ) are shown in Table 2. With temperature increasing from 20°C to 30°C, the biomass productivity and specific growth rate were increased. The highest biomass productivity (0.26 g/l/d) and growth rate (0.88/d) were achieved at 30°C, and the biomass increased by 14 times.

Temperature is one of the major factors that regulate cellular, morphological, and physiological responses of microalgae: generally, higher temperatures accelerate the metabolic rates of microalgae, whereas low temperatures can inhibit microalgal growth [10]. Bernard and Rémond [1] proposed a model describing the effects of temperature and light on microalgal growth. According to this model, over-warming can cause a significant decrease of productivity for most commercial species. The suitable temperature for most microalgal growth is 20–35°C. This study showed that the optimal temperature for *Chlorella* was 30°C. Higher or lower temperature would reduce the growth rate. Interestingly, the highest nitrite transformation rate was also achieved at the optimal temperature (Table 2). There was a positive correlation between nitrite transformation rate and the growth of *Chlorella*, indicating that nitrite transformation was affected by the metabolic activities of algal cells.

The effects of pH. NO_X is widely considered as a toxic gas, because it can cause the acidification of algal culture and thus inhibit the growth of microalgae. However, nitrite toxicity was decreased with the increase in pH of culture suspension [33]. Controlling the pH of culture media is considered to be one of the most effective methods to protect the algal cells from NO_X damage [12,22].

In this study, three pH ranges (7–8, 8–9, and 9–10) were

Table 2. R, P, and μ under different temperatures.

Temperature (°C)	R (mmol/l/d)	P (g/l/d)	μ
20	0.81 ± 0.07d	0.17 ± 0.05c	0.75 ± 0.06b
25	0.96 ± 0.10c	0.24 ± 0.03b	0.85 ± 0.09a
30	1.18 ± 0.23a	0.26 ± 0.09a	0.88 ± 0.15a
35	1.05 ± 0.14b	0.25 ± 0.06ab	0.87 ± 0.08a

Table 3. R, P, and μ under different pHs.

pH	R (mmol/l/d)	P (g/l/d)	μ
7–8	1.18 ± 0.23a	0.26 ± 0.09a	0.88 ± 0.15a
8–9	0.88 ± 0.09b	0.24 ± 0.03a	0.85 ± 0.06a
9–10	0.44 ± 0.05c	0.14 ± 0.02b	0.68 ± 0.08b

set to explore the effects of pH on the growth of *Chlorella* and the transformation rate of nitrite. As shown in Table 3, the transformation rate of nitrite was decreased with increasing pH, and the growth of *Chlorella* was inhibited significantly when the pH value reached above 9. The highest biomass productivity was achieved (0.88 g/l/d) at pH 7–8. However, there was no significant difference between pH 7–8 and pH 8–9 ($p > 0.05$).

Besides temperature and light, the culture pH is another important factor influencing growth and metabolism of microalgae, by affecting the CO_2 availability in photosynthesis, utilization efficiency of organic carbon source in respiration activity, absorption and utilization of ion in culture medium by algal cells, and recycle and toxicity of metabolites [9, 14]. Most microalgal species favor neutral pH, whereas some species are tolerant to higher pH (*e.g.*, *Spirulina platensis* at pH 9) or lower pH (*e.g.*, *Chlorococcum littorale* at pH 4). Khalil *et al.* [9] reported that the maximum dry weight was achieved at pH 7.5 for *Dunaliella bardawil*, but at 9.0 for *Chlorella ellipsoidea*. In this study, an on-line pH automatic control system was used to maintain the algal suspension in stable pH ranges. The results showed that *Chlorella* sp. XQ-20044 grew very well below pH 9, and nitrite was also assimilated efficiently under this condition. Generally, the optimum pH is different for different algal species [9], depending on the original habitat of the species, and it is one of the physiological characters for that species. It was reported that the optimum pH for photosynthesis of *Chlorella* sp. XQ-20044 was 8.0, and the photosynthetic O_2 evolution dropped slightly at both pH 7.0 and pH 9.0, and dropped significantly when pH was further increased to above 9.0 [37]. The effect of pH on growth and nitrite transformation of *Chlorella* sp. XQ-20044 was consistent with the effect of pH on photosynthesis, reflecting the physiological characters of this strain in pH adaptability.

It was interesting that a suitable pH gave a higher biomass productivity and growth rate, and also a higher nitrite transformation rate, which indicated that the effect of pH on nitrite transformation rate was also reflected by the effect of the growth rate of *Chlorella* on nitrite transformation rate.

The effects of $NaHCO_3$ concentration. In this study, the optimal concentration of $NaHCO_3$ for algal growth was 2 g/l (Table 4). At this concentration, the nitrite transformation rate reached the maximum value of 1.28 mmol/l/d. However, the growth of microalga had no difference between $NaHCO_3$ concentrations of 2 and 6 g/l. The lowest growth rate (0.84) was observed when the $NaHCO_3$ concentration was 10 g/l.

$NaHCO_3$ in the algal suspension not only provides an inorganic carbon source for algal growth, but also forms a buffer system to keep the pH stable, which helps cells take up and utilize CO_2 more efficiently. Compared with the culture without $NaHCO_3$, addition of 2.0 g/l $NaHCO_3$ significantly increased both the algal growth and nitrite transformation rate, suggesting that a suitable amount of $NaHCO_3$ benefited the growth of *Chlorella* and nitrite transformation. However, excessive $NaHCO_3$ not only inhibited the growth of *Chlorella*, but also reduced the nitrite transformation rate. As shown in Table 4, the optimal concentration of $NaHCO_3$ was 2.0 g/l for both growth of *Chlorella* and nitrite transformation by *Chlorella*. It was reported that a high concentration of $NaHCO_3$ may form ion stress to algal growth of *Ankistrodesmus braunil* 202-7c and *Chlorella pyrenoidosa* 211-15 [8]. Therefore, the inhibition effects of high $NaHCO_3$ concentration on growth and nitrite transformation observed in this study might also be related to ion stress.

The effects of initial cell density. Four levels of initial cell density (0, 0.01, 0.06, 0.15 g/l) were set to study the effect of initial cell density on nitrite transformation. As shown in Table 5, a higher initial cell density gave a higher nitrite transformation and higher biomass productivity, but a lower specific growth rate. The highest nitrite transformation (1.36 mmol/l/d) and biomass productivity (0.29 g/l/d) were achieved when the initial cell density was 0.15 g/l, whereas the highest specific growth rate (1.42) was obtained when the initial cell density was 0.01 g/l.

In general, a higher initial cell density can shorten the lag phase of algal growth and enhance the tolerance of microalgae to toxic compounds [12, 35]. Therefore, it is reasonable to predict that a higher inoculation of cell

Table 4. R, P, and μ under different $NaHCO_3$ concentrations.

$NaHCO_3$ concentration (g/l)	R (mmol/l/d)	P (g/l/d)	μ
0	1.18 ± 0.13b	0.26 ± 0.09a	0.88 ± 0.05a
2	1.28 ± 0.17a	0.27 ± 0.03a	0.89 ± 0.08a
6	1.14 ± 0.11b	0.26 ± 0.05a	0.88 ± 0.11a
10	0.92 ± 0.14c	0.23 ± 0.05b	0.84 ± 0.07b

Table 5. R, P, and μ under different initial cell densities.

Initial cell density (g/l)	R (mmol/l/d)	P (g/l/d)	μ
0	0	0	0
0.01	1.08 ± 0.16b	0.24 ± 0.05b	1.42 ± 0.13a
0.06	1.18 ± 0.23b	0.26 ± 0.09ab	0.88 ± 0.15b
0.15	1.36 ± 0.12a	0.29 ± 0.07a	0.63 ± 0.05c

density will promote the growth of *Chlorella* and further enhance the transformation of nitrite, which was exactly what we observed in this study (Table 5). When the initial cell density was kept the same, nitrite transformation was positively correlated to both biomass productivity and growth rate. In contrast, when initial cell densities varied, nitrite transformation was only positively correlated to biomass productivity, indicating that nitrite transformation was dependent on the amount of cells, not on how quick the cells were dividing.

In conclusion, *Chlorella* suspension is very efficient in transforming nitrite into biomass, and the transformation rate is positively correlated with the algal biomass accumulation. Therefore, all environmental factors affecting the growth of *Chlorella* have indirect effects on the nitrite transformation rate. The more advantageous the conditions are to growth, the higher nitrite transformation rate will be achieved.

The Positive Correlation Between Nitrite Transformation Rate and Algal Growth, and the Nature of Nitrite Transformation

It was interesting that the physicochemical factors giving higher growth rates also produced higher nitrite transformation rates, except that a higher initial cell density gave a higher nitrite transformation rate but a lower specific growth rate. Based on the data in Tables 1–5, the relationship between nitrite transformation rate and biomass productivity is displayed in Fig. 5.

Nitrite transformation rate (mmol/l/d) can be described as the function of biomass productivity (g/l/d) by the regression equation $y = 4.3764x$ with high significance ($R^2 = 0.9665$). When using mg/l/d as the unit of nitrite transformation rate, the above regression equation can be transformed into $y = 61.3x$ ($R^2 = 0.9665$), meaning that 61.3 mg of N element was assimilated by 1.0 g of dry biomass of algae on average. This number (61.3 mg/g) was close to the nitrogen content (6.95%) in *Chlorella* grown in medium with nitrite as the sole nitrogen source, indicating that the nitrite was completely assimilated by *Chlorella* and converted into organic nitrogen. The results of this study demonstrated that the nature of nitrite transformation by *Chlorella* is the consumption of nitrite as nitrogen source for growth and reproduction of *Chlorella*.

Feasibility of Using Flue Gas for Mass Production of *Chlorella* sp. XQ-20044 Without Preliminary Removal of NO_X

Typical flue gas contains 10–15% CO_2, 0.03% SO_X (mainly SO_2), and 0.03% NO_X (mainly NO and NO_2) [13, 29]. In water solution, SO_2 is hydrated to sulfurous acid, and dissociates protons to yield sulfite [25]. Similarly, NO_2 is dissolved in water solution to form nitrite. Although the chemical reaction of NO dissolution and transformation in water solution is more complicated, nitrite is the main form existing in water solution [29]. According to Liang *et al.* [14], the two basic requirements for using flue gas for mass production of microalgae without preliminary removal of SO_2 are (i) the alga can tolerate high concentration of sulfite, and (ii) The alga can use sulfite as the sole sulfur source for growth [14]. Similarly, two basic requirements, tolerance to high concentration of nitrite and ability to use nitrite as sole nitrogen source, also need to be met when using flue gas for mass production of microalgae without

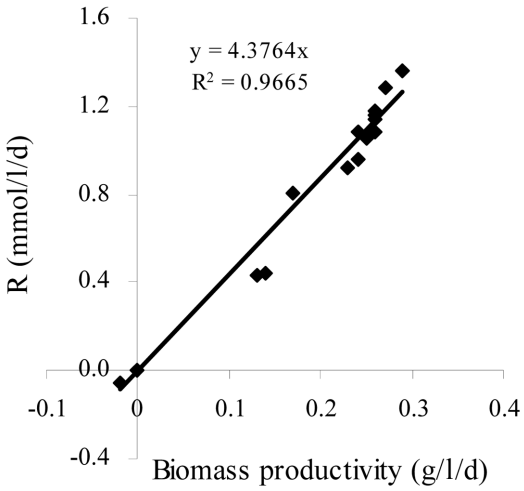

Fig. 5. The relationship between nitrite transformation rate (R) and biomass productivity.

preliminary removal of NO_X.

The results of this study demonstrated that *Chlorella* sp. XQ-20044 could tolerate as high as 8 mmol/l nitrite, and used nitrite as the sole nitrogen source for growth, converting nitrite completely into organic nitrogen. Furthermore, a related study in our laboratory indicated when simulated flue gas containing 0.03% NO was bubbled into an 80-cm-deep *Chlorella* sp. XQ-20044 suspension with pH controlled to 7.0–8.0, about 85% NO was absorbed (unpublished data). This result was also consistent with previous studies. Yoshihara *et al*. [35] supplied 0.03% NO and 15% CO_2 at a speed of 150 ml/min into a 4 L algal suspension of marine microalga NOA-113, and observed removal rates of 10 mg/l/d and 0.875 g/l/d for NO and CO_2, respectively. In another study, Nagase *et al*. [19] supplied simulated flue gas into an algal suspension of *Dunaliella tertiolecta* at depth 2 m, and found that NO was assimilated by *Dunaliella* under illumination, and the NO removal rate was kept at 50–60% for 15 days. As discussed above, the lipid-rich *Chlorella* sp. XQ-20044 is able to tolerate a high concentration of nitrite and use it as the sole nitrogen source for growth, and the alga suspension of *Chlorella* sp. XQ-20044 can remove NO from simulated flue gas efficiently. Therefore, it is feasible to use flue gas for the mass production of feedstock for biodiesel using *Chlorella* sp. XQ-20044, without preliminary removal of NO_X, assuming there is adequate control of the pH.

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