

Growth Hormones Promote the Growth of Microalgae in Simulated Flue Gas

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Abstract: Acidic pollutants in the flue gas have been known as strong toxicity to the environment. Besides, the high concentration of CO₂ from flue gas might contributed to the greenhouse effect. Using *Chlorella* sp as effective biomaterials for fixation of high concentration of CO₂ (15%, V/V), this study was to investigate the tolerance to moderate SO₂ and NO₂ gas derived from self-installation devices with the help of Gibberellin (GA) and triacontanol (TA). The cultures were exposed to SO₂-air mixture (1500 mL, 50 mg/kg) and NO₂-air mixture (1500 mL, 50 mg/kg) per day respectively, and the results showed that the biomass increased by 1.32 times and 1.27 times for GA addition, and increased by 1.22 times and 1.05 times for TA addition, respectively. Besides, the volumes of the cells became larger when growing with addition of GA and TA. The quantification of biochemical composition including protein, lipid, carbohydrate were investigated and the results showed that GA and TA had the same positive effects on the lipid accumulation and the maximal content of lipid, which amounted to 0.62 mg/mg of dry weight. Nevertheless, the content of protein had no obvious changes with the help of GA and TA. High concentration of CO₂ had negative effect on carbohydrate production in the algae and the content of carbohydrate was not elevated by the addition of GA and TA. This study reveals the potential of applying *Chlorella* sp and growth hormones GA and TA to flue gas capture.

Key words: chlorella; flue gas; gibberellin; growth stimulators; NO₂; SO₂; triacontanol

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生长激素促进小球藻在烟道气环境下的生长

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摘要: 烟道气中的酸性污染物对环境有着强烈的危害, 并且其中的高浓度 CO₂ 也可能是造成温室效应的原因之一。本实验研究了小球藻作为一种可以有效固定高浓度 CO₂ (15%, V/V) 的生物材料, 在赤霉素 (GA) 和三十烷醇 (TA) 的作用下对 SO₂ 气体和 NO₂ 气体的耐受性机制。研究表明, 当微藻每天暴露于 SO₂-空气 (1500 mL, 50 mg/kg) 和 NO₂-空气 (1500 mL, 50 mg/kg) 的混合气体中时, 小球藻生物量在赤霉素的作用下分别提高了 1.32 和 1.27 倍, 在三十烷醇的作用下生物量分别提高了 1.22 和 1.05 倍。除此之外, 细胞的体积也变得更大。通过对藻细胞的蛋白质、脂质、碳水化合物等生化成分的定量测定发现, GA 和 TA 均有利于脂质积累和代谢, 并且藻细胞的最大脂肪含量都可达到 0.62 mg/mg 干重, 但对蛋白质的生成都没有明显的作用。高浓度的 CO₂ 会抑制藻类的碳水化合物的生成, 但 GA 和 TA 不能有效提高碳水化合物的含量。本研究初步揭示了小球藻和生长激素 GA 与 TA 在烟道气捕集和能源材料应用产业中的潜力。

关键词: 小球藻; 烟道气; 赤霉素; 生长因子; NO₂; SO₂; 三十烷醇

If flue gas could be used directly without pretreatment, the cost for alga cultivation will be reduced in the actual production process^[1,2]. But extreme condition of CO₂ (above 15%) and the inhibitory compounds like SO₂ and NO_x have great harmful

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influence to algal growth^[3-5]. Flue gas is the gas mixture with more interest for CO₂ capture using microalgal cultures^[6-9]. However, it contains not only CO₂ but also sulfur and nitrogen oxides. These compounds may be toxic for the cultures growth by reducing pH in the medium solution and thus caused inhibition^[10-12]. *C. caldarium* was selected due to its ability to grow in highly acidic media and elevated temperature, which is advantageous one prior to that of other species for CO₂ capture from flue gas, but it was completely inhibited

when the flue gas contained 200 mg/kg SO₂,^[13]. Negoro et al^[14] evaluated the SO₂ and NO_x effects on the growth of ten strains of marine and halotolerant microalgae, and found that the growth of *Nannochloris* sp. and *Nannochloropsis* sp. were not affected by 50mg/kg of SO₂. When at 400 mg/kg of the same gas, the pH dropped and the growth stops after 20 h of cultivation. Despite of absence of significant change of pH value, both growth was affected by this air pollutant. Some acidophilic algal stains have been tested to overcome the inhibition effects caused by the toxic compounds^[15]. The tolerances of several acidophilic algal strains, isolated from a hot water spring, among of three strains showed good growth at 50 mg/kg NO aeration and only one strain proliferate under 50 mg/kg SO₂ aeration. Considering that actual flue gas from industrial sources contains about 100-300 mg/kg NO_x and SO_x, algal stains could not be used for direct CO₂ fixation from flue gas in reported literature^[4]. Selection of *Chlorella* sp. was based on previous reports, indicating its excellent tolerance to high concentration of dissolved CO₂^[16-18]. A method by adding growth stimulator triacontanol into culture solution for improving the tolerance of SO₂ and NO₂ have been tested for resolving this problem. It was reported that the growth of two alga strains exposed to SO₂ or NO₂ for 10', 15' and 20' periods at the 10th day of incubation could be improved by addition of triacontanol and sodium biocarbonate to the culture medium^[19]. In this study, we investigated the effect of a growth stimulator Gibberellin (GA) which was compared with triacontanol (TA) on the algae growth under moderate concentration of SO₂ (50 mg/kg, 1500 mL) and NO₂ (50mg/kg, 1500 mL) air mixed gas. The research on the choice of effective growth stimulators will benefit for improving the tolerance of actual flue gas by the algae in production scene.

1 Materials and Methods

1.1 Strain and culture medium

The alga *Chlorella vulgaris* (FACHB-1227) seed suspension was purchased from Chinese Academy of Sciences Hydrobiology Institute (Wu Han, China) and later was maintained by transferring the seed suspension to BG11 medium. The BG11 medium had the following

composition (in mg/L): NaNO₃ 1500; K₂HPO₄ 40; MgSO₄·7H₂O 75; CaCl₂·2H₂O 36; Citric acid 36; Ammonium ferric citrate 4; EDTANa₂ 1; Na₂CO₃ 20; and 1 mL A₅ solution (in mg/L): H₃BO₃ 2860, MnCl₂·4H₂O 1810, ZnSO₄·7H₂O 222, CuSO₄·5H₂O 79, Na₂MoO₄·2H₂O 21, Co(NO₃)₂·6H₂O 500.)

GA Solution: 0.1 g Gibberellin was dissolved in 1 L 95% ethanol/water solution (0.1 mg/mL)

TA solution: 0.5 g triacontanol was dissolved in 100 mL 95% ethanol/water solution (5.0 mg/mL)

1.2 pH and light measurement

Sample pH was directly determined using a pH meter (PHS-3C, China). The pH meter was calibrated daily using pH 4 and 7 solution. Light intensity was measured using a basic quantum meter (TES 1330A, Taiwan).

1.3 Pre-culture of alga cell

The seed suspension was incubate in 150 mL fresh medium in 250 mL flask and cultured for 2 weeks at 25±1 °C. The initial pH of the medium was adjusted to 7.0 before sterilization. Illumination was provided by fluorescent light and light intensity was about 36 μmol/m²/s at the surface of the flask. The speed of orbital shaker was set 120 r/min.

1.4 Determination of biomass and specific growth rate

The biomass was determined by dry weight measurement. Microalgal dry weight (g/L) was measured according to the following method: the microalgal pellets were removed by centrifugation, washed (distilled water), recentrifuged again for three times, after drying the microalgal pellet at 105 °C to constant, the dry weight was measured.

The initial specific growth rates were determined during the exponential growth phase of batch cultures in 200 mL flask. The exponential growth phase was confirmed by cell count board associated with microscope. Specific growth rate (μ/d) was calculated from the equation: $\mu = \ln(N_2/N_1)/(t_2-t_1)$, where N₁ and N₂ were the biomass (g/L) on days t₁ and t₂, respectively^[20].

1.5 Composition analysis

The dried biomass was analysis for lipid, carbohydrate, protein. Lipid was determined by SPV method^[21]. While phenol-sulfuric method^[22] was used for total carbohydrate analysis. Lowry method combined with the method described by Pruvost et al. were used for protein determination^[23,24].

1.6 Concentration analysis of SO₂-air mixed gas and NO₂-air mixed gas

A series of SO₂-air mixed gas and NO₂-air mixed gas with different volume concentration were prepared according to the below method 1.8. Then these mixed gas were detected by detector tube (Gastec, Japan) give exact concentration values.

1.7 Alga cultivation in photo-bioreactor

The acclimated algae cell suspension was incubated into 250 mL flask containing 200 mL BG11 medium. The flasks were placed on an orbital shaker at 27±1 °C, under continuous, cool white, fluorescent light. Light intensity was approximate 72 μmol/m²/s at the surface of the flask. Filtered ambient air mixed without/with CO₂, SO₂ or NO₂ were prepared by a self-made device (Fig.1). The concentration of CO₂ in mixed gas was controlled 15% (V/V) and the flow rate of mixed gas was kept 1.5 L/min in all operation processes. SO₂ was prepared by dropping of concentrated H₂SO₄ solution into Na₂SO₃ solution and NO₂ was produced by dropping concentrated HNO₃ solution into Cu powder (Fig.2). NO₂ was collected by collection bag when the color of gas turned brown at the outlet gas of flask and SO₂ was just captured by another collection bag as the gas from the outlet of SO₂ generator could be tested by color fading of Fushsine solution. Culture solution in the flask was aerated with the CO₂ mixture gas continuously at a certain gas flow velocity (0.25 vvm). SO₂ and NO₂ gas collected from generators were filled into two gas collecting bags (Fig.2) were later transferred into algal solution through silica gel at certain flow velocities (one bubble per second) and the residual exhaust gas were absorbed by NaOH solution. Alga solution samples were removed daily from the flask to determine the algal growth and pH values. The algal

growth detection depends on cell counting. The pH of culture was directly determined by using pH meter. In order to maintain normal pH environment, the pH of the algal solutions after aerated with acidic gas was adjusted to 7.0 with NaHCO₃ solution to eliminate the effect of pH on the algal growth.

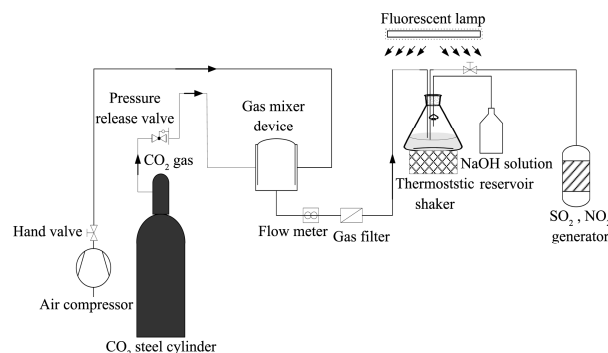


Fig.1 The diagram of route for algae cultivation

Note: The mixture gases with different CO₂ concentrations were prepared by gas mixer device. External illumination of light intensity was provided by a continuous, cool white fluorescent light.

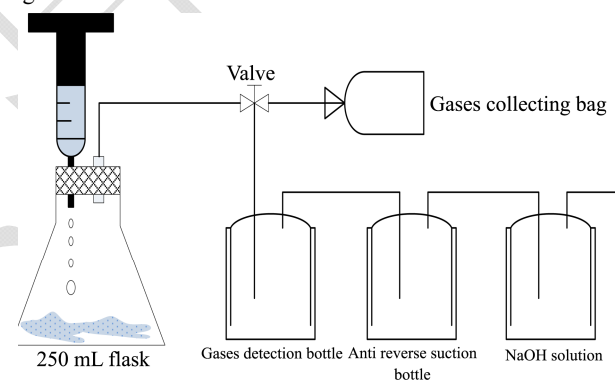
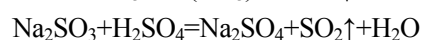


Fig.2 The schematic diagram of SO₂ and NO₂ generator

Note: The amounts of SO₂ and NO₂ gases generated from reactors were captured by collection bags.

1.8 Preparation and quantities of SO₂ and NO₂ gas

The SO₂ and NO₂ can be produced by generator described in Fig.2, and the chemical reaction equation:



The experiment design has the high sensitivity to produce pure NO₂. the beginning of reaction can be easily judged by the color changes of gas (brown color of gas appears). Pure SO₂ could be tested by color fading of Fushsine solution (pink color disappear). The preparation

of SO₂-air mixed gas and NO₂-air mixed gas were easily done by sucking exact volumes of pure SO₂ gas and NO₂ gas and air gas with graduated injector, then mixed them into a collection bag to form certain volume concentration (mg/kg) of SO₂-air and NO₂-air mixed gas respectively. The exact volume concentration of mixed gas was produced according to the analysis by using detection tube (see 1.6). According to the SO₂ and NO₂ concentration data provided by a local thermal power plant, the maximal concentration will not higher than 50 mg/kg when the exhaust gas was handled by specific purification process. Therefore, the concentration of the two mixed gas could be prepared exactly as 50 mg/kg in the following experiments.

1.9 Statistical analysis

Each sample consisted of three replicates in the cultivation process. The results were expressed as the means±standard error of means (SEM). Statistical differences were calculated using student's t-test to analyze the differences between parallel samples every day. The critical p value was set at 0.05, a probability value of $p < 0.05$ was considered to be statistically significant.

2 Results and discussion

2.1 Effect of GA on the growth of alga

It can be seen that GA has the obvious positive effect on the growth of algae (Fig.3). The different concentration of GA in the culture can promote the cell concentration in 11 days and the most obvious cell increase potential could be seen in the culture containing 0.12 mg/L GA compared with other concentration of GA in the culture. The data of biomass (Fig.4) were also consistent with the growth curve in Fig.3 which confirmed the above results that the maximal biomass (11.07 g/L) was gained in 0.12 mg/L GA culture after 11 days later. The cell concentration and biomass could be achieved at the peak in 15% CO₂-air aeration with 0.12 mg/L in the culture, while the cell concentration cultivated with GA almost the same as these grown without GA in acidic gas aeration conditions (see Fig.5 and Fig.6). Above all, the biomass were improved in acidic gas aeration conditions with addition of GA in the

culture, the biomass increased 31.9% (50 mg/kg SO₂), 27.0% (50 mg/kg NO₂), 14.0% (50 mg/kg SO₂+50 mg/kg NO₂) respectively (See table 1). It could be concluded that the acidic gas constrained the cell growth and decreased the rate the cell division, but interestingly, the stimulator GA caused the volume of the cells expanded which was observed in Fig.14.

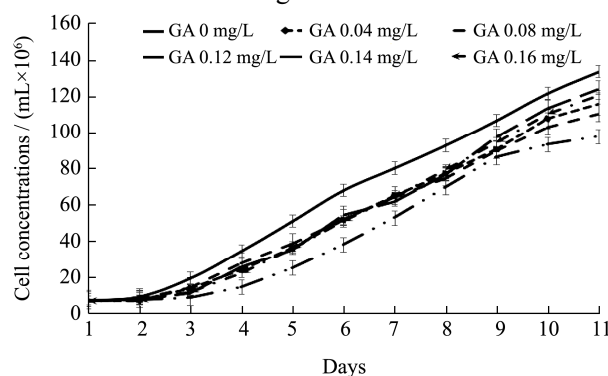


Fig.3 The effect of GA with different concentrations in the culture on the growth of algae

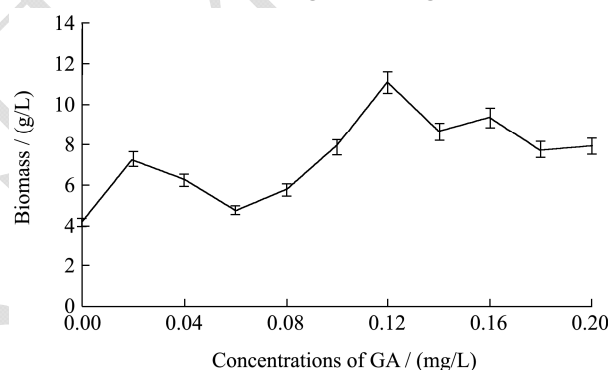


Fig.4 The effect of GA with different concentrations in the culture on the biomass of algae

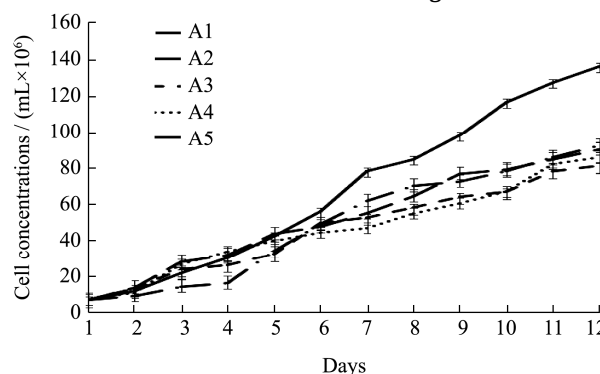


Fig.5 The growth of algae by adding GA (0.12 mg/L) in the culture under different cultivated conditions

Note: A1: GA 0.12 mg/L, no aeration; A2: GA 0.12 mg/L, 15% CO₂, aeration; A3: GA 0.12 mg/L, 15% CO₂, aeration, 50 mg/kg SO₂; A4: GA 0.12 mg/L, 15% CO₂, aeration, NO₂; A5: GA 0.12 mg/L, 15% CO₂, aeration, 50 mg/kg SO₂, 50 mg/kg NO₂.

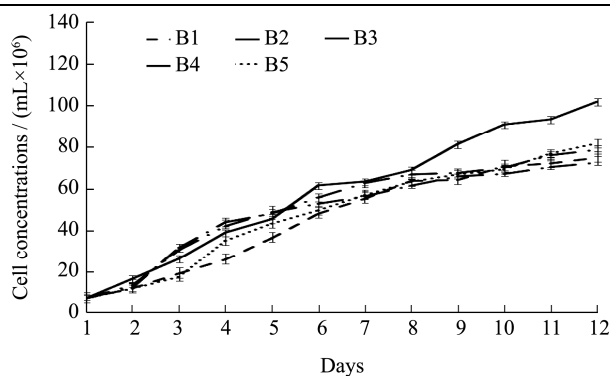


Fig.6 The growth of algae without adding GA and TA under different cultivated conditions (control group)

Note: B1: no aeration; B2: 15% CO₂, aeration; B3: 15% CO₂, aeration, 50 mg/kg SO₂; B4: 15% CO₂, aeration, 50 mg/kg NO₂; B5: 15% CO₂, aeration, 50 mg/kg SO₂; 50 mg/kg NO₂.

2.2 Effect of TA on the growth of alga

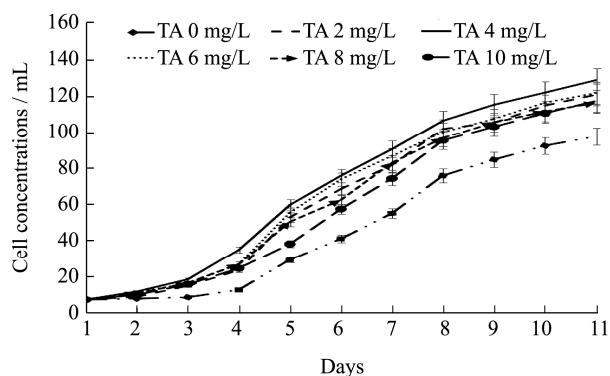


Fig.7 The effect of TA with different concentrations on the growth of algae

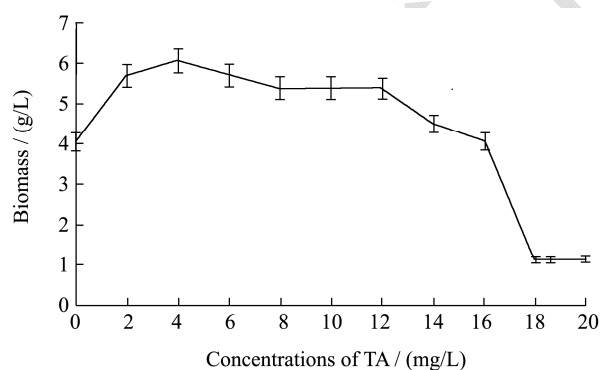


Fig.8 The effect of TA with different concentrations on the biomass of algae

Similarly, It can be seen that TA also has the obvious positive effect on the growth rate of algae (Fig.7), which had been proved that TA increased the tolerance to SO₂ and NO₂ acidic gas in the cultivation process of *Chlorella* sp.^[19]. The different concentration of TA in the culture contributes to the increase of cell concentration in 11

days and the most prominent cellular increase potential could be seen in the culture containing 4.00 mg/L TA compared with other concentration of TA in the culture. The data of biomass (Fig.8) were also consistent with the growth curve in Fig.7 which confirmed the relative results that the maximal biomass (6.00 g/L) was gained in 15% CO₂-air aeration with 4.00 mg/L TA in the culture, and the cell concentration cultivated with TA almost the same as these grown without TA in acidic gas aeration conditions (see Fig.6 and Fig.9). However, the biomass were improved in acidic gas aeration conditions with the adding TA in the culture, the biomass increased 21.9% (50 mg/kg SO₂), 5.63% (50 mg/kg NO₂), 3.58% (50 mg/kg SO₂+50 mg/kg NO₂) respectively (See table 1). The reasons could be explained as the same as the effect of GA on the growth and the cell size (See Fig.15).

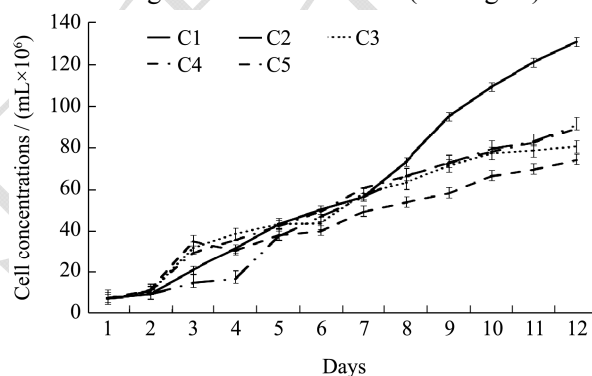


Fig.9 The effect of TA on the growth of algae in different cultivated conditions

Note: C1: TA 4 mg/L, no aeration; C2: TA 4 mg/L, 15% CO₂, aeration; C3: TA 4 mg/L, 15% CO₂, aeration, 50 mg/kg SO₂; C4: TA 4 mg/L, 15% CO₂, aeration, NO₂; C5: TA 4 mg/L, 15% CO₂, aeration, 50 mg/kg SO₂, 50 mg/kg NO₂.

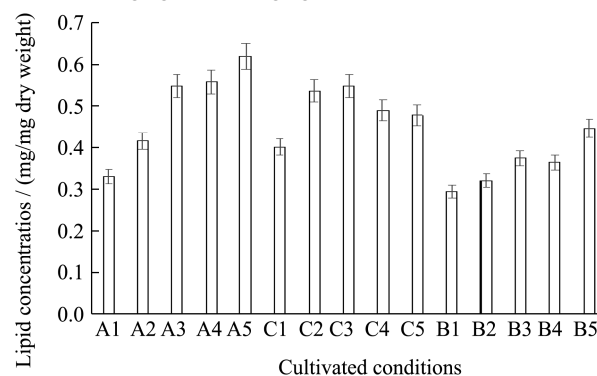


Fig.10 The effect of GA and TA on the concentrations of lipid in the alga

Note: A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, C1, C2, C3, C4, C5 represent the same conditions as these in Fig.5, Fig 6, Fig 9.

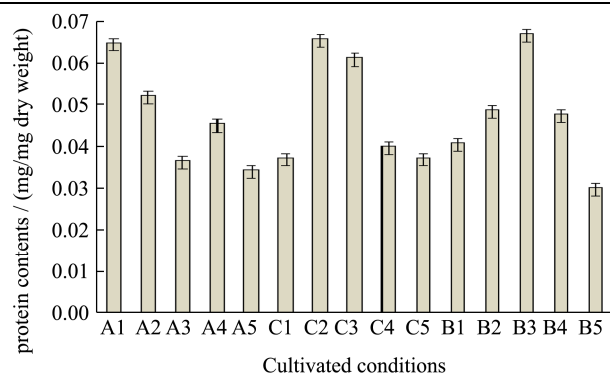


Fig.11 The effect of GA and TA on the concentrations of protein in the alga

Note: A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, C1, C2, C3, C4, C5 represent the same conditions as these in Fig.5, Fig 6, Fig 9.

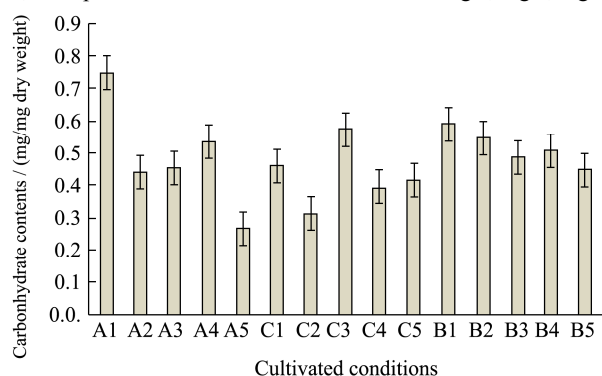


Fig.12 The effect of GA and TA on the concentrations of carbohydrate the alga

Note: A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, C1, C2, C3, C4, C5 represent the same conditions as these in Fig.5, Fig 6, Fig 9.

2.3 Effect of GA and TA on the component concentration of alga

It was seen from the data of Fig.10 that the lipid contents of the algae cultivated in the 14 kinds of cultivated conditions have been improved greatly compared to the control one (B1) which was cultivated without any stimulators and aeration. The results showed that 15% CO₂ aeration particularly contributes the lipid accumulation of these algae cells when cultivated with GA and TA. Among of them, A5 has the maximal content of lipid which reached 0.62 mg/mg dry weight. Therefore, GA and TA have not only accelerated the cell division and growing, but have the obvious function on converting the inorganic CO₂ into lipids, which is the required organic carbon component in energy algae. Compared to TA, GA had the more distinguish effect on lipid accumulation of algae, when cultivated under high

concentration of CO₂ aeration with moderate SO₂ and NO₂. The content of protein averagely were lower than lipid in all of the algae, which range from 0.03~0.07 mg/mg dry weight, even so, GA and TA have not the obvious effect on the increase of protein contents in algae cells with 15% CO₂ aeration (Fig.11). The content of protein were 0.05 mg/mg dry weight (A2) and 0.07 mg/mg dry weight (C2) which were a little higher than that of control one (B2) (0.05 mg/mg dry weight). On the contrary, the content of carbohydrate of the algae (except A1) have a little drop compared to the control one (B2). It is can be inferred that the high concentration of CO₂ have negative effect on the carbohydrate accumulation and GA and TA have no special effect on improving the content of carbohydrate (Fig.12).



Fig.13 The algal cells were cultivated for 12 days in 15% CO₂-air mixed gases

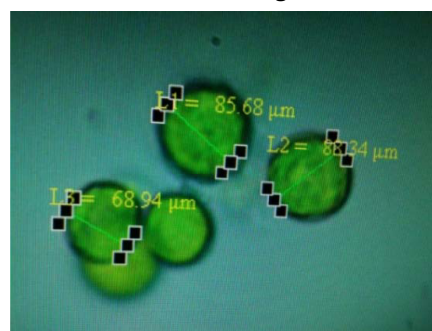


Fig.14 The algal cells were cultivated for 12 days in 15% CO₂-air mixed gases with addition of 0.12 mg/L GA in the culture

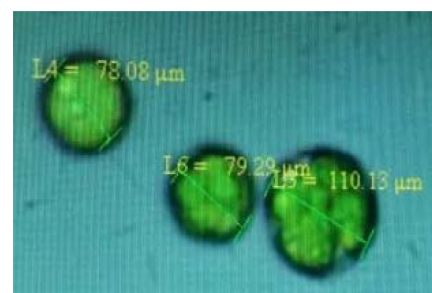


Fig.15 The algal cells were cultivated for 12 days in 15% CO₂-air mixed gases with addition of 4 mg/L TA in the culture

Table 1 Effects of NO₂, SO₂ on the compositions of chlorella

Conditions	Indicators	No aeration	15% CO ₂ -air	15% CO ₂ -air, 50 mg/kg SO ₂	15% CO ₂ -air, 50 mg/kg NO ₂	15% CO ₂ -air, 50 mg/kg NO ₂ and SO ₂
No addition with	^b Biomass/(g/L)	3.28±0.01	4.07±0.02	3.10±0.01	3.37±0.02	3.77±0.02
GA and TA	^a Specific growth rate/d ⁻¹	0.11	0.13	0.10	0.11	0.12
Addition with GA	Biomass/(g/L)	4.35±0.02	11.15±0.03	4.09±0.04	4.28±0.02	4.30±0.02
	Specific growth rate/d ⁻¹	0.13	0.22	0.13	0.13	0.13
Addition with TA	Biomass/(g/L)	3.97±0.02	6.04±0.03	3.78±0.04	3.56±0.02	3.91±0.01
	Specific growth rate/d ⁻¹	0.13	0.16	0.12	0.12	0.12

Note: ^a Specific growth rate was obtained on the growth phase by day during the cultivation. The growth phase was from 1 to 11 days batch with different concentrations of CO₂ aeration. ^b Each data indicates the mean±SD, which were measured from three independent cultures.

3 Conclusion

The success of a method to use microalgae *Chlorella* sp. to capture CO₂ in the conditions of high concentration of CO₂, moderate SO₂ and NO₂ gas by improving biomass and lipid accumulation of the algae with the help of growth stimulators GA and TA. The percentages of simulated flue gas composition approached to that of actual flue gas, because the relative data was collected from a thermal power plant in the local place. Therefore, the simulated flue gas partly approaches the effect of real flue gas on the growth of algae. The effects of simulated flue gas associated with growth stimulators GA and TA on *chlorella* sp. were investigated below:

3.1 Our experiments point out that stimulated flue gas (15% CO₂ aeration, 50 mg/kg SO₂, 50 mg/kg NO₂) had the different effects on the growth of the algae. First, 15% CO₂ aeration had the positive effect on the growth of algae, but 50 mg/kg SO₂ and 50 mg/kg NO₂ could slightly restrain the growth of algae.

3.2 GA and TA contribute to the growth of the algae which were cultivated with 15% CO₂ aeration without other acid gas. Moreover, the two growth stimulators had stimulated the cell increase almost twice in diameter. Therefore, GA and TA had some effect on the growth rate of algae when aeration with SO₂ and NO₂ gas, and also they had contributed the algae to grow much bigger and high yield.

3.3 GA and TA have the three different effective results on the lipid, protein and carbohydrate accumulation. The results showed that GA and TA had the same positive on the accumulation of lipid, but the content of protein had no obvious changes with the help of GA and TA. High

concentration of CO₂ had negative effect on carbohydrate production which have not been elevated with addition of GA and TA.

3.4 Although SO₂ and NO₂ has a little negative effect on the growth of algae when used independently, the combination of them reduced the negative effect of acid gas on the growth of algae, and the result may be relative to the metabolites of SO₃²⁻ and NO₃⁻ in the algae, and these metabolites neutralized each other to maintain the pH neutral.

3.5 Compared to TA, GA has the more distinct effect on the growth of algae, the biomass affected by GA also reached 11.15 g/L which was much higher than the algae affected by TA (6.04 g/L). Moreover, in view of the price of GA is lower than TA in the market, GA is a wide accepted stimulator applied in the cultivation of algae with flue gas in the future.

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