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Mg$^{2+}$ improves biomass production from soybean wastewater using purple non-sulfur bacteria

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ABSTRACT

Soybean wastewater was used to generate biomass resource by use of purple non-sulfur bacteria (PNSB). This study investigated the enhancement of PNSB cell accumulation in wastewater by Mg$^{2+}$ under the light-anaerobic condition. Results showed that with the optimal Mg$^{2+}$ dosage of 10 mg/L, biomass production was improved by 70% to 3630 mg/L, and biomass yield also was improved by 60%. Chemical Oxygen Demand (COD) removal reached above 86% and hydraulic retention time was shortened from 96 to 72 hr. The mechanism analysis indicated that Mg$^{2+}$ could promote the content of bacteriochlorophyll in photosynthesis because Mg$^{2+}$ is the bacteriochlorophyll active center, and thus improved adenosine triphosphate (ATP) production. An increase of ATP production enhanced the conversion of organic matter in wastewater into PNSB cell materials (biomass yield) and COD removal, leading to more biomass production. With 10 mg/L Mg$^{2+}$, bacteriochlorophyll content and ATP production were improved by 60% and 33% respectively.

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Introduction

Purple non-sulfur bacteria (PNSB) are rich in high-value biochemical substances (Kobayashi and Kurata, 1978; Sabourin-Provost and Hallenbeck, 2009; Kobayashi and Tchan, 1973). The biomass is regarded as a raw material for producing high-value substances. At the same time, PNSB can treat a variety of organic wastewaters (Myung et al., 2004; Nagadomi et al., 2000). Therefore, organic wastewaters can be used to generate biomass resources by PNSB. Soybean wastewater is nontoxic, and rich in nutrients for microorganism growth (Yu et al., 1998). Thus, it can be used as a substrate to produce biomass resources by PNSB.

However, when organic wastewaters have been used as substrates, biomass production was much lower than with culturing mediums (Kobayashi and Tchan, 1973). A key to promoting PNSB production from wastewaters is to improve the conversion efficiency from the organics in wastewaters into production of bacterial cells. According to an analysis of PNSB metabolic activity, adding Mg$^{2+}$ into wastewater might enhance the accumulation of PNSB biomass. This is because the main physiological functions of Mg$^{2+}$ are as enzyme/pigment active sites or activation of enzyme/pigment activity, and regulation of energy metabolism and cellular material synthesis (Hakobyan et al., 2012). Therefore, Mg$^{2+}$ directly affects PNSB growth and metabolism.

The purposes of this study are to enhance the PNSB biomass production from soybean wastewater through adding Mg$^{2+}$ and optimizing the dosage, and to investigate potential mechanisms. The light-anaerobic condition was used since PNSB usually grow best under this condition (Kobayashi and Tchan, 1973).
1. Materials and methods

1.1. Materials

PNSB strain (Rhodobacter sphaeroides Z08) was used (He et al., 2010). It was cultured in improved RCVBN medium for 48 hr at 26–30°C (Wu et al., 2012). Afterwards, PNSB at logarithmic growth phase was inoculated.

Soybean wastewater from Harbin Soybean Products Machining Factory, Harbin, China was used. The characteristics of soybean wastewater were as follows: Mg2+ 0 mg/L, COD 10,000 mg/L, and protein 2300 mg/L.

1.2. Methods

Glass flasks were used as bioreactors and were sterilized before use. The inoculation concentration of PNSB was 160 mg/L. After inoculation, the wastewater pH was 6.9, near neutral. The light anaerobic condition was set as follows: the reactor was illuminated by two incandescent lamps from the left and right sides. The light intensity at the surface of the reactor was controlled at 3000 lx. After saturating with nitrogen (the purity of nitrogen was 98.0%), the bioreactor was sealed with a sealing membrane to maintain the anaerobic condition.

1.3. Analysis methods

Samples containing wastewater and PNSB were collected at 0, 24, 48, 72, and 96 hr and were centrifuged at 9000 r/min for 10 min. The supernatant (wastewater) was used to test COD. The sediments (PNSB) were used to measure biomass production. The collected PNSB was used to measure the bacteriochlorophyll content and adenosine triphosphate (ATP) production. Biomass production and COD were tested by American Public Health Association standard methods. Biomass yield was calculated based on biomass production and COD removal as follows: biomass yield = dry weight of biomass increase / COD-removal.

Bacteriochlorophyll content (Edelenbos et al., 2001) and ATP production (Veclin-Bogues et al., 1997) were measured using HPLC (Agilent 1200, Agilent Technology Inc., Santa Clara, California, USA).

1.4. Statistical analysis

Statistical analysis was performed by means of the SPSS Statistical Software Package. In each experiment, parallel samples were conducted in triplicate. Significant difference was identified using the t-test.

2. Results and discussion

2.1. Effects of Mg2+ concentration on COD removal and biomass production

Effects of different Mg2+ (MgSO4) concentrations on cell accumulation and COD removal were examined. The results are presented in Fig. 1. Under all conditions investigated, wastewater COD removals increased gradually with time. Addition of Mg2+ enhanced the wastewater COD removal with a significant difference (p < 0.05) compared to the control group. The optimal Mg2+ dosage was 10 mg/L, and COD removal reached 86% (Fig. 1a).

Meanwhile, it can be seen from Fig. 1a that with optimal Mg2+ dosage of 10 mg/L, COD removal after 72 hr was higher than that of the control group after 96 hr. To achieve the same COD removal, addition of 10 mg/L Mg2+ could shorten the hydraulic retention time of soybean wastewater from 96 to 72 hr, resulting in an improvement in the efficiency and a decrease in cost and energy consumption.

Addition of Mg2+ not only promoted COD removal, but also improved cell accumulation. At all Mg2+ dosages, biomass production was improved with significance (p < 0.05) compared to the control group. The highest biomass production of 3630 mg/L appeared at the 10 mg/L Mg2+ dosage, which was improved by 70% compared to the control group (Fig. 1b).

Fig. 1 – Effects of different Mg2+ concentrations on COD removal (a) and biomass production (b) in purple non-sulfur bacteria (PNSB) soybean wastewater treatment under light anaerobic condition, 3000 lx, Mg2+ concentrations followed by * are significantly different at p < 0.05, compared to the control group.
Biomass yield was calculated according to Fig. 1. For the control group, 5, 10, and 15 mg/L Mg^{2+} group, biomass yield was 0.25, 0.36, 0.4 and 0.34 respectively. With optimal Mg^{2+} dosage of 10 mg/L, biomass yield was improved by 60% compared to the control group. The increase of biomass yield was very important since it meant that more PNSB biomass could be obtained with the same amount of wastewater COD.

2.2. Potential mechanisms of Mg^{2+} improvement of biomass production and COD removal

The above results clearly show the enhancing effects of Mg^{2+} on biomass production and COD removal (Fig. 1). According to the literature, the most important function of intracellular Mg^{2+} is to participate in energy metabolism (Hakobyan et al., 2012). Thus, the enhancement may be related to Mg^{2+} regulation of PNSB energy metabolism. Since the light-anaerobic condition was adopted, the energy production pathway of PNSB in this work was photosynthesis. Based on the above results and literature analyses, we proposed the potential mechanisms of Mg^{2+} enhancement shown in Fig. 2.

Mg^{2+} improved ATP production through up-regulating the content of bacteriochlorophyll in photosynthesis (Fig. 2 steps one and two). The improvement of ATP production not only directly enhanced biomass production, but also increased COD removal (steps three and four). Moreover, the increase of COD removal meant that more organics were degraded, which provided more raw materials for the synthesis of PNSB cellular substances. This further enhanced PNSB biomass production. As a result, COD removal and biomass production were simultaneously improved by addition of Mg^{2+}.

2.3. Mg^{2+} improved bacteriochlorophyll content and ATP production

In order to prove the hypothesis proposed in Fig. 2, bacteriochlorophyll content and ATP production were measured under all investigated conditions. The results are summarized in Fig. 3.

Addition of Mg^{2+} enhanced the bacteriochlorophyll content with significance ($p < 0.05$) compared to that of the control group. With optimal Mg^{2+} dosage of 10 mg/L, bacteriochlorophyll content was improved by 60% compared to that of the control group (Fig. 3a). This is because Mg^{2+} is the active center of bacteriochlorophyll and plays an important role in capturing light, converting light into electron energy and...
initiating electron transfer (Sandmann and Malkin, 1983; Horton et al., 2002).

With the increase in bacteriochlorophyll content, ATP production was also improved with significance \((p < 0.05)\) compared to that of the control group. The best Mg\(^{2+}\) dosage for ATP production was 10 mg/L, and the ATP production increased by 33% compared to that of the control group (Fig. 3b). The reason was that bacteriochlorophyll played important roles in photosynthesis (Sandmann and Malkin, 1983). Thus, the magnitude of bacteriochlorophyll content determines the energy/ATP production in the photosynthesis pathway, which then determines the activity of PNSB.

ATP plays a very important role in the growth and reproduction of microbes (Horton et al., 2002). The synthesis of intracellular material (proteins, nucleic acids, lipids, and polysaccharides) involves the consumption of a large number of ATP molecules. Thus, the amount of intracellular ATP directly affects the biomass yield and production. At the same time, an increase of ATP production enhanced COD removal. This was because energy (ATP) was needed for the degradation of organic pollutants (protein) and for trans-membrane transport of small molecule substances. The increase of biomass yield is very important since it means that PNSB can synthesize more biomass with the same amount of COD.

3. Conclusions

Addition of Mg\(^{2+}\) was successful in enhancing the PNSB biomass production and COD removal in wastewater treatment. Results showed that with the optimal Mg\(^{2+}\) dosage of 10 mg/L, biomass production was improved by 70% to 3630 mg/L; biomass yield was improved by 60%; COD removal reached over 86% and hydraulic retention time was shortened by 25%. The reason was that Mg\(^{2+}\) improved ATP production through promoting the content of bacteriochlorophyll in photosynthesis. Increased ATP production then enhanced the biomass production and COD removal.

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