Magnesium ions improving the growth and organics reduction of *Rhodospirillum rubrum* cultivated in sewage through regulating energy metabolism pathways

Chang-Ru Xu, Pan Wu, Lang Lang, Ri-Jia Liu, Jian-Zheng Li and Yu-Bin Ji

ABSTRACT

Rhodospirillum rubrum has the potential for biomass resource recycling combined with sewage purification. However, low biomass production and yield restricts the potential for sewage purification. This research investigated the improvement of biomass production, yield and organics reduction by Mg^{2+} in *R. rubrum* wastewater treatment. Results showed that with optimal dosage (120 mg/L), biomass production reached 4,000 mg/L, which was 1.5 times of that of the control group. Biomass yield was improved by 43.3%. Chemical oxygen demand (COD) removal reached over 90%. Hydraulic retention time was shortened by 25%. Mechanism analysis indicated that Mg^{2+} enhanced the isocitrate dehydrogenase and Ca^{2+}/Mg^{2+}-ATPase activities, bacteriochlorophyll content on respiration and photophosphorylation. These effects then enhanced ATP production, which led to more biomass accumulation and COD removal. With 120 mg/L Mg^{2+} dosage, the isocitrate dehydrogenase and Ca^{2+}/Mg^{2+}-ATPase activities, bacteriochlorophyll content, ATP production were improved, respectively, by 33.3%, 50%, 67%, 41.3% compared to those of the control group.

Key words | biomass production, Mg^{2+}, *R. rubrum*, respiration, photophosphorylation, wastewater treatment

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INTRODUCTION

Rhodospirillum rubrum (*R. rubrum*) is a purple non-sulfur bacteria, and widely distributed in rivers, ponds, lakes and oceans (Kobayashi & Tchan 1973; Kobayashi & Kurata 1978). Like other purple non-sulfur bacteria, it has the superior ability to combine wastewater treatment with biomass resource recovery to generate high-value biochemicals (Kobayashi & Tchan 1973; Kobayashi & Kurata 1978; Myung *et al.* 2004; Sabourin & Hallenbeck 2009). *R. rubrum* biomass has been utilized as raw materials in materials, aquaculture and health industrial products (Nagadomi *et al.* 2000). Furthermore, *R. rubrum* wastewater treatment technology avoids excess sludge problems because generated bacterial mass can be reutilized as a resource.

However, when organic wastewaters were used as substrates, biomass production was low (Nagadomi *et al.* 2000). The key to promote *R. rubrum* production from wastewaters is to improve the conversion efficiency from organics in wastewaters into cells. According to the analysis for *R. rubrum* metabolic activity, adding Mg^{2+} may accelerate conversion efficiency. This is because Mg^{2+} participates in many of the biochemical activities of *R. rubrum* (Ferreyra *et al.* 2002; Hakobyan *et al.* 2012).

In previous studies for *R. rubrum*, effort concerning the improvement of the conversion efficiency from organics in wastewater into cells was rare in *R. rubrum* organic wastewater treatment system. Thus, little work was done on Mg^{2+} simultaneously enhancing biomass production and organics reduction in *R. rubrum* organic wastewater treatment.

Soybean processing wastewater (SPW) is a typical non-toxic, high nutrient solution, rich in substrates for microorganism growth (Yu *et al.* 1998). Thus, it can be used for *R. rubrum* culture for biomass recovery and undergoes purification at the same time.

The purpose of the study was: (i) to enhance biomass accumulation and yield in order to accomplish biomass resource recycling through selecting Mg^{2+} dosage in *R. rubrum* SPW treatment under natural light micro-oxygen conditions; and (ii) to investigate the mechanism of Mg^{2+}

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enhancing biomass accumulation and organics reduction simultaneously through regulation of *R. rubrum* energy metabolism pathways.

MATERIALS AND METHODS

Materials

In this work, *Rhodospirillum rubrum* (*R. rubrum*) was adopted. The strain was stored at 4 °C in a refrigerator and cultured with modified Sistrom minimal (RCVBN) medium in a thermostat shaker (120 rpm, 32 ± 2 °C) for approximately 48 hours before the experiment.

SPW from Harbin Soybean Products Machining Factory was used (Harbin, China). The characteristics of SPW were as follows (all in mg/L): Mg^{2+} 1, chemical oxygen demand (COD) 10,000, and protein 2,300.

Experimental setup

One-liter glass flasks were used as bioreactors and were sterilized before use. The inoculation concentration of *R. rubrum* was 190 mg/L. After inoculation, the wastewater (600 ml) pH was 6.9, near neutral. Various dosages of $MgSO_4$ (i.e., 60, 120, 240, 360 mg/L Mg^{2+}) were added into the wastewater.

Natural light micro-oxygen conditions were adopted. Illumination was achieved by incandescent lamps (60 W) on both sides. In the daytime, observed light intensity at the surface of the reactor was 5,000 lux. At night, there was no light. Micro-oxygen conditions were realized by micro aeration; air of 98.5% was supplied. The dissolved oxygen (DO) concentration in the bioreactor was kept at 0.09 mg/L.

Analysis

Triplicate samples were collected from different bioreactors and were centrifuged at 9,000 *g* for 10 minutes (4 °C) before analysis. The supernatants were used to test COD in SPW. The collected *R. rubrum* was used to measure biomass production. The collected 1 g *R. rubrum* cells were used to measure isocitrate dehydrogenase (IDH), Ca^{2+}/Mg^{2+}-adenosine triphosphate (ATP)ase activities, bacteriochlorophyll content and ATP production. Biomass (dry cell weight) and COD were tested by APHA (2005) standard methods. ATP production, bacteriochlorophyll content (Edelenbos *et al.* 2001) were measured by high performance liquid chromatography (HPLC) (Agilent 1,200, Agilent Technologies, Inc., Santa Clara, CA, USA).

IDH and Ca^{2+}/Mg^{2+}-ATPase activities were measured spectrophotometrically (ThermoSpectronic, Rochester, NY, USA) against an appropriate blank using the IDH and Ca^{2+}/Mg^{2+}-ATPase activities assay kits (purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Extraction of ATP was conducted at a low temperature (4 °C). Perchloric acid, potassium dihydrogen phosphate, potassium hydroxide and PBS were previously cooled. We collected 3 g *R. rubrum* cells in centrifuge tubes and mixed them with 200 mL 0.16 mol/L perchloric acid. The centrifuge tube was quickly put into liquid nitrogen. After 5 minutes, the tube was taken out and thawed on ice. After thawing, cells were centrifuged at 12,000 *g* for 10 minutes. Supernatant was collected and 1 mol/L potassium hydroxide was added until pH reached 6.5. The sample was filtered by 0.22 μm filter membrane. ATP production was measured using HPLC (Agilent 1,200, Agilent Technologies, Inc., Santa Clara, CA, USA) (Vectian-Bogues *et al.* 1997).

Statistical analysis

Statistical analysis was performed using the SPSS Statistical Software Package. Results are based on one experiment, each with three replicates per treatment. Bars indicate standard error of the mean. The significant difference was identified by T-test.

RESULTS AND DISCUSSION

Effects of Mg^{2+} concentrations on biomass production and organics reduction

In order to clarify the effects of Mg^{2+} concentrations on the removal of organic matter and biomass accumulation in *R. rubrum* SPW treatment, COD removal and biomass production were tested under all given doses as presented in Figures 1 and 2.

The addition of Mg^{2+} enhanced biomass production and COD removal showed significantly different ($P < 0.05$) compared to the control group. With 120 mg/L Mg^{2+} dosage, COD removal reached its highest level (90%). The highest biomass production was 4,000 mg/L under 120 mg/L Mg^{2+} dosage, which was 1.5 times of that of the control group. Furthermore, Mg^{2+} is not only a trace element, but also a transition metal (Ferreyra *et al.* 2002; Hakobyan *et al.* 2012), meaning that too high or too low Mg^{2+} does not support *R. rubrum* growth.

Biomass yield under all given dosages was calculated according to COD removal and biomass production

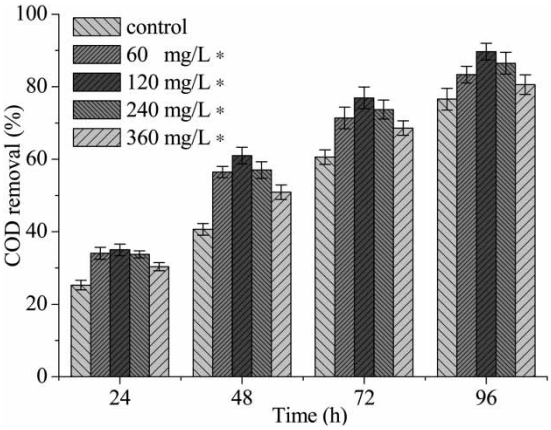

Figure 1 | Effects of different Mg^{2+} concentrations on COD removal in *R. rubrum* SPW treatment under natural light micro-oxygen conditions. Mg^{2+} concentrations followed by asterisks are significantly different at $P < 0.05$, compared to the control group.

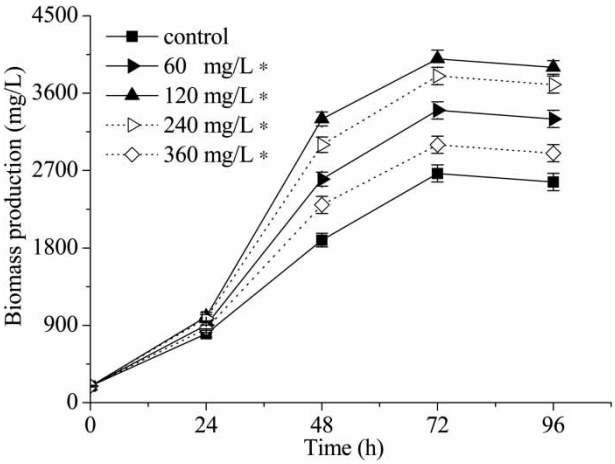

Figure 2 | Effects of different Mg^{2+} concentrations on biomass production in *R. rubrum* SPW treatment under natural light micro-oxygen conditions. Mg^{2+} concentrations followed by asterisks are significantly different at $P < 0.05$, compared to the control group.

(Figures 1 and 2). Biomass yield was defined as biomass-increase/COD-removal. The value was 0.3, 0.35, 0.43, 0.38, 0.32 mg-biomass(dry cell weight)/mg-COD-removal with Mg^{2+} dosage of control, 60 mg/L, 120 mg/L, 240 mg/L, and 360 mg/L, respectively. Biomass yield reached the highest level of 0.43 mg-biomass(dry cell weight)/mg-COD-removal with the addition of 120 mg/L Mg^{2+}, which was improved by 43.3% compared to that of the control group.

Mechanism of Mg^{2+} enhancing biomass production and COD removal through regulating energy metabolism pathways

It was clearly observed that the addition of Mg^{2+} improved COD removal and biomass production in *R. rubrum* SPW treatment (Figures 1 and 2). This might be related to the effect of Mg^{2+} on energy production. This was because intracellular Mg^{2+} participated in energy metabolism and composited in enzyme active sites or the activation of enzyme activity as activators on the electron transport chain (ETC), based on the literature (Ferreyra *et al.* 2002; Horton *et al.* 2002; Hakobyan *et al.* 2012). In this work, natural light micro-oxygen conditions were adopted. Thus, *R. rubrum* can generate ATP through photosynthesis and respiration. Based on the above results and literature analyses, we proposed the potential mechanisms of Mg^{2+} improvement as shown in Figure 3.

The Mg^{2+} improved ATP production through enhancing the content of bacteriochlorophyll in photosynthesis and IDH, and Ca^{2+}/Mg^{2+}-ATPase activities in respiration. The improvement of ATP production not only directly enhanced biomass production and yield, but also increased COD removal. Moreover, the increase of COD removal meant that more organic matter was degraded into small molecules, which provided more raw materials for *R. rubrum* cellular substances' accumulation.

Mg^{2+} improved bacteriochlorophyll content, IDH, Ca^{2+}/Mg^{2+}-ATPase activities and ATP production

In order to prove that Mg^{2+} improved ATP production to enhance biomass production and COD removal through regulating photosynthesis and respiration, the changes of bacteriochlorophyll content, IDH, Ca^{2+}/Mg^{2+}-ATPase activities and ATP production were measured against the control and 120 mg/L groups, respectively.

Figures 4 and 5 show that, with the addition of 120 mg/L Mg^{2+}, the IDH and Ca^{2+}/Mg^{2+}-ATPase activities and bacteriochlorophyll content were improved by 33.3%, 50% and 67%, respectively, and were significantly different ($P < 0.05$) compared to the control group. On the one hand, Mg^{2+} is the activator of IDH and Ca^{2+}/Mg^{2+}-ATPase (Ferreyra *et al.* 2002; Horton *et al.* 2002; Hakobyan *et al.* 2012); IDH and Ca^{2+}/Mg^{2+}-ATPase are active with the participation of Mg^{2+}. Therefore, the magnitude of Mg^{2+} determines IDH and Ca^{2+}/Mg^{2+}-ATPase activities. Conversely, Mg^{2+} is the active center of bacteriochlorophyll, plays an important role in bacteriochlorophyll capturing light, converting light into electrons and transferring electrons (Sandmann & Malkin 1983; Horton *et al.* 2002). The magnitude of Mg^{2+} determines bacteriochlorophyll content.

At the same time, bacteriochlorophyll, IDH, Ca^{2+}/Mg^{2+}-ATPase also regulated energy metabolism. In respiration, IDH is the most important dehydrogenase because

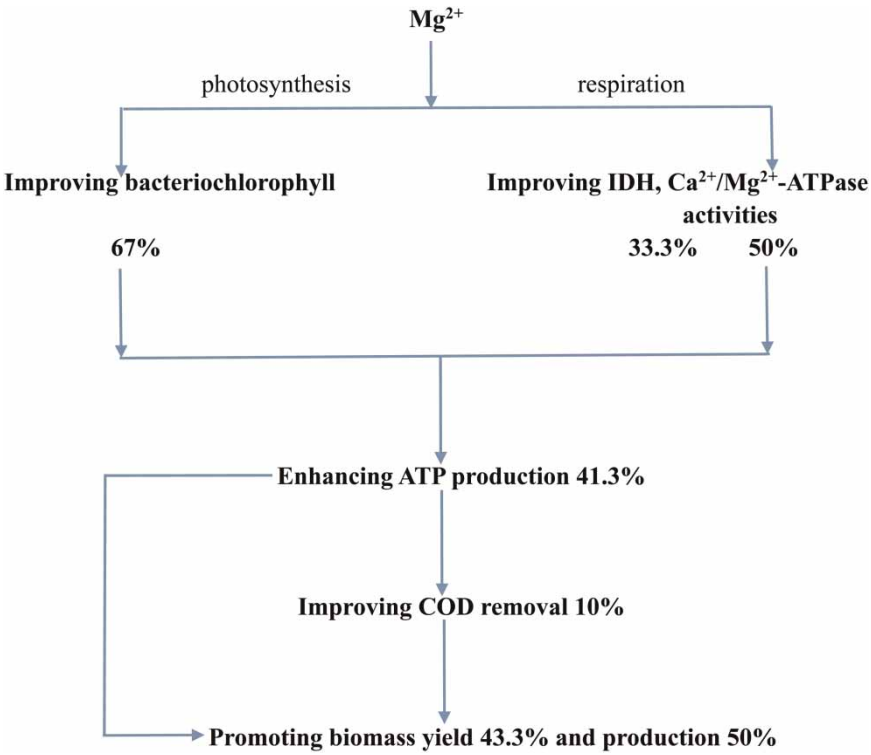

Figure 3 | Mechanism of Mg^{2+} enhancing biomass production and COD removal through regulation for energy metabolism pathways under natural light micro-oxygen conditions.

it is the rate-limiting enzyme (Sandmann & Malkin 1983; Horton *et al.* 2002). In photosynthesis, bacteriochlorophyll play an important role in capturing light, converting light into electrons and transferring electrons (Sandmann & Malkin 1983; Horton *et al.* 2002). For the use of energy, Ca^{2+}/Mg^{2+}-ATPase is the most important. With the participation of Ca^{2+}/Mg^{2+}-ATPase, ATP can be hydrolyzed and converted into ADP, and then energy is used by *R. rubrum* (Sandmann & Malkin 1983; Horton *et al.* 2002). Thus, the magnitude of IDH and Ca^{2+}/Mg^{2+}-ATPase activities and bacteriochlorophyll content (Mg^{2+}) determines ATP production, which then determines the conversion efficiency from organics in wastewater into biomass by *R. rubrum*. As a result, with the addition of 120 mg/L Mg^{2+}, intracellular ATP production was improved by 41.3%, and was significantly different ($P < 0.05$), compared to that of the control group (Figure 6).

Increase of ATP production enhancing *R. rubrum* biomass production and yield in SPW

ATP plays a very important role in the growth and reproduction of microbes (Horton *et al.* 2002). The synthesis of cellular material (protein, nucleic acid, lipid, polysaccharide) needs to consume a large amount of ATP. For example, in the Calvin cycle, ATP is consumed continuously to fix carbon dioxide and to synthesize glucose and carbohydrates (Sandmann & Malkin 1983). Moreover, feedstocks of many intracellular biological macromolecules are produced in the ATP generation process (TCA cycle). So, the amount of intracellular ATP directly affects biomass accumulation of *R. rubrum*.

As Figures 2 and 6 demonstrate, the increase of ATP production enhanced biomass yield (43.3%) with optimal 120 mg/L Mg^{2+} dosage. The increase of biomass yield meant that more *R. rubrum* biomass could be obtained using the same amount of wastewater COD. The conversion efficiency from organics in SPW into cells by *R. rubrum* was improved. Therefore, with optimal 120 mg/L Mg^{2+} dosage, biomass production was enhanced by 67% in *R. rubrum* SPW treatment.

Increase of ATP production promoting the degradation of organic pollutants in SPW

Simultaneously, Figures 1 and 6 showed that COD removal was also enhanced with the increase of ATP production. As Figure 1 showed, after 72 hours of treatment, COD removal with 120 mg/L Mg^{2+} dosage was higher than that of the control group after 96 hours of treatment, indicating that removal of COD was accelerated. To achieve the same COD removal, the addition of 120 mg/L Mg^{2+} shortened the

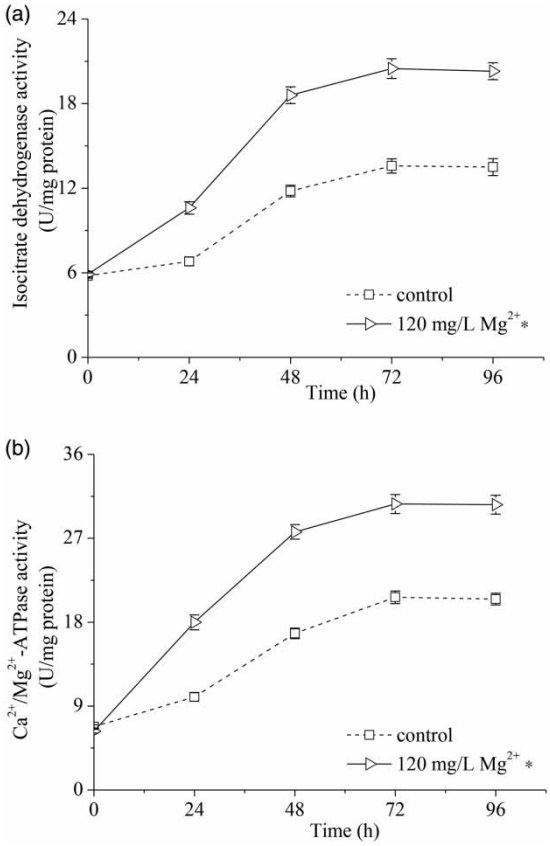

Figure 4 | Effects of different Mg^{2+} concentrations on IDH, Ca^{2+}/Mg^{2+}-ATPase activities of *R. rubrum* under natural light micro-oxygen conditions. Mg^{2+} concentrations followed by asterisks are significantly different at $P < 0.05$, compared to the control group (a) IDH activity (b) Ca^{2+}/Mg^{2+}-ATPase activity.

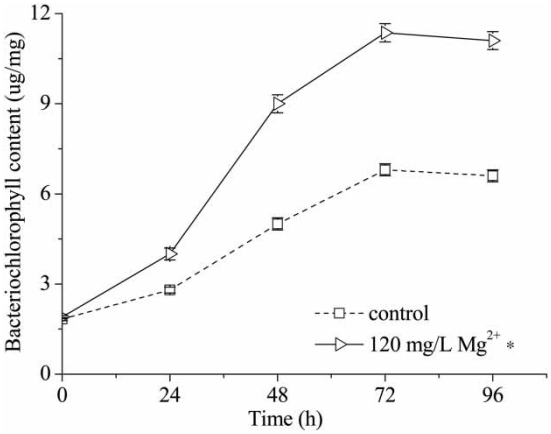

Figure 5 | Effects of Mg^{2+} concentrations on bacteriochlorophyll content of *R. rubrum* under natural light micro-oxygen conditions. Mg^{2+} concentrations followed by asterisks are significantly different at $P < 0.05$, compared to the control group.

hydraulic retention time of wastewater from 96 hours to 72 hours, which not only improved the efficiency of *R. rubrum* treating SPW, but also lowered the cost and energy consumption.

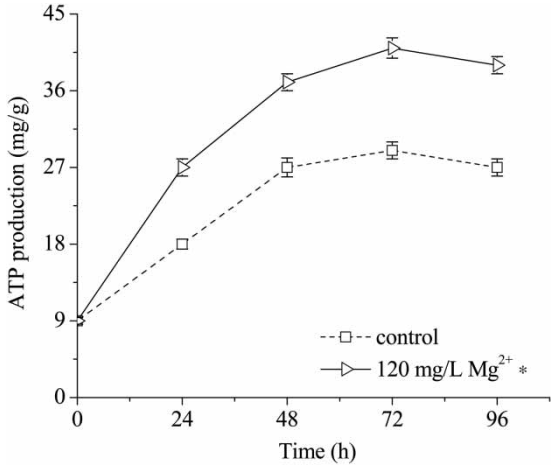

Figure 6 | Impacts of Mg^{2+} concentrations on ATP production of *R. rubrum* under natural light micro-oxygen conditions. Mg^{2+} concentrations followed by asterisks are significantly different at $P < 0.05$, compared to the control group.

This was because the degradation of organic pollutants (protein) in SPW, synthesis and secretion of extracellular enzymes (protease), trans-membrane transport of small molecule substances all need energy (ATP) for *R. rubrum* treatment of SPW. Thus, the increase of intracellular ATP greatly affects COD removal.

CONCLUSIONS

The addition of Mg^{2+} improved biomass production, yield and organics reduction in *R. rubrum* SPW treatment. Results showed that with optimal dosage (120 mg/L), biomass production was enhanced by 50%. Biomass yield was improved by 43.3%. COD removal reached 90%. Mg^{2+} enhanced ATP production through regulating the IDH and Ca^{2+}/Mg^{2+}-ATPase activities, bacteriochlorophyll content on respiration and photophosphorylation. Then, biomass accumulation and COD removal were improved. With 120 mg/L Mg^{2+} dosage, the IDH and Ca^{2+}/Mg^{2+}-ATPase activities, bacteriochlorophyll content, and ATP production were improved by 33.3%, 50%, 67%, and 41.3%, respectively, compared to those of the control group.

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