

## Effect of Salinity on Hydrogen Production and Growth of *Lyngbya* sp.

Yoshinori KUWADA and Yoshiyuki OHTA

*Laboratory for Microbial Biochemistry, Faculty of Applied Biological Science,  
Hiroshima University, Higashi-Hiroshima, 724, Japan*

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**Abstract** Hydrogen production by *Lyngbya* sp. (no. 108) immobilized on calcium alginate was affected by the salinity of the reaction mixture and growth medium. Free cells grown in 3% NaCl produced less hydrogen than cells grown in lower salt concentrations. Hydrogen production by immobilized cells increased with increasing salinity of growth medium. Calcium alginate gel also seems to protect these cells from osmotic shock.

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### INTRODUCTION

It has been known that photosynthetic bacteria (GEST and KAMEN, 1949), cyanobacteria (BENEMAN and WEARE, 1974) and green algae (HEALY, 1970) produce hydrogen. While photosynthetic bacteria evolve more hydrogen than cyanobacteria, they require organic compounds as electron donors (GEST and KAMEN, 1949). On the other hand, cyanobacteria produce hydrogen in the absence of added organic compounds, but need longer cultivation time (RAO *et al.*, 1982). For practical hydrogen production, more hydrogen evolution and less cultivation time is desirable. We have reported that cells which have lost the ability to produce hydrogen can be reactivated in fresh medium (KUWADA and OHTA, 1989). We have also reported the optimum conditions for cultivation of immobilized *Lyngbya* sp. strain no. 108 on calcium alginate (KUWADA and OHTA, 1989). This strain produced hydrogen when it used storage carbohydrate as electron and proton donor (PRINGSHEIM and WIESSNER, 1960), and the amount of storage carbohydrate is affected by the salinity of the growth medium (PRINGSHEIM and WIESSNER, 1960). Previously, we showed optimum conditions for the growth of immobilized cyanobacterium (*Lyngbya* sp. no. 108) (KUWADA and OHTA, 1987), but we did not investigate the effect of salinity on immobilized cells. In this paper, we report the effect of salinity on growth of this strain and hydrogen production.

### MATERIALS AND METHODS

#### *Microorganism*

The cyanobacterium *Lyngbya* sp. (no. 108) was isolated from the estuary of the Ashida river on the coast of the Seto Inland Sea. This strain is a filamentous, nonheterocystous, ensheathed cyanobacterium which has a straight trichome. Other characteristics and optimum culture conditions have been reported in a previous paper (KUWADA *et al.*, 1988).

#### *Estimation and Measurement of hydrogen production*

Estimation and measurement of hydrogen production were also as described in a previous paper (KUWADA *et al.*, 1988). The reaction mixture was a modified PRINGSHEIM and WIESSNER'S medium (pH 6.5, Na<sub>2</sub>EDTA 0.03 g/l, KCl 1.0 g/l, CaCl<sub>2</sub> 0.1 g/l, KH<sub>2</sub>PO<sub>4</sub> 0.02 g/l, MgSO<sub>4</sub>·7H<sub>2</sub>O 5.0 g/l, FeSO<sub>4</sub> 15 mg/l, H<sub>3</sub>BO<sub>3</sub> 43 mg/l, MnCl<sub>2</sub>·4H<sub>2</sub>O 5.4 mg/l, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 1.25 mg/l, ZnCl<sub>2</sub> 0.4 mg/l, CuSO<sub>4</sub> 3.95 μg/l, CoCl<sub>2</sub>·6H<sub>2</sub>O 17.5 μg/l), and no NaCl was included.

#### *Immobilization of whole cells and Quantitation of immobilized cells*

Whole cells were immobilized in calcium alginate gels. Detail conditions and methods are described in the previous paper (KUWADA *et al.*, 1988).

Calcium alginate gel was suspended in 1 M K<sub>2</sub>HPO<sub>4</sub> (pH 9.5) to measure the amount of cells. Detail methods have been described already (KUWADA *et al.*, 1987). Carbohydrate content was measured by phenol-sulfuric acid method (DUBOIS *et al.*, 1956).

## RESULTS AND DISCUSSION

Figure 1 shows the effect of salinity on hydrogen production in the reaction mixture. As has been shown previously (PRINGSHEIM and WIESSNER, 1960), hydrogen production by free cells is affected by the salinity of the reaction mixture. In 0% NaCl, immobilized cells produced 830 ml hydrogen/g dry cells in 10 days. The hydrogen production of immobilized cells decreased with increasing NaCl concentration. In 5% NaCl, immobilized cells produced 20 ml hydrogen/g dry cells. The hydrogen production of free cells also decreased with increasing NaCl concentration. Thus salinity affected the hydrogen production of immobilized cells as it affected that of free cells. However, after 10 days of hydrogen production, these affected cells could grow in the normal culture medium. Therefore, immobilized cells were not killed by the high concentration of NaCl. *Lyngbya* sp. (no. 108) can grow in 5% NaCl growth medium (KUWADA *et al.*, 1988).

Figure 2 shows the effect of salinity of the growth medium on hydrogen production.

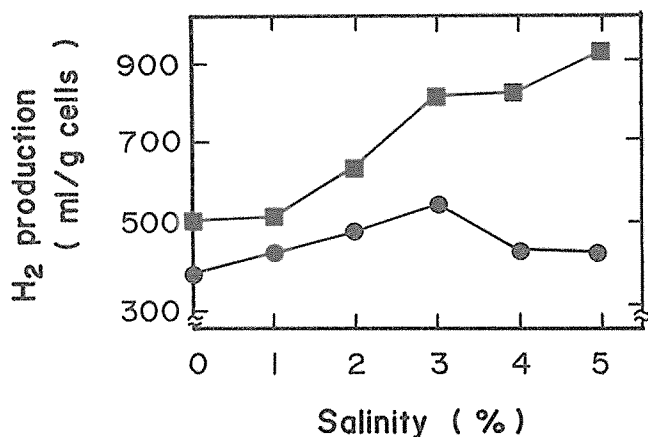


Fig. 1. Effect of salinity of reaction mixture on Hydrogen production.

(●) Free cells and(■) immobilized cells produced hydrogen in 0 to 5% NaCl reaction mixture. Cells were grown in 3% NaCl culture medium.

As we reported previously, cells grew best in the absence of added NaCl (KUWADA *et al.*, 1988), and the optimum salinities for growth and for hydrogen production were not the same. When the cells were immobilized in calcium alginate gel, those grown in 5% NaCl produced the most hydrogen (910 ml/g dry cells). Maximum hydrogen production of free cells was 510 ml/g dry cells, and these cells were grown in 3% NaCl growth medium.

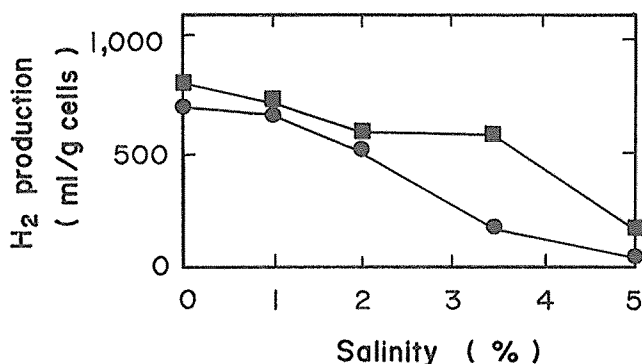


Fig. 2. Effect of salinity of growth medium on hydrogen production.

The cells were grown in 0 to 5% NaCl culture medium (nitrogen poor conditions) under 2,000 lux, at 30°C. These cells were immobilized in calcium alginate gel. Free cells (●) and immobilized cells (■) produced hydrogen in reaction mixture with 0% NaCl under 2,000 lux, at 30°C.

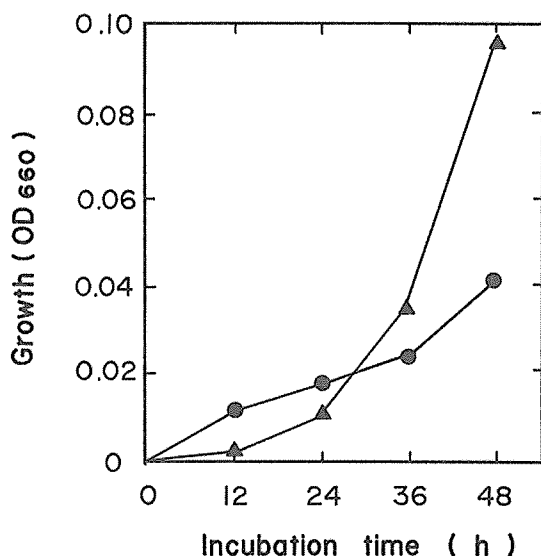


Fig. 3. Effect of salinity of precultured medium on growth.

Cells grown in 5% NaCl culture medium were used to inoculate culture medium containing 5% NaCl (●) and 0% NaCl (▲) culture medium. The growth of these cells was measured spectrophotometrically at 660 nm.

The carbohydrate content of immobilized cells increased with increasing salinity of the growth medium (KUWADA and OHTA, 1989). Therefore, cells grown in 5% NaCl growth medium would have higher potentials for hydrogen production than the cells grown in 0% NaCl growth medium. Some cyanobacteria accumulate carbohydrate against osmotic stress (MACKAY *et al.*, 1983), and the carbohydrate content of the red algae (*Porphyra purpurea*) is affected by the

salinity of the growth medium (REED *et al.*, 1980). Carbohydrate accumulation by *Lyngbya* sp. (no. 108) might likewise reflect osmotic stress on the culture. When these cells were suspended in 0% NaCl, cells containing much carbohydrate might have been osmotically shocked, and therefore they produced less hydrogen. On the other hand, the cells immobilized in calcium alginate gel might have been protected from the osmotic shock due to the physical support of the cells.

The cells precultured in 5% NaCl were inoculated into 5% NaCl and 0% NaCl growth medium and cultivated for 48 hours. (Fig. 3) The cells grown in 5% NaCl had twice the growth amount (for 12 hours) than in 0% NaCl. The change of NaCl concentration affected the growth rate of these cells. These

results supported the hypothesis that cells grown in 5% NaCl are osmotically shocked when they are suspended in 0% NaCl reaction mixture. When the cells became adapted to the

low osmotic condition, the yield of cells grown in 0% NaCl was 2.5 times larger than that in 5% NaCl in 10 days.

In this report, we have examined the effect of NaCl concentration on hydrogen production and growth of *Lyngbya* sp. (no.108). From the above results, we propose the hypothesis that the cells immobilized in calcium alginate gel might be protected from the osmotic shock. Further investigations are needed to prove this hypothesis.

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## ラン藻 *Lyngbya* sp. no. 108 株の水素生産 および成育に対する食塩濃度の影響

桑田 佳典・太田 欽幸

広島大学生物生産学部，東広島市 724

アルギン酸ナトリウムで固定化したラン藻 *Lyngbya* sp. no. 108 による水素の生産量は，培地および反応液の食塩濃度に影響を受けた。3%以上の食塩濃度の培地で培養した菌体の水素生産量は，3%以下の食塩濃度の培地で培養した菌体の水素生産量より低かった。しかし，これらの菌体を固定化した場合，培地の食塩濃度が増大するとともに，水素の生産量も増大した。これらの結果より，菌体を保持しているゲルが，浸透圧によるショックから菌体を保護していると考えた。