BIOLOGICAL HYDROGEN PRODUCTION BY *ENTEROBACTER AEROGENES*

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Of numerous kinds of microorganisms living in soil, water, animals, etc., some species have been found to produce hydrogen under photosynthetic or anaerobic conditions. For instance, it has long been known that hydrogen is evolved together with carbon dioxide from a fermentation in which *Clostridium acetobutylicum*[2,4] takes part. In recent years, attention has been paid to hydrogen as a clean energy source. Accordingly, biological hydrogen production has also received a great deal of attention and an extensive survey has been made to discover new species of microorganisms with high capabilities of hydrogen production (see Mitsui[1]).

The objective of this short note is to report the hydrogen production rate of *Enterobacter aerogenes*. This microorganism is facultative anaerobic, not affected by the presence of molecular oxygen. Therefore, compared to obligate anaerobic species such as *Clostridia*, *E. aerogenes* may be more easily handled.

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Hydrogen Production by *E. aerogenes*

Many different kinds of grass leaves were first tested: each kind of leaf was ground and stored with water in a 0.1 *l*-flask (with stirring) overnight at 30°C; the space above the broth in the flask was sealed with argon. Vigorous bubbling of a mixture of hydrogen and carbon dioxide was observed in the broth of four-o'clock (*Mirabilis jalapa* L.) leaves grown on our university campus.

A sample taken from this broth was inoculated into an enrichment broth and incubated (with continuous shaking) for 20 h at 37°C. A streak-plate method was applied to isolate the bacteria and grow each species,

in colony, on blood and MacConky agars. Each bacterium thus isolated was then tested in hydrogen production ability by inoculating it again into a sterilized broth of four-o'clock leaves (ground). The hydrogen production microorganism found from the four-o'clock leaves was finally identified, through an API test (of API System S.A., France), as *Enterobacter aerogenes*. (Details of the identification have been published elsewhere.)

To examine quantitatively the hydrogen production capability of this microorganism, preculture was made on Trypto-Soya agar (of Nissui Seiyaku Co., Japan) under aerobic condition for 20 h at 37°C; the cells taken from the preculture agar were washed with sterilized water; the cells were then collected in a centrifuge.

A favorable condition for this species to produce hydrogen was found to be pH 6–7 and a temperature of 38–40°C. Figure 1 shows the gas evolution rate (hydrogen and carbon dioxide) and the increase in number of cells after the medium in a flask was inoculated with the cells collected from the preculture, sealed with argon gas, under the following conditions:

culture medium: 0.8 *l* aqua solution with 14% (weight) K_2HPO_4, 6% KH_2PO_4, .2% $(NH_4)_2SO_4$, 1% $Na_3C_6H_5O_7 \cdot 2H_2O$, 0.2% $MgSO_4 \cdot 7H_2O$, 15% glucose and 5% peptone;

number concentration of the bacterium inoculated: 8×10^{14} per 1 *l* of the culture medium;

temperature: 38°C;

pH: 7.

As shown, the hydrogen evolution rate reaches its peak, 0.20–0.21 *l*-H_2/(h·*l*-culture medium), at 5.5–6.5 h after inoculation. This highest hydrogen production period is in accord with the period of sharp increase in cell concentration.

In the early stage, hydrogen is the chief gas collected in the culture flask. This is considered to come from the fact that carbon dioxide produced is absorbed in the medium. After reaching their peaks, the hydrogen and carbon dioxide evolution rates both decrease with time. During this period the hydrogen-to-carbon dioxide ratio is the lowest, at about 1 : 4, and then it turns upward somewhat, although the evolution rates of hydrogen and carbon dioxide are no longer high.

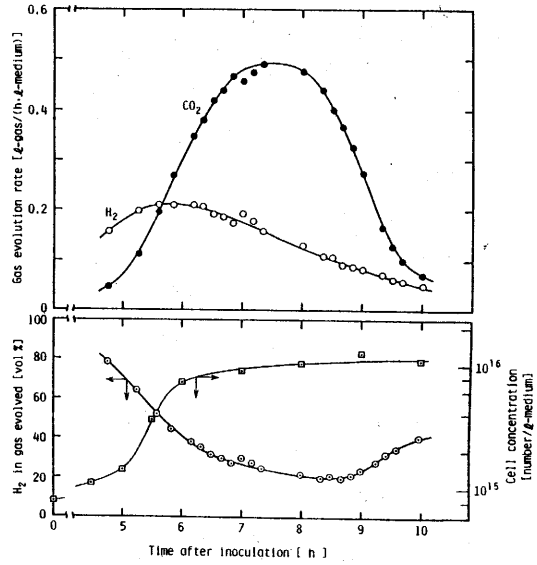

Fig. 1. Gas evolution rate (hydrogen and carbon dioxide) and cell concentration of *Enterobacter aerogenes*.

Suzuki *et al.*[3] have succeeded in continuous gas evolution (hydrogen and carbon dioxide) using immobilized *Clostridium butyricum* in a continuously operated culture tank. Their hydrogen evolution rate is almost the same as above-mentioned rates with *E. aerogenes*. Application of the batch culture with *E. aerogenes* to a continuous process is now being studied in the author's laboratory.

Literature Cited

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