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A MICROBIAL SENSOR FOR URIC ACID

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A microbial sensor for uric acid has been devised by coupling intact yeast cells (*Pichia membranaefaciens* 162300) with a carbon dioxide electrode. The resulting sensor has a markedly longer lifetime than the corresponding isolated enzyme sensor. The response slopes ranging from 37 to 42 mV per decade were obtained over the concentration range 1.0×10^{-4} - 2.5×10^{-3} mol dm^{-3} uric acid during the period 4 - 50 d.

The uricase-catalyzed oxidation of uric acid has been utilized for the determination of this compound by enzyme sensors, in which uricase-coated platinum electrodes are employed to monitor the consumption of oxygen[1-3] and the appearance of hydrogen peroxide.[4] We also presented the enzyme sensor for uric acid based on the potentiometric carbon dioxide gas-sensing electrode.[5,6] Recently, living microbial cells in place of isolated enzymes have been coupled with potentiometric and amperometric electrodes to prepare microbial sensor for the determination of aspartate,[7] arginine,[8] cysteine,[9] BOD,[10] acetic acid,[11] alcohol,[12] glutamic acid,[13] cholesterol,[14] ammonia,[15] and methane.[16] Onishi et al.[17] reported the screening and cultivation of uric acid-utilizing yeasts and found that *Pichia membranaefaciens*, *P. miso*, *Hansenula anomala* and *Shizosaccharomyces octosporus* etc. showed rapid growth and high cell yield in a 0.1% uric acid medium. Among them, we found that *P. membranaefaciens* 162300 was useful to the microbial sensor for uric acid. In this paper, a suitable sensor and its application for the determination of uric acid are described in comparison with the corresponding enzyme sensor.[5,6]

P. membranaefaciens 162300 was cultivated in a medium of 1.17% Bacto yeast carbon base containing 0.1% uric acid as a sole nitrogen source and 2% glucose as a carbon source at pH 6.6 - 6.8 (phosphate buffer) and 30 °C. After 3 d-cultivation, the yeast cultures were centrifuged and washed with water several times. A sensor was constructed by coating the gas-permeable membrane of an Orion Model 095-02 carbon dioxide electrode with *ca.* 9 mg of the washed paste-like cells and

covering the sensing assembly with moistened cellophane to secure the cells. The resulting microbial sensor was conditioned in the pH 6.5 phosphate buffer solution containing uric acid overnight before use, and stored in the same buffer solution between measurements. All potentiometric measurements were done with a Corning Model 12 pH/mV meter in conjunction with a Hitachi Model 056 potentiometric recorder. Measurements were carried out in a thermostatted glass vessel at 33.0 ± 0.1 °C under oxygen atmosphere.

First, the productivity of uricase by 17 yeast-strains and the applicability of these yeasts to the microbial sensor were examined. These results are summarized in Table 1. As can be seen, *P. membranaefaciens* and *P. miso* were found to be useful to the sensor. *P. membranaefaciens* was used in this work.

Table 1. Production of uricase by yeasts in a medium containing uric acid and applicability of yeasts to microbial sensor

Strain	Uricase activity after 3 d (U/g, dry)	Applicability to the sensor
Saccharomyces rouxii N 28	0	-
S. rouxii E 7	1.41	-
S. rouxii var. halomembranis A 31	1.20	-
Saccharomyces acidifaciens S 9	3.42	-
S. acidifaciens var. halomembranis H 3	0.15	-
Saccharomyces fragilis ATCC 12424	0.41	+
Pichia membranaefaciens 162300	1.92	+++
P. miso ATCC 20210	3.29	+++
Hansenula anomala NRRL Y 366	3.59	+
H. subpelliculosa H 79	3.13	+
H. subpelliculosa NRRL Y 1683	4.83	-
H. petersonii NRRL Y 3808	1.59	-
Debaryomyces subglobosus PRL RS 4	0.24	-
D. hansenii H 70	0	-
Schizosaccharomyces octosporus PRL F 2	0.10	+
Endomycopsis capsularis PRL R2A	1.48	-
Trichosporon behrendii E3a	0.77	-

Typical dynamic response curves of the sensor with *P. membranaefaciens* are shown in Fig. 1. Freshly prepared sensors showed a little response, but the dynamic response increased gradually with repeating trial. After 4 d, almost linear response with a slope of 37 mV/decade for a calibration curve could be obtained over the concentration range 1.0×10^{-4} - 2.5×10^{-3} mol dm^{-3} uric acid.

The reaction rate for the enzyme-catalyzed oxidation of uric acid and the response of the carbon dioxide electrode depended on the pH of the solution and the reaction temperature.[5,6] The effect of pH on the response of the sensor was ex-

amined at 33 °C. A maximum reaction rate was obtained at pH 6.3 - 6.5, decreasing on both sides of this range. This result is identical to that of the enzyme sensor for uric acid.[5,6] The effect of temperature on the potential difference of the sensor is shown in Fig. 2. A maximum potential difference was observed at around 33 °C. The activity of the yeast seemed to increase with increasing temperature up to 33 °C and decreased at higher temperatures, while the initial rate of the enzyme sensor increased with increasing temperature up to 45 °C.

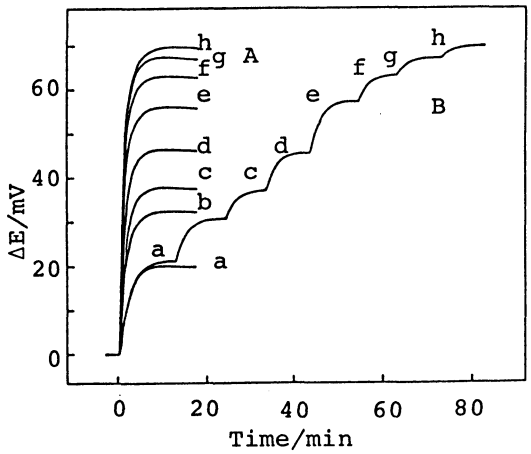

Fig. 1. Dynamic response curves with time.

Uric acid concentration (mol dm^{-3})

a: 9.8×10^{-5}, b: 1.9×10^{-4},
c: 2.8×10^{-4}, d: 4.6×10^{-4},
e: 8.3×10^{-4}, f: 1.2×10^{-4},
g: 1.4×10^{-3}, h: 1.7×10^{-3}.

Uric acid solution was added into the buffer solution, separately (A) and successively (B).
All at 33 °C and pH 6.5.

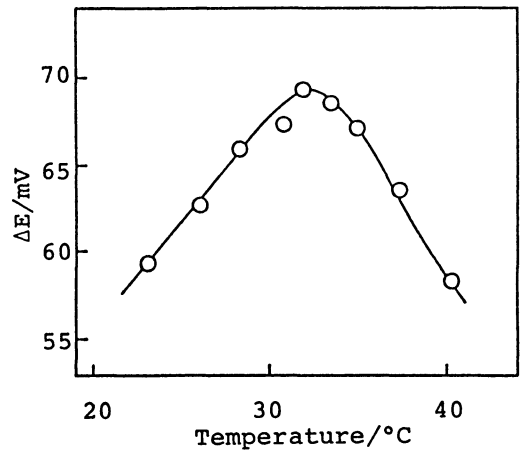

Fig. 2. Effect of temperature.
Uric acid: 8.3×10^{-4} mol dm^{-3}.
All at pH 6.5.

Typical calibration curves for uric acid are shown in Fig. 3. Under the experimental conditions of pH 6.5 at 33°C, almost linear response slopes ranging from 37 to 42 mV per decade were obtained over the concentration range 1.0×10^{-4} - 2.5×10^{-3} mol dm^{-3} uric acid. The sta-

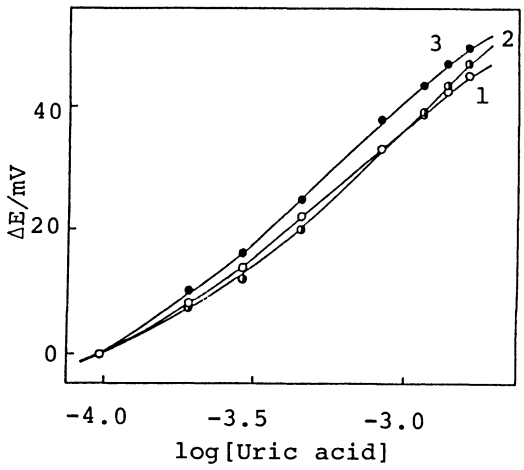

Fig. 3. Calibration curves for uric acid.

Curve (1), 10 d; (2), 20 d; (3), 30 d.

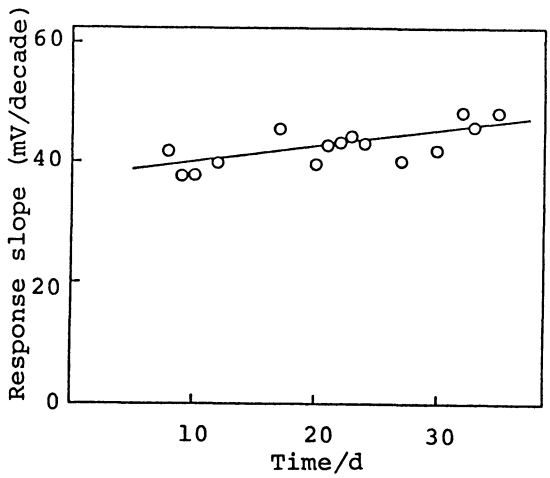

Fig. 4. Stability of the sensor.

bility of the sensor between the 7th and 33rd d are shown in Fig. 4. As is seen, the response slopes tended to increase with aging, possibly because of regrowth of fresh cells on the electrode surface.[18] The lifetime of the sensor could be extended to 50 d longer than that of the corresponding enzyme sensor,[5,6,19] and no response was entirely observed after 57 d.

Although the selectivity study was not evaluated in detail, 5.0×10^{-3} mol dm^{-3} of glucose gave rise to a potential difference equivalent to 1.9×10^{-4} mol dm^{-3} of uric acid. Detailed and further studies are underway for evaluating the proposed microbial sensor for the practical analysis.

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19) It was reported in Refs. 1 and 4 that the immobilized uricase-coated enzyme sensors based on amperometric detection showed long stability. One is useful for the determination of uric acid in blood serum even after 100 d and the other 1000 assays for 17 d, although the enzyme activity decreases gradually with time (d).

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