An organic pollution sensor based on surface photovoltage

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Abstract

A surface photovoltage (SPV) device, which is sensitive to surface pH, was applied to the fabrication of an organic pollution sensor. *Trichosporon cutaneum*, designated for use as a biochemical oxygen demand (BOD) sensor by the Japanese Industrial Standard, was employed as the immobilized biocatalyst. A flow cell of the system consists of an SPV device and a microbial membrane immobilized with *T. cutaneum* between membrane filters. The pH measurement by the SPV device and fabrication of SPV-based sensor is discussed and characterized by comparison with the 5 day BOD test (BOD5) and sensors used in the measurement of BOD (BODs). Response time was 25 min, and a microbial membrane can be used more than 14 weeks. Though there is a different substrate specificity, the system was applicable to BOD measurement of some real waste water and shows good agreement with BODs and BOD5. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Biochemical oxygen demand; Surface photovoltage; Light-addressable potentiometric sensor; *Trichosporon cutaneum*; Biosensor

1. Introduction

Biosensors consist of biomaterial to sense analytes selectively and a transducer to transduce the chemical/physical information produced by the biomaterial into electric information. The recent development of biosensor technology has employed various types of transducers such as dissolved oxygen electrodes, hydrogen peroxide electrodes, ion selective electrodes, photodiodes, and ion sensitive field effect transistors. A silicon based surface photovoltage (SPV), or light-addressable potentiometric sensor (LAPS) device is a transducer that measures the surface potential of the device, especially the pH of the solution near the surface [1–4]. The device can easily be prepared by using a silicon chip. One side of the chip has an insulator layer such as silicon dioxide and/or silicon nitride as sensor side. The other side (back side) has two regions. The first region consists of a deposited metal layer for ohmic contact. The other region is not coated and remains transparent. Illumination from the back side of the SPV device induces a photovoltage. When only the sensor side of the SPV device is immersed in analyte solution and the potential of the solution is biased against the bulk silicon of the device using the ohmic contact, the silicon/solution-system acts as MIS (metal insulator semiconductor) structure. Because the depletion layer at the surface of the device has a certain capacitance depending on the surface potential, the modulated photovoltage induces a photocurrent that depends on the sum of the bias potential and the surface potential of the device. Thus, frequently modulated illumination induces an AC photocurrent. The SPV device has advantages compared to other pH sensors. For example, 1) the fabrication process is simple, 2) by multiplexing different light sources in different locations, the device can be a multisensor without additional process complexity, and 3) encapsulation is easier and less critical. SPV devices have been used in some applications including chemical sensors for quantification of enzyme-
linked immunoassays [5,6], measurement of taste compounds [7], hydrogen [8], and the metabolism of animal cells [9–13].

Metabolism of microorganisms or cells produces acidic substances such as the carbonate ion and organic acids. Microbial metabolism is affected by many factors in a cell’s environment. Changes in the biological, chemical and physical environment of a cell are reflected in the production of acidic compounds. Thus, when microorganisms or cells are immobilized on the pH sensor side of the SPV device, the system is expected to sense the biological information of the analyte solution.

The BOD (biological oxygen demand) sensor is the most widely used microbial sensors [14]. BOD evaluates the organic pollution in wastewater and is a typical parameter of the pollution. The 5 day BOD test (BOD$_5$) has remained a standard pollution monitoring parameter in wastewater and is a typical microbial sensor [14]. BOD evaluates changes in the biological, chemical and physical environment of a cell’s environment. Changes in the biological, chemical and physical environment of a cell are reflected in the production of acidic compounds. Thus, when microorganisms or cells are immobilized on the pH sensor side of the SPV device, the system is expected to sense the biological information of the analyte solution.

The BOD (biochemical oxygen demand) sensor is the most widely used microbial sensors [14]. BOD evaluates the organic pollution in wastewater and is a typical parameter of the pollution. The 5 day BOD test (BOD$_5$) has remained a standard pollution monitoring tool since 1936 [15]. In practice, the BOD$_5$ test requires a 5 day incubation period at 20°C and demands skill in determination. More rapid and simple BOD measurement methods for estimating of BOD$_5$ are still required for pollution control. Recently many sensors have been developed for the measurement of BOD (BOD$_5$). The sensors have consisted of microbial cells immobilized on an oxygen electrode and have measured the current decrease resulting from a decrease in dissolved oxygen [16–24]. Some other methods have been also reported [25–27]. Both the 5 day method and the oxygen electrode method use oxygen consumption as a parameter of the activity of the microorganism. Other parameters of microbial metabolism are potentially useful in constructing a microbial pollution sensor. These parameters may be measured with an appropriate transducer.

Not only accuracy and reproducibility, but portability at low cost are required for microbial sensors to measure environmental parameters in the future. Multi-channel type sensors employing different characteristics of metabolism of each microorganism are also required to deal with solutions containing many kinds of materials. The SPV device is useful to develop a portable sensor which can measure multiple analyte at low cost.

No microbial sensor, however, has used the SPV device. Therefore, we applied the SPV device to the fabrication of a novel BOD sensor. In this research, we fabricated and characterized a novel BOD sensor combining microorganisms with the SPV device. Trichosporon cutaneum, designated for use in BOD sensors by the Japanese Industrial Standard (JIS) committee [14], was employed as immobilized microorganism. First, we will discuss pH measurement by using the SPV device. Second, fabrication of the SPV-based pollution sensor is described and is compared with BOD$_5$ and BOD$_5$.

2. Experimental

2.1. Materials

*Trichosporon cutaneum* IFO10466(AJ4816) was kindly donated by Nissin electric corporation. BOD standard solution containing glucose (150 mg l$^{-1}$) and glutamic acid (150 mg l$^{-1}$) was employed as a model waste water of 220 mgO$_2$ l$^{-1}$ according to Japan Industrial Standard (JIS) committee. Waste water was sampled from a septic tank for sewage of an institute (JAIST), and a brewery. Water was used after deionization and distillation. All other chemicals were laboratory grade and used without further purification.

2.2. Cultivation and storage of microbe

*Trichosporon cutaneum* IFO10466 (AJ4816) was cultured in GP medium containing 20 g of glucose, 5 g of polypeptone, 2 g of yeast extract, 1 g of KH$_2$PO$_4$, 0.5 g of MgSO$_4$ per liter at 37°C for 36 h. The cells were collected by centrifugation (3000 rpm, TMA-6, TOTOM). Phosphate buffer and glycerol as cryoprotectant were added to the cells to be 20% of glycerol and 28 g l$^{-1}$ of wet cell weight. A 2 ml aliquot of the suspension in a cryogenic bial (Nalgene) was gradually frozen at $-25$°C, and stored at $-80$°C.

2.3. Immobilization of the cells

The cell stock was melted in a water bath at 37°C. The cells were washed three times with phosphate buffer by centrifugation. A double-sided adhesive tape with a 4 mm hole was put on an acetylcellulose membrane filter (DISMIC—25 cs, pore size 0.8 μm, Advantec). 1 mg of the cells were filtered through the membrane as shown in Fig. 1. The same type of membrane then was used to cover cells so as to form a ‘sandwich’ of two membranes with cells and adhesive tape as the center layer. The size of the membrane was adjusted to the silicon device (Fig. 1).

2.4. Flow system

An SPV device (Shindengen, Japan) consists of a silicon nitride layer of 50 nm deposited by CVD on a thermally oxidized layer. The thickness of the oxidized layer is 50 nm at a sensor window of 1 × 1 mm, or 300 nm at the other surface. The microial membrane was placed on the SPV device and held between the device and a silicone sheet to give a flow cell as shown in Fig. 2. Because a reference electrode in the flow cell was platinum to reduce the complexity of the cell, the response will be differentially determined by pH and redox potential. Thus, a Ag/AgCl electrode was used in a waste reservoir if required. A peristaltic pump was
employed to flow carrier and sample solution from a carrier or a sample reservoir to the flow cell. The flow cell was connected to an SPV controller (SE1030, Technolgue, Japan) and a computer (9801ns/t, NEC, Japan). A carrier solution (1 mM phosphate buffer with 0.15 M NaCl) was pumped at a flow rate of 250 μl min⁻¹ until the photocurrent became stable. The photocurrent was first measured at increasing bial potentials to obtain a capacitance versus voltage (C–V) curve. The bias potential between the bulk silicon of the device and the solution in the flow cell was adjusted to be centered on the voltage of maximal slope of the C–V curve. All of artificial sample solution’s pH was adjusted to 7 after adequate dilution with the same solution as the carrier solution. After 10 min data acquisition, the carrier reservoir was replaced with a sample reservoir, and the photocurrent was monitored for 25 min. The difference between the average value of the photocurrent from 5 min before the replacement to the time of the replacement and from 20 to 25 min after the replacement was treated as the sample response.

### 2.5. Determination of 5 day BOD

The 5 day BOD value of BOD standard solution and various waste water samples was determined by the Japanese Industrial Standard (JIS) method [15]. After appropriate dilution of the sample water, incubator flasks including BOD regent, sample and the microbes were cultured at 20°C for 5 days under dark conditions. Before and after the cultivation, dissolved oxygen concentration was measured by a dissolved oxygen electrode.
3. Results and discussion

The response of SPV device depends on its surface potential. The pH of the solution is a major factor determining the surface potential. However, other factors, such as ionic strength and physical adsorption, can potentially affect the response. First, the response to pH and other factors were evaluated. The phosphate buffer flowed at 250 μl min⁻¹ and the photocurrent was measured at increasing bias potentials. A sigmoidal curve was obtained as shown in Fig. 3. When there was something wrong, such as a pinhole in the insulation layer or bubbles in the cell, no sigmoidal curve was obtained. Thus, the system was checked with the curve before use. Fig. 3 also shows the sigmoidal curves at various pH values and calibration curves at various bias potentials. The highest sensitivity and reproducibility were obtained at the center point of the sigmoidal curve of pH 7. There are two methods to measure the pH using the SPV device. One is the difference of the photocurrent at the fixed bias potential and the other is the difference of the bias of the center point of its sigmoidal curve. The fixed bias potential method is suitable for real time monitoring. Thus, the potential was fixed for photocurrent measurement at the center potential of the sigmoidal curve of the buffer solution in the following experiment. When the surface potential of the device is under equilibrium, the potential should follow the Nernst equation:

\[ E = E^0 + \frac{RT}{F} \ln [H^+] \]

\[ = E^0 + \frac{8.31 \times 293}{9.65 \times 10^4} \cdot \log [H^+]/\log e \quad \text{for} \quad T = 20°C \]

\[ = E^0 + 58\text{(mV)} \cdot \text{pH} \]

where \( E \) is the surface potential, \( R \) gas constant, \( T \) is temperature, and \( F \) is Faraday constant. Experimental value of the surface potential change was 46 mV pH⁻¹ in Fig. 4, though it is calculated to be 58 mV pH⁻¹ from the Nernst equation. The difference may due to the existence of other ions in the buffer [28]. The same tendency was reported in the research of ISFET that had similar MIS structure as SPV. When the bias was fixed and the pH of the buffer was reduced from seven to four, the photocurrent began to decrease, and then showed a steady response after 6 min. The volume of the tube from the reservoir to the sensor chamber was about 410 μl and that of the chamber was about 130 μl. Because the flow rate was 250 μl min⁻¹, it took about 2 min to replace the buffer in the chamber after the replacement of the reservoirs. The average values of the response from 5 min before the replacement to the time of the replacement and from 25 to 30 min after the
Fig. 3. CV characteristics and calibration curves at various fixed bias against various pH. CV characteristics (a) were obtained at the condition of 250 μl min⁻¹, 10 mM buffer. Calibration curves (b) were calculated from (a) at the fixed bias of 1.0 (A), 1.3 (B), and 1.5 (C) V.

replacement was calculated to reduce error for the estimation of the difference as a response.

The sample of sugar, amino acid, organic acid, or protein at the concentration of 200 mg l⁻¹ or 1000 mg l⁻¹, in the buffer (pH 7) was examined as interfering substance. Almost every sample influenced to the response as shown in Table 1, though no pH change should have occurred. Other proteins such as glucose oxidase (pI = 3.8), bovine serum albumin (4.9), casein (6.4), hemoglobin (8.0), alcohol dehydrogenase (9.2), and cytochrome c (11) also can influence the response at pH 7. No dependence on their isoelectric points was observed. However, the addition of NaCl at the concentration range from 0.1 to 0.2 M removed the interfering substances, and stabilized the response. Thus, the effect was partially due to ionic strength in the flow cell. The addition of NaCl less than 5 mM or more than 300 mM had no effect on stabilizing the response. Fig. 4 shows a transition of the sigmoidal curve depending of the concentration of dissolved salt. Addition of NaCl reduces a photocurrent at the reversed phase of the MIS characteristics, but has no effect on the voltage. The result simply means that lower concentration of the salt enhances the sensitivity of the device. Dependence of the response on the concentration of salt in the buffer was also examined. MOPS buffer solution of 1 mM (pH 7) with various concentration of sodium chloride flowed at 250 μl min⁻¹. Lower concentrations of the salt than 10 mM reduced the sensitivity, and made the response unstable due to the difference between the concentration of the buffer and sample. Higher concentration of the salt also showed instability. The result explains the influence of ionic compounds as interfering substances. Thus, 0.15 M of NaCl was added to both carrier and sample solution in further experiments.

Various kinds of metal ions were also tested at the concentration 0.1 mM. FeCl₂ strongly influenced in the response, though FeCl₃ had no effect. AgNO₃ also influenced it slightly. When 0.1 mM of ascorbic acid was applied to the system, a strong decrease of the photocurrent, though other organic acids such as acetic acid and lactic acid had no effect. Other reducing agents, such as ferrocenyl ethanol, nitrotetrazolium blue, Meldola’s blue, and NAD+, and the waste water from a brewery diluted with the buffer also induced the response, though NAD⁺ showed no response. To confirm the redox effect of the sample, a platinum reference electrode in the flow cell was replaced with Ag/AgCl electrode at a waste reservoir. The system with Ag/AgCl electrode showed no response to the reducing agents listed above. The result suggests that the reducing agent is oxidized at the Pt electrode in the chamber, and shifts the surface potential.

These results suggest that the system with Ag/AgCl electrode adding 0.15 M of NaCl to the carrier and sample solution responds only to the pH of the solution. The Ag/AgCl electrode, however, makes the flow cell complex because of the requirement of internal solution. As described bellow, we finally employed a...
Table 1
Influence of various substances without biochemical reaction

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Photocurrent [nA]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 mg l⁻¹ without NaCl</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.8</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>6.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>2.0</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.0</td>
</tr>
<tr>
<td>Albumin (egg)</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Microbial membrane on the device. The membrane should change the pH only on the device and downstream of the membrane. Then the Pt electrode in the flow cell is unaffected by the activity of the microorganism in the membrane. Thus, the Pt electrode was used as reference to reduce the complexity when a sample contains no or ignorable interfering substances such as ferrous ion and ascorbic acid. The system showed linear response to pH in the range of 2–12 even though a platinum electrode was used as the reference. The sensitivity was 9.9 nA pH⁻¹.

Next, some immobilization methods of microbes were evaluated with the response to 1000 mg l⁻¹ of glucose in the buffer. First, the microbes were suspended in the test solution, and flowed as suspension. The response was small and unstable. The suspension sometimes choked up the tube. Thus, this method is unsuitable for the purpose. Second, the microbes were immobilized in alginate gel. *T. cutaneum* mixed with 4% of sodium alginate, and the mixture was dropped into 0.05M of calcium chloride solution at 37°C through a needle (4mm in diameter), and allowed to equilibrate overnight at 4°C. Some ratio of the microbial weight in wet was examined, and 50 wt%, maximum ratio, gave the maximum response. However, the method was unsuitable for repeated use due to destruction of the gel. To reduce the destruction, calcium ion was added to the buffer, but it reacted with phosphate in the buffer. Finally, the microbe was immobilized using an acetylcellulose membrane. Several kinds of the immobilized amount were examined as shown in Fig. 5. More than 0.5 mg microbes gave a higher response than the alginate gel method with 50 wt% microbes. More than 1 mg gave a maximal response with no further increase with increasing organisms. Thus, 1.0 mg of the microbe was immobilized for further experiments. Because both sides of the microbial membrane were acetylcellulose membrane, the membrane was easy to handle and store. Long term stability of a microbial membrane was evaluated for 14 weeks. A microbial membrane was stored in the 1 mM phosphate buffer (pH 7) containing 0.15 M of NaCl at 4°C, and used once a two weeks. No significant decrease or increase in response was observed in the period examined. Thus, the membrane can be used more than 14 weeks.

Biosensors that measure pH shift have a large dependence on the buffer ability. High buffer ability inhibits the pH shift at the sensor surface and reduces the sensitivity, though low buffer ability makes the response unstable. Fig. 6 shows the dependence of the response on the concentration of the buffer. Lower concentration of the buffer gave larger response, and even the lowest concentration, 1.0 mM, showed enough stability.

Fig. 7 shows the dependence of the response on the flow rate. Generally speaking, slower flow makes the response larger, but it takes a longer time to become stable. The response time for the flow rate of 50 µl min⁻¹, about 70 min, was too long, comparing to the
Fig. 6. Dependence of the response on the concentration of the buffer. After stabilization of the response with the flow of 1mM phosphate buffer (pH 7) containing 0.15 M NaCl at 250 μl min⁻¹, carrier solution was changed to sample solution containing 1 g l⁻¹ glucose in various concentration of phosphate buffer. *T. cutaneum* was 1.0 mg. The time when the solution changed is zero in horizontal axis. The concentration of phosphate in buffer was 1.0 ( ), 2.0 ( ), 5.0 ( ), 10 ( ) mM. Control sample was 1.0 mM of phosphate buffer without glucose in the sample ( ).

Fig. 7. Dependence of the response on flow rate. After stabilization of the response with the flow of 1mM phosphate buffer (pH 7) containing 0.15 M NaCl at various flow rate, carrier solution was changed to sample solution containing 1 g l⁻¹ glucose in the buffer. *T. cutaneum* was 1.0 mg. The time when the solution changed is zero in horizontal axis. The flow rate was 50 ( ), 250 ( ), 300 ( ), 500 ( ) mM. Control experiment was at 250 μl min⁻¹ without glucose in the sample ( ).

Table 2
Comparison of BOD values of various organic samples

<table>
<thead>
<tr>
<th>Substrate</th>
<th>SPV</th>
<th>BOD₅</th>
<th>BOD₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.66</td>
<td>0.72</td>
<td>0.50–0.78</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.73</td>
<td>0.54</td>
<td>0.71</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.45</td>
<td>0.36</td>
<td>0.49–0.76</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.04</td>
<td>0.06</td>
<td>0.45–0.72</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>0.07</td>
<td>0.07</td>
<td>0.22–0.71</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.36</td>
<td>0.45</td>
<td>0.52–0.55</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.40</td>
<td>0.70</td>
<td>0.64</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.34</td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.39</td>
<td>1.77</td>
<td>0.34–0.88</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.18</td>
<td>0.72</td>
<td>0.63–0.88</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.14</td>
<td>0.17</td>
<td>0.40</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.49</td>
<td>2.90</td>
<td>0.93–1.67</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.44</td>
<td>0.51</td>
<td>0.62–0.83</td>
</tr>
</tbody>
</table>

a Values are expressed in mg O₂/mg Substrate. The SPV method was performed at the condition of 250 μl min⁻¹, 1 mM phosphate buffer, pH 7, containing 0.15 M of NaCl, and 1.0 mg cell weight in wet. Substrate was 100 mg l⁻¹, pH 7 in the buffer.

b Calculated data from [18].
method, comparing with reported data of BOD$_5$ and BOD$_s$ for the compounds [18]. For the organic substances including lactose, soluble starch, and lactic acid, the SPV-based sensor and the sensor based on the oxygen electrode show lower BOD values compared with the BOD$_5$. Because these two methods employ short-time metabolism of the same kind of microorganism, the compounds that are difficult to degrade would have smaller BOD values than BOD$_5$.

The SPV-based sensor, however, gives lower values to some compounds such as acetic acid, citric acid, and ethanol. One of the most likely explanation is that the buffer ability of the compounds itself reduces the sensitivity. The concentration of phosphate in the dilution and running buffer was in the same order of the acidic compounds tested in these experiments. Because each pH of the test solution was adjusted before injection, the solution might show higher buffer ability than other test solution. As described above, high buffer ability reduces the sensitivity. When the phosphate concentration was 2 mM, the sensitivity was about 80% of 1 mM. However, the difference between BOD$_{SPV}$ and BOD$_s$ against the acidic compounds were larger than the reduction by buffer ability. Therefore there should be another reason to explain the small response against the acidic compounds.

The other explanation is that the compounds the system mainly responds to, are the acidic compounds produced by the microbe in its early metabolism stage. For example, a microbe metabolizes glucose to produce acidic compounds such as pyruvic acid, and lactic acid through glycolysis. Glycolysis is generally faster than TCA cycle, and the major factor of the response might be glycolysis in the short response time. There is no consumption of oxygen in glycolysis, and only two molecule production of NADH requires a small amount of oxygen in the following reaction of ATP production. Compared to TCA cycle, glycolysis has only a small contribution to oxygen consumption in the oxygen electrode method, though it produces a certain amount of acidic compounds. Thus, relatively small amounts of response might be obtained for the compounds that is metabolized only in TCA cycle. The mechanism suggests that the method can be used in anaerobic condition. Microorganism that metabolizes certain material specifically in anaerobic condition would be applicable to microbial sensor combining it with SPV-based sensor.

Fundamentally, the SPV method measures not the ‘oxygen demand’, or the consumption of oxygen, but the production of acidic compounds, while the reference methods measure the consumption of oxygen. Acidic compounds produced by microbe are mainly carbon dioxide and carbonic acid. Oxidation of substrate with oxygen produces acidity. Thus, the production of acidic compounds has some correlation to oxygen consumption. The difference of principle can induce different substrate specificity though they utilize the same microbe. Strictly speaking, BOD value estimated by the SPV method has different characteristics from BOD$_s$ and BOD$_5$. There is also the difference between BOD$_s$ and BOD$_5$. The 5 day method utilizes any kind of microorganisms allowing them to metabolize for a long time, while the sensor method utilizes the designated microorganism allowing it to metabolize for short time. We have to pay close attention when comparing the BOD values when the sample contains compounds of different specificity. Fortunately, environmental monitoring often treats the sample from fixed sites or the same kind of sample, and one can consider the difference before analysis.

Subsequently, SPV-based BOD values of some waste water samples were determined after adequate dilution. The conventional 5 day BOD was also determined by the standard dilution method. In this procedure, the concentration of dissolved oxygen before/after the incubation was determined with an oxygen electrode. The waste water samples tested were treated and untreated waste water collected from a septic tank of an institute and a brewery. Each of the waste water samples was diluted appropriately with the same solution as the carrier solution prior to use. A good agreement between the SPV-based sensor and BOD$_5$ methods was obtained for the test sample (Fig. 9). The correlation coefficient between BOD$_{SPV}$ and BOD$_s$ was 0.979 and 0.976 for the sample from the institute and the brewery, respectively. Though the sample from the brewery may contain redox compounds such as ascorbic acid, the sensitivity without extra reference electrode is almost the same as that of the sample from the institute.

4. Conclusion

In this research, we employed the SPV method for pH measurement, and combined it with a microbial
membrane to construct an organic pollution sensor system. The system responds only to the pH change in the range of 2–12 with certain amount of salt, though extra reference electrode is required to avoid the effect of redox compounds, if any. The system is applicable to organic pollution measurements and shows good agreement with BOD$_5$. There is, however, a certain different substrate specificity that has to be taken into consideration as one does for the difference between BOD$_5$ and BOD$_3$.

The results expand the applicability of SPV method to the field of microbial sensors, though this research only treats BOD measurement. In principle, many microbial sensors measure the activity of immobilized microorganism, and the method used in this research was less specific to BOD measurement. Thus, the method should be applicable to other microbial sensors only by the replacement of the immobilized microorganism from *T. cutaneum* to other that is specific to certain analyte and some optimization.

**References**


**Biographies**

Yuji Murakami received his Ph.D. degree in engineering from the University of Tokyo in 1995. He has been a research associate at the School of Materials Science of Japan Advanced Institute of Science and Technology, Hokuriku (JAIST, Hokuriku) since 1995. He is interested in miniaturized biosensors and biotechnological devices by micromachining.
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Yoshitaka Ito graduated from Fukushima National College of Technology in 1967. Since 1967 he has been employed by Research and Development Center of Shindengen Electric MFG,CO.,LTD., in Japan. His fields of expertise are development of IC processes, designing and fabrication of silicon devices. Since 1977 his main fields of his interest are development of ISFET. He was awarded the first Seiyama Award of Japan Association of Chemical Sensors in 1998 for development ISFETs and commercialization of ISFET-pH meters. His research subject still concern the further development of ISFETs and VLSI chemical sensors based on silicon planer technology.

Masataka Takiue received his BE degree in engineering from Nihon university in 1975. He has been employed by Teknologue co., Ltd. since 1977. His fields of expertise are development of potentiogalvanostat, LAPS, and some other instruments. His current is interested in the development of LED color selector and IrDA tester.

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Teruaki Katsube received his Ph.D. degree in engineering from the University of Tokyo in 1974. He served as a lecturer at the department of electric engineering of Saitama University in 1974. He has been a professor at the department of information science of Saitama University from 1988. He is interested in microelectronics and sensing systems.

Eiichi Tamiya received his Ph.D. degree in engineering from Tokyo Institute of Technology in 1985. He served as a research associate, and lecturer at Research Laboratory of Resources Utilization of Tokyo Institute of Technology, and associate professor at the Research Center for Advanced Science and Technology, the University of Tokyo in 1985, 1987, and 1988, respectively. He has been an professor at School of Materials Science of JAIST, Hokuriku since 1993. He is interested in precise understanding of biological functions with the use of advanced biotechnology.