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# Determination of Sulfur Dioxide in Wine and Dried Fruits Using a Microbial Sensor

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## Abstract

The concentration of free sulfur dioxide in wine and dried fruits was determined using a microbial sensor method and the modified Rankine's method. The values obtained by the sensor method were more accurate than those by the modified Rankine's method. Pigments in the red wine did not disturb the measurements with the sensor. The sensor was also possible to determine the sulfur dioxide in extracts of dried fruits accurately even though the extracts contained 7 % of sugar. Moreover, the microbial sensor method was more profitable economically from the point of view of reducing the time and the materials for analysis especially when the measurement of many samples were performed.

Previously we have reported that a microbial sensor of immobilized *Thiobacillus thiooxidans* S3 viable cells was an advantageous method to determine sulfur dioxide in wine(1). It is well known that sulfur dioxide dissolved in water exists in three forms, bisulfite ($HSO_3^-$), sulfite ($SO_3^{2-}$), and molecularity sulfurous acid ($SO_2$) depending on the liquid pH(2).

In the microbial sensor method, sulfur dioxide was separated from sample solution by making use of the state change of $HSO_3^-$, $SO_3^{2-}$ and $SO_2$, and then specifically detected by Thiobacilli cells employed as molecular recognition elements.

This paper describes that the microbial sensor method is applicable to not only wine but also solid samples containing sugar such as dried fruits.

## Theoretical Background

Sulfur dioxide dissolved in water exists in three forms, $HSO_3^-$, $SO_3^{2-}$, and $SO_2$, depending on the pH value. Employing the pK values suitable for the dissociation state of sulfur dioxide in wine(2), the existence ratio of the three forms was calculated as shown in Fig. 1 according to the Henderson-Hasselbach's equation(3). Figure 1 shows the lower pH value gives the higher existence ratio of sulfur dioxide. On the other hand, *Thiobacillus thiooxidans* S3 performs the maximum oxidation of $SO_3^{2-}$ at pH 5.0. Generally speaking, the oxidation mechanism of sulfur compounds by *T. thiooxidans* is still unclear because the enzymological study has not been

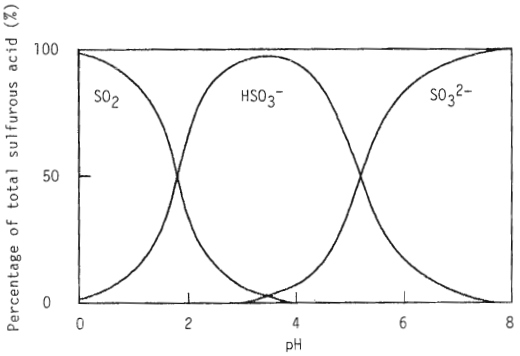


Fig 1. Effect of pH on form of sulfurous acid in water. The values were calculated from the equation ; log (oxidized form/reduced form)=pH−pK. The pH value for the equilibrium $SO_2 \rightleftarrows HSO_3^-$ is 1.77, and the equilibrium $HSO_3^- \rightleftarrows SO_3^{2-}$ is 5.30 (Amerine *et al.* 1972).

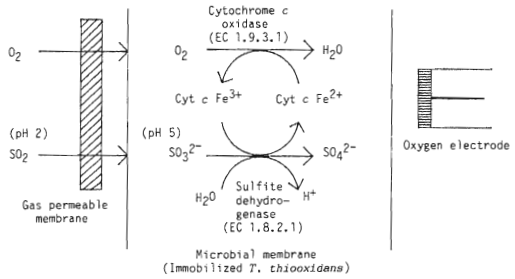


Fig 2. Mechanism of sulfur dioxide determination by microbial sensor.

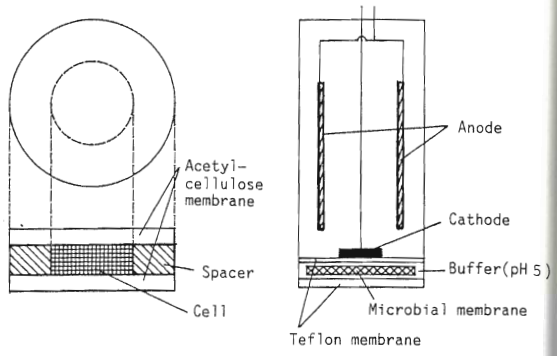


Fig 3. Preparation methods of the microbial membrane and the electrode for free sulfur dioxide determination.

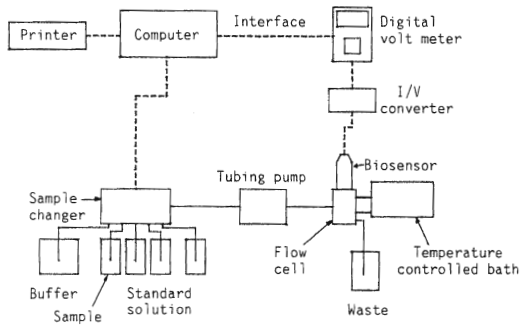


Fig 4. Schematic diagram of the computer aided analytical system for free sulfur dioxide.

enough until now. In this study, the mechanism of sulfur dioxide determination by the microbial sensor was supposed as shown in Fig. 2 on the assumption that sulfite dehydrogenase (EC 1. 8. 2. 1) and cytochrome *c* oxidase (1. 9. 3. 1) take part in the reaction. When the pH of sample solution is adjusted to 2.0, 37 % of the free sulfite exists in $SO_2$ form (gas). Therefore the $SO_2$ gas can pass through a gas-permeable Teflon membrane. The $SO_2$ gas passed through the Teflon membrane changes into $SO_3^{2-}$ (aq.) because the buffer solution soaking the membrane immobilized *T. thiooxidans* S3 has a higher pH value of 5.0 than the outside pH of the Teflon membrane. The immobilized *T. thiooxidans* S3 oxidizes $SO_3^{2-}$ into $SO_4^{2-}$ accompanied with the reduction of cytochrome *c*, and then reduced cytochrome *c* is oxidized with oxygen. The amount of $SO_2$ will be calculated from the amount of consumed dissolved oxygen measured by an oxygen electrode.

## Materials and Methods

**Bacterial strain** *Thiobacillus thiooxidans* S3(4) was used in this study. Cultivation was done according to a previous paper(1).

**Preparation of microbial membrane and microbial electrode**

Acetylcellulose membranes (Millipore Ltd., Massachusetts, USA, type HAWP 02500) was used to immobilize *T. thiooxidans* cells by the sandwich method as shown in Fig. 3. A piece of the microbial

membrane was set onto oxygen electrode (Denki Kagaku Keiki Co. Ltd., Tokyo) and soaked in 0.1 M sodium citrate buffer (pH 5.0) covered with a gas-permeable Teflon membrane.

**Measuring equipment** For automatic measurement the experimental hardware was assembled as shown in Fig. 4. The microbial electrode was placed into a flow cell apparatus and connected to a digital volt meter via I/V converter. The system was controlled by a computer (NEC Co. Ltd., Tokyo, Type PC-9801 VX21). Five millimole per liter sulfuric acid solution (pH 2) passed at a flow rate of 2.7 ml/min through the flow cell which was kept at 30℃. When the output current of the sensor became constant, a sample solution (pH 2.0) was introduced into the system for 2 min by a sample changer. Current decrease was caused in proportion to the concentration of sulfur dioxide, and calibrated by use of a sodium sulfite solution(1).

The sulfur dioxide concentration of the sample solution was automatically calculated by the computer.

**Sample preparation** Wine (red and white) and dried fruits (papaya, pineapple, and apricot) were purchased on the market. All sample solutions were diluted with the arbitrary concentration of sulfuric acid solution in order to match the concentration of sulfur dioxide in the samples with a calibration curve and adjust the pH of samples at 2.0.

## Results and Discussion

**Optimum conditions for determination of sulfur dioxide** We investigated the various conditions for the determination of sulfur dioxide with the microbial sensor method. Optimum conditions were found as shown in Table 1. Under the conditions, the total required time for measurement of one sample was 10 min : 2 min for sampling and 8 min for washing.

**Determination of sulfur dioxie in wine** Free sulfur dioxide in wine (white and red) was determined by the apparatus shown in Fig. 4. Results are shown in Table 2. Sulfur dioxide in red wine was quantified as 2.4 mg/ℓ in every run. On the other hand, the conventional modified Rankine's method gave the measurement values

Table 1. Optimum conditions for sulfur dioxide determination with the microbial sensor.

| | |
|---|---|
| Inner buffer | 0.1 M Na citrate-NaOH buffer (pH 5.0) |
| Carrier solution | 5mM $H_2SO_4$ soln. (pH 2.0) |
| Temperature | 30℃ |
| Flow rate | 2.7 ml/min |
| Immobilized cell wt. | 0.25 mg/membrane |
| Storage conditions for microbial membrane | 4℃, 0.1 M Na citrate-NaOH buffer (pH 6.0) |

Table 2. Determination of sulfur dioxide in wine with the microbial sensor and the modified Rankine's method.

| Sample | | Sensor(mg/ℓ) Free-$SO_2$ | Rankine (mg/ℓ) Free-$SO_2$ | Rankine (mg/ℓ) Combined-$SO_2$ |
|---|---|---|---|---|
| Red wine | Run 1 | 2.4 | 2.0 | 26.0 |
| | Run 2 | 2.4 | 3.3 | 26.0 |
| | Run 3 | 2.4 | 4.0 | 26.0 |
| White wine | | 36.8 | 36.1 | 118.5 |

ranging from 2.0 to 4.0 mg/ℓ (average : 3.1 mg/ℓ). As mentioned in our paper(1), the relative standard deviation for the microbial sensor method was smaller than for the modified Rankine's method. In this study the sensor method also gave more accurate results, compared with the Rankine's method. Therefore, a number of runs for analysis are necessary for the Rankine's method to obtain accurate values. Increasing the number of the assay, the Rankine's method requires longer time than the sensor for measurement. If we have 20 samples, 230 min should be required in the sensor method including the three standard samples, but the Rankine's method needs at least 400 min for analysis : 15 min for sample preparation and 5 min for titration.

Sulfur dioxide in white wine was also determined. The sensor gave the measured value of 36.8 mg/ℓ. The result obtained by the sensor method was reasonable and similar comparing the measured value in the Rankine's method.

From the above results, the microbial sensor method for the determination of sulfur dioxide was quite promising for practical use from the point of view of accuracy, stability, and reducing the time for analysis. Pigments(5) in the wine did not disturb the measurements.

**Determination of sulfur dioxide in extracts of dried fruits** Sulfites were added to various solid foods for the purpose of bleaching and protection from undesirable oxidation and microbial spoilage. The microbial sensor method has been found to be feasible for determination of sulfur dioxide in wine. Here, the microbial sensor method was also applied for the determination of sulfur dioxide in solid foods in order to expand the field of its application. We employed dried fruits as the typical solid food containing sulfites. Sulfites were extracted with 0.05 M NaOH solution from three kinds of dried fruits under mild condition of room temperature for 4 hours. The concentration of sugar in the extract was about 7 %.

Table 3 shows the results measured by the sensor and Rankine's method. The sensor could determine the concentration of sulfur dioxide in the extracts of dried fruits as well as the Rankine's method. Measured values by the sensor and Rankine's method agreed well in the case of papaya and apricot, but the sensor gave a little higher values than the Rankine's method in pineapple sample. No combined sulfite was detected in the extracts of papaya and pineapple. The extraction method conducted in this study was supposed to be unsuitable for papaya and pineapple because the swelling of samples was not enough to release the combined sulfite. As to the extract solution, however, direct measurement was possible by the microbial sensor without especial pretreatment.

From the above results, the microbial sensor method was found to be accurate enough to determine free sulfur dioxide in solid foods such as dried fruits containing sugar.

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Table 3. Determination of sulfur dioxide in extracts of dried fruits with the microbial sensor and the modified Rankine's method.

| Sample | | Sensor(mg/ℓ) Free-$SO_2$ | Rankine (mg/ℓ) Free-$SO_2$ | Rankine (mg/ℓ) Combined-$SO_2$ |
|---|---|---|---|---|
| Papaya | Run 1 | 10.0 | 9.8 | 0 |
| | Run 2 | 10.0 | 9.8 | 0 |
| Pineapple | Run 1 | 6.0 | 4.6 | 0 |
| | Run 2 | 5.5 | 4.9 | 0 |
| Apricot | | 8.5 | 8.9 | 39.9 |

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