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The Bio-deodorizing Effects of Acidithiobacillus thiooxdans SOB5VT1

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Abstract

A microorganism harmless to men was used to screen bacteria that could remove nine odorous substances, including H_2S, NH_3, and volatile organic compounds (VOCs), which are most likely to be contained in noisome odor gases, and could grow at low pH and to assess efficiency of a bio-deodorizer. *A. thiooxidans* SOB5VT1, which serves as a deodorizer for noisome odor gases, was used to assess the level of deodorization. SOB5VT1 was cultured in an MW medium containing S powder (1%) and the medium contained KH_2PO_4 (3g), $MgSO_4$ (0.5g), $CaCl_2$ (0.3g), $FeSO_4$ (0.01g) T.B (0.02g), and S powder (1%) per 1 L. According to the composition of the medium, the microorganism was cultured in volumes of 30 mL, 300 mL, 3 L, 20 L, 1 t, and 10 t; after the inoculation in a deodorizer, the efficiency of deodorization was assessed by the change of color from yellowish red to red and by pH variation, as determined using a pH electrode, in order to identify its growth along with sulfur precipitation as it was absorbed by sulfur. As a result, among noisome odor gases, 99.88% of hydrogen sulfide and up to 95.73% of ammonia were removed. The other seven odorous substances were also decomposed and the concentration of emitted gases was lower than the permissible level of odor emission, as determined by the Air and Environment Preservation Law established by the Ministry of Environment.

Keywords: *A. thiooxidans SOB5VT, bio-deodorizer, H_2S, NH_3, and volatile organic compounds (VOCs)*

1. Introduction

The odor caused by the release of environmental pollutants, such as H_2S and NH_3, into the air, which is now a social problem, can displease and disgust us, stimulate mucosa in the respiratory system or eyes, change blood pressure or pulsation, cause anorexia, vomit, and insomnia, and lower the quality of air.[1～3] Moreover, as cities have become larger in recent times, the lots of odor generated from the pulp industry, food processing plants, and public environmental facilities, such as domestic sewage treatment plants, in residential areas and in the neighborhood of commercial areas becomes a target of public grievance. Although stricter regulations with the air pollution law in South Korea have increased the market size of the air emission treatment industry, poor development of deodorization techniques prevents it from contributing to the improvement in human health and the quality of life [3～9]. The methods to remove odorous substances can largely be divided into two categories: the physicochemical method, which involves absorption, combustion, and washing, and the biological method. The former is able to treat pollutants in a stable way but may cost much in maintenance and, most of all, cause secondary pollutants. To this contrary, bio-deodorization using the process of microorganism oxidation, which is the biological method, may cost less in maintenance than other types of treatment and cause no secondary pollution. However, most producers fail to apply the characteristics of various microorganisms to a bio-filter or be fully effective in removing odorous substances due to poor management of microorganisms and gases generated from them [3,4,5,7].

Now the biggest problem of the bio-filter system is that H_2S dissolves in water to become sulfuric acid, thus drastically lowering pH and creating the environment in which most bacteria cannot grow. Most of the bacteria used as a deodorant may grow in neutral conditions with pH ranging from 6 to 8 and their growth rate may drop as pH drops. To solve this problem, it is necessary to develop microorganisms that can grow well and be cultured with low pH [10 ～ 12]. This study aimed to assess the deodorizing effects of *A. thiooxidans* SOB5VT1[13], which grows with low pH, in order to improve the deodorization efficiency of a bio-filter deodorizer. An attempt was made to culture *A. thiooxidans* SOB5VT1 and assess the noisome odor gas decomposition rate to determine how well it grew in the actual environment.

How to apply the microorganism according to the variation in the concentration of waste gases and the efficiency of the deodorizer were examined.

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2. Method

2.1 Microorganisms screening

Two principal odorous gases generated from spaces with environmental pollution are H_2S and ammonia and such spaces may have pH drop to 2 to 4 due to oxidation of these chemicals. Biological deodorization requires efficient degassing of acidifying compounds, which may grow well in such an acidified environment. So this study aimed to examine *Thiobacillus sp*. with sulfur oxidizing power in an acid condition with pH ranging from 2 to 4 and to screen *A. thiooxidans* SOB5VT1 out [Fig. 1].

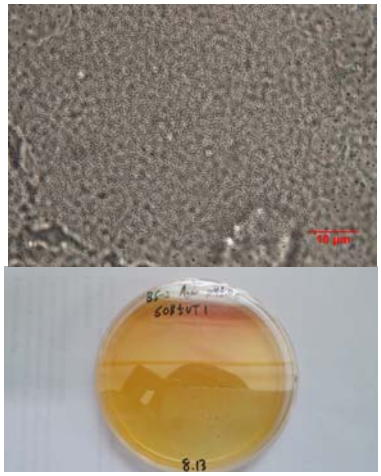

Fig. 1. SOB5VT1 strain

2.2 Modified Waksman (MW) Medium Composition

The method developed by Kim et always modified to use an MW medium containing S (1%) to culture SOB5VT1[11,13,15,16]. Wacksman's modification (MW) method medium contained KH_2PO_4 (3 g), $MgSO_4$ (0.5 g), $CaCl_2$ (0.3 g), $FeSO_4$ (0.01 g), T.B. (0.02 g), and S (1%) per 1 L. According to this composition, 30 mL, 300 mL, 3 L, 20 L, 1 t, and 10 t media were prepared to culture it in small and large volumes.

2.2.1 SOB5VT1 inoculation (30mL, 300mL, 3L)

To culture 30 mL SOB5VT1, a 500 mL conical flask was filled with 100 mL distilled water to dissolve T.B. (0.002 g). KH_2PO_4 (JUNSEI, Germany, 0.3 g), $MgSO_4$ (JUNSEI, Germany,0.05 g), $CaCl_2$ (JUNSEI, Germany, 0.03 g), and $FeSO_4$ (JUNSEI, Germany, 0.001 g) were dissolved in the flask, which was then divided into 30 mL flasks. These flasks were then put in an autoclave at 121°C for 15 minutes. 0.3 g S (1%) in 30 mL was put in a clean bench, which was then put in a 30 mL conical flask sterilized for about 24 hours. 3 mL (10%) of SOB5VT1 was inoculated in the prepared MW medium. SOB5VT1 grows best at 30°C. So the 30 mL conical flask in which it was inoculated was put in a medium to culture at 30°C for 4 to 5 days.

2.2.2 Mass-production of SOB5VT1

3 L of the grown microorganism was cultured in 20 L in a different process. SOB5VT1 is an aerobe, up to 3 L of which can be raised in a conical flask; however, 20 L of it was cultured in a large-volume flask possibly provided with oxygen to keep it aerobic. Since dissolved oxygen is important in a large-volume flask, the appropriate MW medium composition was provided to the 20 L flask to which 1% S was added; then, 2 L cultured SOB5VT1 was put to culture it. To maintain the oxygen saturation amount, a system providing oxygen constantly with an air pump was built to culture the microorganism.

2.3. SOB5VT1 inoculation and performance test in actual bio-filter

Yusung Engineering Co., LTD, a bio-deodorization company, was asked to set the gas inflow rate of the bio-filter at 350[m^3/min] and the temperature was kept at 30°C to create an environment in which SOB5VT1 grew well. 1 t SOB5VT1 was inoculated in an actual bio-filter along with 10 t according to the MW medium composition. For a few days after that, we observed SOB5VT1 for color variation in a circulate flask for microorganisms, assured that sulfur dissolved well, and examined pH variation. We measured pH with ORION3 star pH Benchtop manufactured by Thermo (U.S.) and examined variation of the nine water-soluble odorous substances, including H2S and ammonia, in the inlet and the outlet, respectively, in order to assess performance of SOB5VT1. MultiRAE plus manufactured by RAE SYSTEMS (U.S.) and Derector Tube by Gastek Co. (Korea) were employed in the measurement.

3. Result

3.1 MW Medium Composition

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According to the experiment method, we succeeded in using KH_2PO_4 (3 g), $MgSO_4$ (0.5 g), $CaCl_2$ (0.3 g), $FeSO_4$ (0.01 g) T.B. (0.02 g), and S (1%) per 1 L to prepare the first medium [Fig. 6]. For continuous SOB5VT1 culture, a guaranteed reagent (GR) was used in small volumes (30 mL, 300 mL, 3 L, and 20 L) and a commercial grade reagent was used in large volumes (300 L, 1 t, and 10 t) to prepare a medium.

Fig. 2. Modified Waksman broth media

We inoculated small volumes of SOB5VT1 (30 mL, 300 mL, 3 L, and 20 L) in the MW medium and took it out of the medium five days later to see if it was cultured well. The yellowish red color as shown in Fig. 3 before the culture and red after the culture demonstrated that SOB5VT1 had decomposed sulfur in the medium, generated sulfuric acid, and lowered pH in the medium, as could be observed with naked eye. No notable color change with visually observable pH variation in the medium was found in those cases of 30 mL, 300 mL, and 3 L [Fig. 4]. The drop in pH may suggest good growth of the microorganism and the pH variation by color changes can be summarized as follows: [Fig. 5][Table 1]. We used ORION3 star pH Benchtop manufactured by Thermo (U.S.) to get five measurements, which were then averaged. This result is consistent with the growth characteristics of SOB5VT1, as described by Kim et al.,[13] and suggests that the microorganism has no biochemical degeneration.

Fig. 3. After inoculation of SOB5VT1 with 1% sulfur (L), color changes after culture (R)

Fig. 4. Inoculation of 30 mL SOB5VT1 in 300 mL MW medium (L), inoculation of 300 mL SOB5VT1 in 3 L MW medium (R)

Fig. 5. Color changes of SOB5VT1 over time

Table 1. pH and color variation over time in Fig. 9

	Standard	2 weeks	3 months	6 months
pH	4.5	2.3	1.4	0.9
Color	Yellow-redish	Red	Pink	Purple

3.2 SOB5VT1 culture in large volumes (20 L, 1 t)

3 L of cultured SOB5VT1 was cultured in 20 L in a different process from the small-volume culture and the medium was prepared according to the MW medium composition for large volumes.

To maintain dissolved oxygen in the medium, we put an oxygen generator in 20 L one, provided MW medium composition according to the volume, and added 1% sulfur to culture [Fig. 6]. Four to five days later, we observed the same color as in small volumes [Fig. 7] and succeeded in mass-producing SOB5VT1 with similar pH. We produced 1 t SOB5VT1, which was 10% of the volume of the deodorizer (10 t) actually used in Yusung Engineering Co., LTD [Fig. 8].

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Fig. 6. Inoculation of 20 L SOB5VT1 in 20 L MW medium

Fig. 7. Culture 20 L SOB5VT1

Fig. 8. Mass-culture with air pump for air supply

3.3 Assessment of SOB5VT1 growth in actual bio-filter

We inoculated SOB5VT1, put an MW medium, and determined if it grew well in an actual bio-filter (Yusung Engineering Co., LTD). To do this, we opened the circulate flask of the bio-filter to compare pH; then, we found that organisms had their color changed from yellowish red to red over time and that they were absorbed by sulfur and precipitated on the ground after the inoculation. The pH variation over a few days, as presented in Table 2, is identical with that of the afore-mentioned SOB5VT1 growth and suggests that SOB5VT1 has grown well in an actual bio-filter.

Table 2. Daily pH variation of SOB5VT1

	1 day	4 day	7day	10day	13day	16day	19day
PH	5.4	4.1	3.7	3.4	2.9	2.4	2.0

3.3 Assessment of SOB5VT1 growth in actual bio-filter

We inoculated SOB5VT1 in an actual bio-filter, assured that the microorganism was cultured, and determined how much the nine water-soluble odorous substances, including H_2S and ammonia, among flowed-in noisome odor gases were removed. The gas volume in the inlet and the outlet of the deodorizer and the measurements of H_2S from the outlet to the tube of Gastek [Table 3], the ammonia removal rate [Table 4], and the other seven degassing amounts [Table 5] were checked. We could use a gas tube and a measurement instrument to determine how much the nine odorous gases SOB5VT1 could dissolve were removed: on average, 99.88% of H_2S and 95.73% of ammonia were removed along with other seven [Table 5] gases. This result confirms that it is lower than the permissible level of odor emission, as determined by the Air and Environment Preservation Law established by the Ministry of Environment[14].

Table 3. H_2S removal rate

Date (time)	Inlet (ppm)	Outlet (ppm)	Removal (%)	Date (time)	Inlet (ppm)	Outlet (ppm)	Removal (%)
10/13	16	0.04	99.75	10/27	15	0.02	99.86
10/14	34	0.02	99.94	10/28	30	0.02	99.33
10/15	40	0.03	99.92	10/29	28	0.03	99.89
10/16	16	0.02	99.87	10/30	25	0.02	99.92
10/17	21	0.04	99.8	10/31	12	0.02	99.83
10/20	30	0.02	99.93	11/03	38	0.03	99.92
10/21	22	0.04	99.81	11/04	29	0.03	99.89
10/22	19	0.04	99.78	11/05	36	0.02	99.94
10/23	20	0.03	99.85	11/06	15	0.04	99.73
10/24	13	0.03	99.76	11/07	30	0.04	99.86
-				Ave.	24.45	0.029	99.88

Table 4. NH3 removal rate

Date (time)	Inlet (ppm)	Outlet (ppm)	Removal (%)	Date (time)	Inlet (ppm)	Outlet (ppm)	Removal (%)
10/13	10.5	1	90.48	10/27	13.5	0.5	96.29
10/14	12	ND	100	10/28	11	0.5	95.45
10/15	13.5	0.5	96.29	10/29	12	0.5	95.83
10/16	11	0.7	93.63	10/30	10.5	ND	100
10/17	13	0.3	97.69	10/31	13	0.5	96.15
10/20	14	0.5	96.42	11/03	10	1	90
10/21	12.5	0.5	96	11/04	15	0.5	96.66
10/22	13	1	92.31	11/05	13	0.3	97.69
10/23	12	0.5	95.83	11/06	14	0.5	96.42
10/24	11	0.7	93.63	11/07	11.5	0.5	95.65
-				Ave.	12.3	0.525	95.73

Table 5. Degassing amount of other seven gases

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Variety of Gas	Amount of Gas (ppm)	
	Inlet	Outlet
Methylmercaptans	0.031	0.002
Acetaldehyde	0.044	ND
Propionaldehyde	0.003	ND
Butylaldehyde	0.033	ND
n-valeraldehyde	0.008	ND
Methylethylketone	0.002	ND
VOC	38	ND

4. Discussion

In this study, we aimed to investigate the characteristics of *A. thiooxidans* SOB5VT1, which could remove both H_2S and NH_3, two of the most frequent noisome odor gases, and has sulfur-oxidizing power, apply it to an actual bio-filter, and measure the rate of odor gas removal[17,18,19]. *A. thiooxidans* was cultured in an MW medium containing sulfur (1%) and inoculation of 10% SOB5VT1 in this medium may lead to sulfur decomposition and sulfuric acid (H_2SO_4) emission due to oxidation according to the characteristics of the microorganism. Such a phenomenon lowered pH with its color changed from yellowish red to red according to the characteristics of the MW medium, as observed with naked eye. 1% S was first found to float on the reagent; then, the microorganism got absorbed and precipitated. These two phenomena were also found in an actual bio-filter, confirming that the microorganism was inoculated and cultured well. Of the odorous gases, 99.88% of H_2S and 95.73% of ammonia were removed. The other seven odorous substances were also decomposed. Regardless of the concentration of gases in the inlet, that in the outlet was lower than the permissible level of odor emission, as determined by the Air and Environment Preservation Law established by the Ministry of Environment. To put the results together, SOB5VT1 grew well in an actual bio-filter and the bio-filter system removed the nine water-soluble odorous substances, including H_2S and ammonia, all at once. It is expected that the bio-deodorant, SOB5VT1, can be used for deodorization in a large volume.

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