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SCHOLARENA

Journal of Environmental Pollution and Management

RESEARCH ARTICLE

ISSN: 2639-7269

Characteristics of the Production of Extracellularly Secreted Substances and the Removal of Gaseous Toluene by Microorganisms Cultivated with Gaseous Toluene as a Substrate

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Citation: Takashi Higuchi, Tomohiro Takahashi (2024) Characteristics of the Production of Extracellularly Secreted Substances and the Removal of Gaseous Toluene by Microorganisms Cultivated with Gaseous Toluene as a Substrate, J Environ Pollut and Manage 6: 103

Abstract

We conducted a basic study on the influence of substances extracellularly secreted by microorganisms on gas removal by microorganisms. Toluene-degrading bacteria were the target microorganisms, which were cultured in inorganic liquid culture media using gaseous toluene as a substrate. Additionally, the components secreted into the extracellular liquid phase during culturing were analyzed as protein components and saccharide components. After culturing, the biomass was collected in a thin layer on a membrane filter and left at the bottom of a sealed container, and the post-culturing supernatant was sprinkled on top of the biomass layer to form a liquid film. The toluene gas removal velocity due to the biofilm on the filter plane prepared using the above method was observed using various combinations of biomasses and liquid films. Experimental results showed that an appropriate presence of extracellularly secreted substances promoted toluene gas removal velocity, but high concentrations conversely inhibited it. In other words, the state of the liquid film that exists between the gas phase and biomass had a large effect on the substrate uptake rate for microorganisms that use gaseous toluene as a substrate.

Keywords: Biofiltration; Toluene; Extracellularly Secreted Substances; Liquid Film; Saccharide; Protein

Introduction

Microorganisms take in various substrates into their bodies, including many gaseous substances. The most common gaseous substrate is carbon dioxide, and many microorganisms assimilate carbon through photosynthesis and chemical synthesis. Meanwhile, many other gaseous carbon compounds are volatile organic compounds (VOCs) that also serve as substrates for microorganisms with metabolic ability. For example, toluene is a major air pollutant that is used in large quantities in human society and released into the atmosphere, but the existence of microorganisms that decompose toluene has long been known [1,2], and their action is also used as a purification process for toluene gas. This process, known as biofiltration, removes various gaseous substances including toluene, and has been studied from a variety of perspectives and put into practice [3-6].

It is much more difficult for microorganisms to take gaseous substances into cells as substrates than to take in solute components in liquids. Normally, the abundance ratio of carbon dioxide and organic substances in the gas phase is low, and gases inherently have low material densities, so microorganisms have difficulty taking in large amounts of these substances in a short period. Furthermore, moisture is essential for the growth environment of microorganisms, so a liquid phase or liquid film usually exists between biofilms that live on the surface of solids that are in contact with the gas phase. The presence of this liquid phase often acts as a barrier for microorganisms to ingest gaseous substrates [7,8]. In biofiltration that uses microorganisms naturally grown on solid surfaces, the treatment rate for hydrophobic gas components is generally lower than that for hydrophilic gas components. This is mainly due to the low mass transfer velocity caused by the solubility in the liquid phase (liquid film) [9].

Meanwhile, many microorganisms and other living organisms have mechanisms for efficiently taking gaseous substances into their cells, but most of these mechanisms involve oxygen and carbon dioxide. Not only do enzymatic reactions that actively capture carbon dioxide exist but so do a series of carbonic anhydrases that catalyze the hydration reaction of carbon dioxide, which contributes to carbon fixation by microorganisms and plants [10,11]. However, gaseous organic substances other than carbon dioxide are usually found at low concentrations in the atmospheric environment, so little is known about the mechanisms that actively incorporate them. Once gaseous organic matter is dissolved in the liquid film from the gas phase, however, the gaseous organic matter is taken in by the microorganisms either by directly being taken into cells through a passive diffusion process, or by being converted into an easily ingestible state by the action of extracellular enzymes, and then taken in [4].

The biggest challenge in biofiltration, which removes gaseous substances, is improving removal capacity. Due to the low removal capacity, current biofiltration systems require large-scale facilities compared to other VOC treatment systems, such as for adsorption and combustion. For instance, general values of contact time for incineration, adsorption and biofiltration process are said to be 0.2-1, 2-4 and 25-60, respectively [12,13]. Therefore, more widespread application of this method requires technical improvements to increase removal capacity. One technical improvement in biological treatment equipment is the application of effective microorganisms. However, microbial flora in actual biofiltration converges to a composition that is adapted to the environment inside the equipment after effective microorganisms are inoculated into the equipment [14], so maintaining a growth environment within the equipment that facilitates the metabolism of gaseous substances that serve as substrates is essential. Therefore, reducing the mass transfer resistance of gaseous substances to microbial flora may be a means of improving equipment performance by increasing the substrate supply rate. Systems that focus on this point and add additional substances to the liquid film have been attempted [15,16]. However, in the liquid film of biofiltration, the presence of substances secreted extracellularly by the microbial flora underneath the film to protect itself is desirable. In practical use of biofiltration, the situation in which secreted substances in liquid film interfere the mass transfer should be avoided, inversely, if these secreted substances promote mass transfer, they had better be positively utilized. The previous studies propose the optimization of the composition of liquid film by adding substances to maximize mass transfer, but this study focuses on whether the microorganisms inhabit into the biofiltration system themselves can play the role of supplier of the substances which maximize mass transfer. As a result, the information that is first needed to improve biofiltration technology, with a focus on this mass transfer, is the relationship between the presence of extracellularly secreted substances in liquid films and the gaseous substance uptake rate into microorganisms.

Therefore, in this study, we prepared a biofilm on a flat surface using microorganisms cultured using toluene gas as a substrate, placed a liquid film containing extracellularly secreted substances on top of the biofilm, and observed the removal velocity of toluene gas in a sealed container. We used these results as a basis to discuss the liquid film composition that is effective for gas removal.

Experimental Method

Culturing of Microorganisms

The microorganisms used in this experiment include *Pseudomonas putida* strain, which was isolated and identified in a previous study as toluene-degrading bacteria [17], as well as a microbial flora present in the circulating irrigation liquid of an experimental biofiltration apparatus [18] that targets toluene gas.

For subculturing using liquid culture media, an inorganic nutrient medium having the composition of the "high concentration media" shown in Table 1 was used; 200 mL of this medium was placed in an Erlenmeyer flask. As shown in Figure 1, the gas phase inside the flask was set so that it could be exchanged with outside air through a membrane filter, and 100 μL of liquid toluene was injected into the test tube placed in the flask once every two days, to create a state filled with toluene vapor. In other words, the liquid phase in the flask was in contact with a gas phase containing toluene vapor in addition to oxygen close to the atmospheric concentration. We inoculated 100 μL of the culture solution, which had already been cultured under the same conditions for about seven days, into this liquid phase, after which a static culture was set at 25 °C. The first culturing without a culture solution involved inoculation with an appropriate amount of *P. putida* strain on an agar medium.

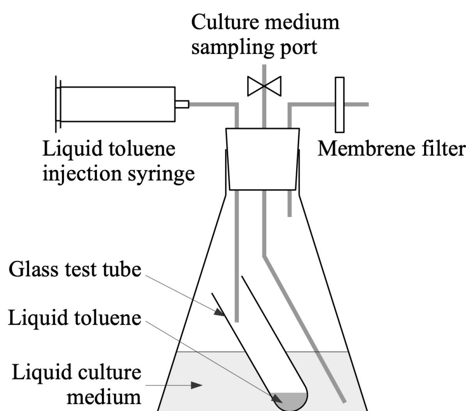


Figure 1: Schematic of the container used for liquid culture

As shown in Table 1, we set two types of liquid culture media for the culturing of microorganisms used in the toluene gas removal test described below: high concentration and low concentration. Two inoculation methods were used: the same method for subculturing as described above, and a method of inoculating a total of 100 μL (50 μL of culture solution + 50 μL of experimental biofiltration apparatus circulating irrigation liquid) and culturing in the same way (henceforth, the microorganisms and microbial flora obtained by the former and latter methods are referred to as the "isolated strain" and "microbial mixture", respectively). Therefore, the four types of microorganisms obtained after culturing are as follows: isolated strain / high-concentration culturing, microbial

mixture / high-concentration culturing, isolated strain / low-concentration culturing, and microbial mixture / low-concentration culturing.

Table 1: Composition of liquid culture media

	Content (mg)	
Compounds	High concentration medium	Low concentration medium
K_2HPO_4	1550	155
$NaH_2PO_4•2H_2O$	850	85
$(NH_4)_2SO_4$	2000	200
$MgCl_2•6H_2O$	100	10
EDTA	10	
$ZnSO_4•7H_2O$	2	
$CaCl_2•2H_2O$	1	
$FeSO_4•7H_2O$	5	
$Na_2MoO_4•2H_2O$	1	
$CuSO_4•5H_2O$	0.2	
$CoCl_2•6H_2O$	0.4	
$MnCl_2•4H_2O$	1	
Distilled water	1.0 L	
pH	7.1	7.2

Meanwhile, the supernatant obtained from the isolated strain culture solution by the method described below was used for the liquid sample containing extracellularly secreted substances used as a liquid film for the toluene removal test. Therefore, the two types of liquid films used are the one obtained from high-concentration culturing of the isolated strain and the one obtained from low-concentration culturing of the isolated strain (henceforth, the former is called “high-concentration liquid film”, and the latter is called “low-concentration liquid film”).

Thus, there are eight conditions in the toluene gas removal experiment, which are obtained by combining the four types of microorganisms obtained by combining the inoculated bacteria and culture medium composition, and the two types of liquid film. The results obtained under each condition were compared and discussed.

The growth status of the microorganisms was observed using absorbance (660 nm wavelength). The toluene gas removal properties of the microorganisms cultured in this experiment were best approximately two days after the start of culturing, so the amount of culture solution needed for the toluene gas removal test described below was collected two days after the start of culturing, and a biofilm was prepared and the supernatant was collected.

Toluene Gas Removal Test

Figure 2 shows an overview of the test. The biofilm used to measure the toluene gas removal velocity and the liquid film on its upper surface (surface in contact with the gas phase) were prepared on a membrane filter (Mixed Cellulose Ester, D25mm PS0.45μm, Advantec). The biofilm was prepared by filtering the culture solution cultured using the above-mentioned method through a mem-

brane filter. The volume of the culture solution at this time was calculated using the following equation from the absorbance of the solution:

$$V_f = 1/abs_{660} \qquad (Equation \;\; 1)$$

V_f: Volume of the culture solution filtered by the membrane filter (mL)

abs_{660}: Absorbance of culture solution at a wavelength of 660 nm

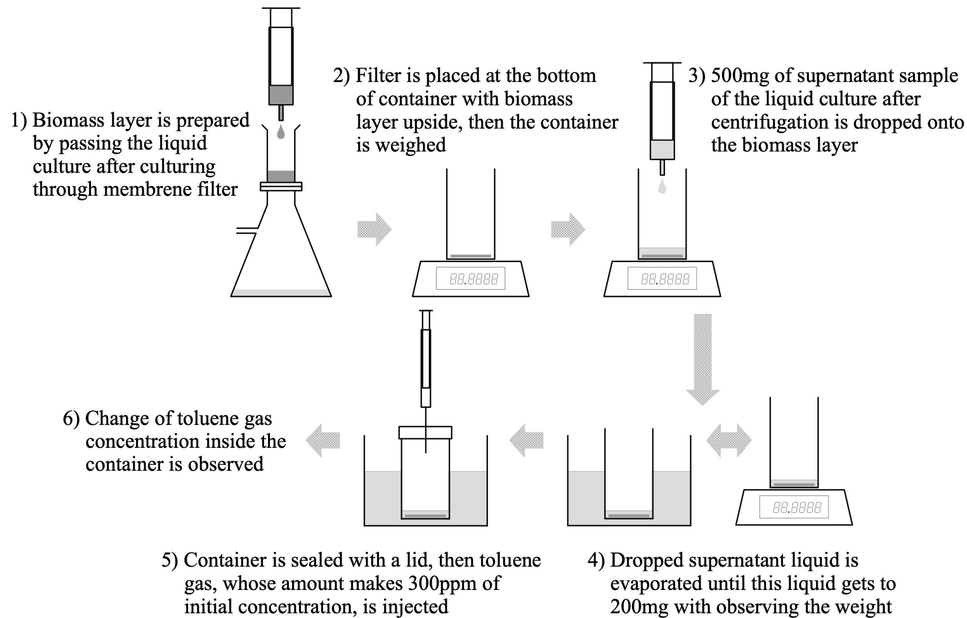


Figure 2: Overview of the toluene gas removal test

This equation means that filtering volume is just inversely proportional to the concentration of microorganisms suspended in the culture solution. Previous research [19] found that the optimal biofilm density on the membrane filter for the same toluene gas removal as this experiment is 0.5–1.0 × 10^{-4} g mm^{-2}, and the filtering volume calculated by the upper equation yields the biofilm with that range of density. For example, culture solution samples whose absorbance of 660nm of wavelength was 0.100 and 0.020 have to be filtered 10 and 50 mL, respectively.

In this experiment, we used the membrane filter at the bottom of the toluene gas removal test container (30 mL Standard PFA Vial, Savillex, henceforth referred to as “gas removal test container”), with the biofilm generated by filtration facing upward. At this point, the gas removal test container was left uncovered in a water bath at 40 °C.

Furthermore, an appropriate amount of the culture solution was centrifuged (CF15R, himac) at 1.4 × 10^4 rpm for 10 min and at 0 °C, and due to make liquid film on the biofilm, 500 mg of the supernatant was evenly sprinkled with a needle-equipped syringe. Then the container was left in a water bath without sealed the top in order to make a concentration of extracellularly secreted substances and to make acclimation of biofilm to newly-supplied liquid film. Evaporated volume of liquid film was checked intermittently by measuring the change of the container’s weight, and after confirming that the irrigation liquid volume had decreased to 200 mg, the container was sealed with a lid.

Toluene gas was prepared by injecting a small amount of a standard liquid toluene reagent (Wako Pure Chemical Industries Ltd.) into a sealed resin container (30 mL Nalgene Narrow-Mouth PPCO Bottle, henceforth referred to as "injection gas container") and allowing it to volatilize. Additionally, the gas removal test container and injection gas container both had holes in the lid so that the gas sample inside the containers could be collected with a gas-tight syringe without opening the lids, and a septum for a gas chromatograph device (plus type for Shimadzu, GL Sciences) was inserted and brought into close contact.

The toluene gas concentration in the air inside the injection gas container was measured under the analysis conditions described below, and the air inside the injection gas container was collected using a gas-tight syringe (MS-GAN100, Ito Seisakusho) and injected into the gas removal test container so that the initial value of the toluene gas concentration in the gas removal test container served as the target value (300 ppm). This injection time was taken as the experiment start time, and the air inside the gas removal test container after 0, 1, 3, 5, and 10 min was sampled using a gas-tight syringe (MS-GAN025, Ito Seisakusho), and the change in toluene gas concentration over time was measured.

The obtained results were used to regress the relationship between the elapsed time and toluene gas concentration using the following equation:

$$lnC\left(t\right) = -kt + lnC_0 \qquad (Equation\ 2)$$

C(t): Toluene gas concentration after t (min) (ppm)

k: Removal velocity coefficient (min^{-1}, > 0)

ln C_0: Intercept in the regression line

(C_0 is the initial concentration found from the regression line)

However, in this experiment, the rate of attenuation of toluene gas concentration decreases over time, and the linear regression in the above equation often does not hold over the entire experimental period. Therefore, linear regression was conducted using only the data of the elapsed time up to the maximum t (min) for which the following two conditions were satisfied to calculate removal velocity from the initial attenuation:

$$\left|r\right| > 0.95 \qquad (Equation\ 3)$$

r: Absolute value of the correlation coefficient of the regression line

$$0.95 \times C\left(0\right) < C_0 < 1.05 \times C\left(0\right) \qquad (Equation\ 4)$$

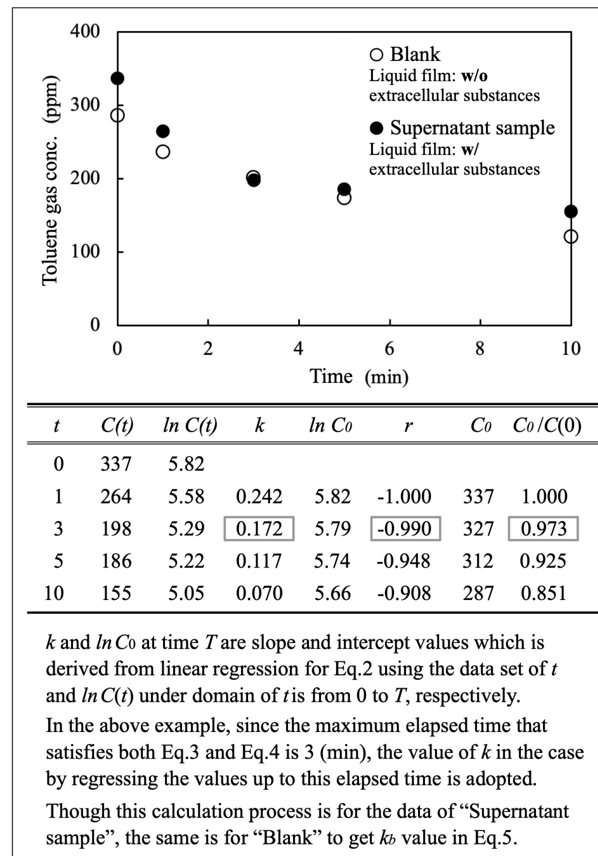
C(0): Initial concentration of toluene gas (actual value, ppm)

Figure 3 shows an example of the process of calculating the removal velocity coefficient. The main observation target in this study was the effect of the state of the liquid film on toluene gas removal, but in this experiment, the toluene removal velocity of the biofilm was expected to vary with each culture. Therefore, during the experiments, as blank tests, we always observed removal velocity in parallel using a liquid film prepared in the medium before culturing (i.e., a liquid film in which no secreted substances derived from microorganisms were present). The results of the blank test were used as a basis to calculate each measurement result for toluene gas removal velocity as a specific removal velocity coefficient with the following equation:

$$k_s = k/k_b \ (Equation\ \ 5)$$

k_s: Specific removal velocity coefficient (dimensionless)

k_b: Removal velocity coefficient in blank test (min^{-1})



t	$C(t)$	$\ln C(t)$	k	$\ln C_0$	r	C_0	$C_0/C(0)$
0	337	5.82					
1	264	5.58	0.242	5.82	-1.000	337	1.000
3	198	5.29	0.172	5.79	-0.990	327	0.973
5	186	5.22	0.117	5.74	-0.948	312	0.925
10	155	5.05	0.070	5.66	-0.908	287	0.851

k and $\ln C_0$ at time T are slope and intercept values which is derived from linear regression for Eq.2 using the data set of t and $\ln C(t)$ under domain of t is from 0 to T, respectively.

In the above example, since the maximum elapsed time that satisfies both Eq.3 and Eq.4 is 3 (min), the value of k in the case by regressing the values up to this elapsed time is adopted.

Though this calculation process is for the data of "Supernatant sample", the same is for "Blank" to get k_b value in Eq.5.

Figure 3: Example of the calculation of removal velocity coefficient in the toluene gas removal test

Analysis Conditions

Toluene gas concentration was measured using gas chromatography with a flame ionization detector (GC-4000, GL Sciences, Column: BX-100, 60/80mesh, 3φ × 5 m, GL Sciences), with a calibration curve using toluene standard gas (Sumitomo Seika Chemicals Co., Ltd.). The culture solution absorbance and the absorbance in the analysis of the saccharide and protein components described below were all measured using a spectrophotometer (UV-1600, Shimadzu Corporation).

For the supernatant of the culture solution, the saccharide and protein concentrations in the solution were detected and quantified using the phenol-sulfuric acid method with glucose as the reference material and the Lowry method with bovine serum albumin as the reference material, respectively.

Results

Protein and Saccharide Concentrations in the Supernatant after Microbial Culturing

Figure 4 summarizes the measurement results of the protein and saccharide concentrations in the supernatant after culturing microorganisms (isolated strain, microbial mixture). These results demonstrate both high-concentration medium and low-concentration medium culturing, and relatively similar saccharide concentrations were observed. Meanwhile, the protein concentration varied greatly by culture.

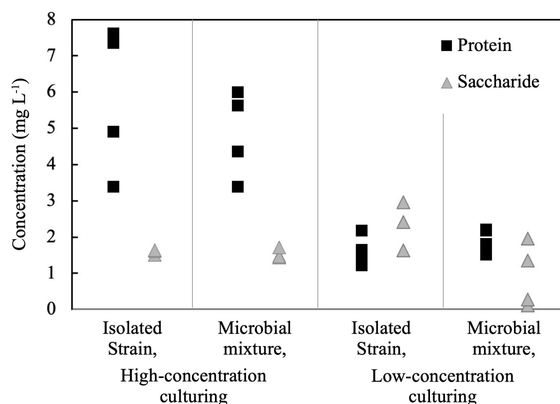


Figure 4: Distribution of secreted substance concentrations in supernatant after culturing

Toluene Gas Removal Velocity

Figure 5 summarizes the specific removal velocity coefficient values obtained from the experiment. In the figure, “high-concentration culturing” and “low-concentration culturing” indicate whether the biofilm was cultured in a high-concentration medium or low-concentration medium, and “high-concentration liquid film” and “low-concentration liquid film” indicate whether the liquid film used was the supernatant of a high-concentration culture or low-concentration culture. The two types of biofilms used are of the isolated strain and the microbial mixture, but all liquid films use the supernatant obtained from culturing the isolated strain.

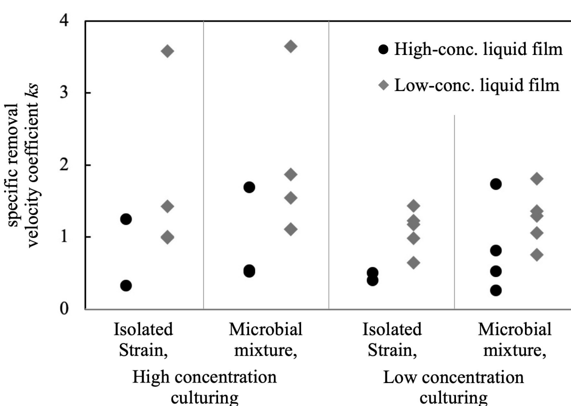


Figure 5: Distribution of specific removal velocity coefficient of toluene gas under each condition

The results of these experiments indicated that the overall toluene gas removal velocity was higher when using a low-concentration culturing liquid film than when using a high-concentration culturing liquid film. The specific removal velocity coefficient k_S is generally less than 1 for high-concentration culturing liquid films and greater than 1 for low-concentration culturing liquid films.

In other words, we judged that the low-concentration and high-concentration culturing liquid films promoted and inhibited toluene gas removal, respectively.

Comparison of Secreted Substance Concentration and Toluene Gas Removal Velocity Between Conditions

The protein and saccharide concentrations in the supernatant after microbial culturing and the toluene gas removal velocity observed under each condition were each compared in the high-concentration medium case and the low-concentration medium case. The results of testing the difference in mean values are shown in Tables 2 and 3, respectively.

Table 2: Comparison of secretion concentrations under different culture conditions

		Protein concentration (mg L^{-1})			Saccharide concentration (mg L^{-1})		
		Isolated strain	Microbial mixture	Total	Isolated strain	Microbial mixture	Total
	n	4	4	8	3	4	7
High-concentration culturing	Mean	5.82	4.85	5.33	1.59	1.52	1.55
	S.D.	2.03#	1.2	1.63#	0.07#	0.13#	0.11#
	n	4	4	8	3	4	7
Low-concentration culturing	Mean	1.63	1.93	1.78	2.33	0.93	1.53
	S.D.	0.41#	0.33	0.38#	0.67#	0.88#	1.05#
T value (test statistic)		4.045*	4.680**	6.006**	1.928	1.337	0.048
t(0.05)		2.353	1.943	1.761	2.132	1.943	1.782
t(0.01)		4.451	3.143	2.624	3.747	3.143	2.681
P value		0.014	0.0017	0.000269	0.097	0.137	0.482

All results are by one-side test.

Welch's test is applied

* $p<0.05$, ** $p<0.01$

The protein concentration of the supernatant after high-concentration culturing was significantly higher than that during low-concentration culturing (5% level for the isolated strain, 1% level for the others). Meanwhile, no significant difference was observed because the number of data under each condition was small and the variation was large, but overall, toluene removal velocity was higher when high-concentration cultured microorganisms were used than when low-concentration cultured microorganisms were used. Furthermore, the specific removal velocity coefficients of the toluene gas observed in each condition were compared between when the supernatant of the high-concentration culturing was used as the liquid film and when the supernatant of the low-concentration culturing was used as the liquid film. The results of testing the difference in mean values are shown in Table 4. Overall, the removal velocity was higher for liquid films from low-concentration culturing (a significant difference was observed at the 5% level for isolated strains at low-concentration culturing and 1% level for overall cases).

A part of toluene gas removal experiment was conducted with the liquid film of which protein and saccharide concentration is not quantified individually, considering that the difference of protein and saccharide concentrations of the liquid films between two conditions of culturing are roughly to be the result of Table 2, and such data are included in Table 3 and Table 4. The influence of protein and saccharide concentration in the liquid film on the toluene gas removal is minutely discussed later using a limited data in which these concentrations were measured for each liquid film individually.

Table 3: Comparison of toluene gas removal velocity of microorganisms obtained under different culture conditions

		Toluene gas specific removal velocity coefficient k_s (dimensionless)				
		High-concentration liquid film		Low-concentration liquid film		
		Isolated strain	Microbial mixture	Isolated strain	Microbial mixture	Total
Microorganisms of high-concentration culturing	n	2	3	4	5	14
	Mean	0.79	0.92	1.75	1.86	1.47
	S.D.	0.65	0.67	1.24 #	1.05	1.01 #
Microorganisms of low-concentration culturing	n	2	4	5	5	16
	Mean	0.45	0.84	1.09	1.26	1
	S.D.	0.07	0.64	0.30 #	0.39	0.47 #
T value (test statistic)		0.725	0.17	1.042	1.194	1.61
t(0.05)		2.92	2.015	2.353	1.86	1.74
t(0.01)		6.965	3.365	4.541	2.896	2.567
P value		0.272	0.436	0.187	0.133	0.063

All results are by one-side test.

Welch's test is applied

Table 4: Comparison of toluene gas removal velocity of microorganisms under different liquid films

		Toluene gas specific removal velocity coefficient k_s (dimensionless)				
		Microorganisms of high-concentration culturing		Microorganisms of low-concentration culturing		Total
		Isolated strain	Microbial mixture	Isolated strain	Microbial mixture	
	n	2	3	2	4	11
High-concentration liquid film	Mean	0.79	0.92	0.45	0.84	0.78
	S.D.	0.65	0.67	0.07	0.64	0.53
	n	4	5	5	5	19
Low-concentration liquid film	Mean	1.75	1.86	1.09	1.26	1.48
	S.D.	1.24	1.05	0.3	0.39	0.82

T value (test statistic)	0.995	1.361	2.880*	1.226	2.526**
t(0.05)	2.132	1.943	2.015	1.895	1.701
t(0.01)	3.747	3.143	3.365	2.998	2.467
P value	0.188	0.111	0.017	0.13	8.74E-03

All results are by one-side test.

* $p<0.05$, ** $p<0.01$

Discussion

The results of Tables 2–4 show that culturing microorganisms in high-concentration liquid culture media can yield microorganisms with high toluene removal velocity, and those microorganisms (both from the isolated strain and microbial mixture) tend to secrete more protein into the liquid phase (liquid film), but also that obtaining high toluene gas removal does not require a high concentration of proteins in the liquid film. This is also reflected in the fact that the high-concentration liquid film, where proteins are present at a high concentration, exhibits a lower toluene gas removal velocity ($k_S < 1$) than in a situation without secreted substances (i.e., blank test). Conversely, the low-concentration liquid film, where proteins are present at a low concentration, exhibits a higher toluene gas removal velocity ($k_S > 1$) than in a situation without secreted substances, so an optimal concentration range of protein in liquid film exists for toluene gas removal. That is, in the biofiltration process for treating toluene gas, biological activity had better be kept by high-concentration of nutrients, at the same time, the concentration of extracellularly secreted substances must be controlled properly.

Saccharide and protein concentrations of the supernatant after culturing indicate the ability of the microorganisms used in the toluene gas removal test to produce saccharides and proteins, respectively, but no direct relationship between these values and k_S was observed. Figure 6 shows the relationship between the protein/saccharide concentration ratio after high-concentration culturing and the k_S observed in the biofilm obtained in that culturing. The figure shows that k_S had an increasing tendency as the concentration ratio increased. This signifies that microorganisms that secrete relatively more protein than saccharide into the liquid film had a more potent ability to remove toluene gas.

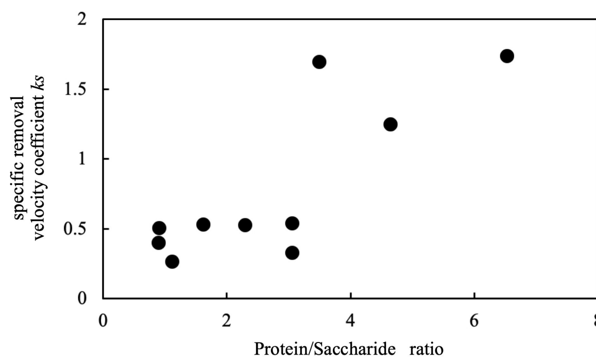


Figure 6: Relationship between the protein/saccharide concentration ratio after culturing and the specific removal velocity coefficient of toluene gas by microorganisms (when using high-concentration liquid film)

Therefore, multiple regression analysis was conducted using saccharide concentration and protein concentration as explanatory variables and k_S as the objective variable. Table 5 shows the results. Although not statistically significant, a multiple regression equation was obtained that agreed with the experimental results, which showed that saccharide secretion and protein secretion by microorganisms inhibited and promoted toluene gas absorption, respectively.

Analysis of Variance

Source of variation	Sum of square	Degree of freedom	Mean square	F value	Significance
Regression	1.194	2	0.597	2.874	p>0.05
Residual	1.662	8	0.208		
Total	16.95	10			

Multiple correlation coefficient	0.265

Multiple Regression

Valuables	Coefficient
Protein concentration	0.136
Saccharide concentration	-0.452
Intercept	0.955

Table 5: Result of multiple regression analysis on toluene gas specific removal velocity in which secretion materials as the explanatory variables

Although the microbial growth environment differed from that in this study, the protein/saccharide concentration ratio was similar to that observed in a study of extracellularly secreted substances from activated sludge [20]. A decrease in the protein/saccharide concentration ratio also leads to a decrease in the degree of hydrophobicity of the cell surface [21]. It was speculated that the secretion of enzymes for toluene assimilation and the possibility that microorganisms that use toluene as a substrate may improve the hydrophobicity of the interface to more actively ingest gaseous toluene may factor in the increase in protein/saccharide concentration ratio promoting toluene gas removal in this study.

Conclusion

In this study, we conducted a basic examination of the biological removal of gaseous VOCs by observing the relationship between toluene gas removal by biofilms and the properties of the liquid film present on the biofilm. Biofilms that produce more proteins than saccharides as substances secreted into liquid films during microbial culture tended to have a stronger toluene gas removal ability. Meanwhile, under the conditions of this study, a liquid film with a small amount of protein secretion promoted toluene gas removal more than a liquid film with a large amount of protein secretion, indicating the possibility of a range of appropriate protein concentrations in a liquid film. Additionally, in this study, the condition of the liquid film had a larger effect on toluene gas removal than the microorganism culturing conditions.

These results suggest that in gas treatment equipment such as those for biofiltration, an appropriate liquid film for the biofilm (i.e., a situation in which extracellularly secreted substances produced by microorganisms are not present at very high concentrations) provides better gas removal properties than the metabolic activity of the microorganisms with respect to the gas to be treated. For example, it may be possible to test operating conditions that have separate periods for stimulating the metabolic activity of microorganisms with a high-concentration nutrient solution and periods for promoting highly efficient gas removal with a low-concentration nutrient solution.

These conclusions can never be derived from the data of practical or experimental biofiltration apparatus because of the complexity of the phenomena occurred in it. The results in this study whose data is obtained by a simplified experiment are entirely unique information and are thought to suggest a further possibility of innovation to biofiltration techniques. Meanwhile, in this study, the only two liquid film conditions that affected toluene gas removal were high concentration and low concentration, and we observed a tendency for the presence of secreted substances at low concentrations to promote gas removal, but more detailed tests under multiple concentration conditions are necessary to obtain optimal conditions. Furthermore, during actual biofiltration, we hope to use an analysis method that enables easy monitoring of secreted substances in the liquid film on the surface of the packed material inside the equipment and in the supplied water (circulated water) to observe the relationship with gas removal velocity. This will allow us to improve operating methods that will contribute to improving equipment performance.

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