

Magnetic Material as a Solid Carrier for Microorganisms

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In this study we tried to develop new type of magnetic material as a solid carrier. At first, in order to establish a method of quantitative estimation of microbes accumulation on the surface of the carrier, quantification system of microbial adsorption to the solid material was investigated. Then the test of accumulation of microorganisms on the carrier was performed using activated sludge as an example. It was found that the magnetic material tested showed almost the same capacity of microbial accumulation as that of certain ceramic material which is practically used as a carrier of microbes in waste water treatment process. Among the materials tested, porous type ferrite material showed a good result, meaning that this material has a possibility of practical use.

Key word: Magnetic Material, Carrier, Microorganisms, Waste Water Treatment

1. INTRODUCTION

The application of magnetism and magnetic material to biological/environmental technology is one of the most promising but undeveloped fertile fields. The action of magnetism to the living things — animals, plants and microorganisms — has attracted a great interest of many researchers for about one hundred years. Examples of scientifically analyzed facts, however, are very few. In exceptional case, it was reported that the behavior of microorganisms does not change remarkably even under very strong magnetic field such as over several teslas, with only a little decrease in death rate observed¹⁾. Relatively popular examples of the application of magnetism are several devices for health such as a magnetic necklace and a bracelet. But the effects of these devices on the health of the user as well as the mechanism have not been analyzed enough.

One example of the application of magnetic material, such as ferrite, as functional powder is carried out in a medical treatment field; e.g. as a carrier in the missile therapy against cancer²⁾. Magnetic force collection is also tried in a biological production process using a material that is wrapped with a high polymer gel³⁾. On the other hand, the application of magnetic material in an environmental technology field is very rare. The authors consider, however, that magnetic material will be able to bring a seed of new technological innovation since it is sensitive to magnetic force which enable non-contact operation. For example, most post-processing stage of biological waste water treatment, that is often a solid-liquid separation process, will be simplified if magnetic material can be used as a carrier of microorganisms. In this context we tried in this study to research on the possibility of magnetic powder as a solid carrier for microorganisms.

Whether or not certain material can be used as a carrier depends on both physical characteristics such as specific gravity, particle diameter, specific surface area etc. and chemical surface condition such as pH, charge etc. which will control the affinity of the material to microorganisms. We tried several kinds of ferrite powder with various characteristics and intended to estimate the degree of the affinity by measuring accumulation of microorganisms on the material using

activated sludge as example.

The first problem was, however, that there was no established method to evaluate the accumulation of microorganisms quantitatively and accurately. Therefore, this study should consist of the following contents.

- (a) To establish an appropriate method that can evaluate quantitatively the accumulation of microorganisms on the magnetic material.
- (b) To achieve the accumulation tests using magnetic material (ferrite) with various characteristics.

2. EXPERIMENTAL PROCEDURE

2.1 Method to estimate the accumulation quantity of microorganisms on a solid surface

There were few attempts to evaluate conveniently and precisely the quantity of the microorganisms that absorbed on a solid particle surface. In many cases, an adsorption quantity was evaluated with a decrease of a number of microorganisms during the experimental period. However, only an adhesion of microorganisms in a short period can be measured by this method because the number of microorganisms will change by growth or death with time. Moreover, an error factor such as the adhesion of microorganisms to the surface of experimental devices may be rather large.

In this study, microorganisms that absorbed on the sample material were lysed with NaOH solution. And proteins released from microbial body to aqueous phase were measured quantitatively by Lowry method⁴⁾.

The measurement of released proteins was carried out with the following procedure.

- (1) The relation between drying mass of microorganisms and absorbance obtained from Lowry method is determined, and the relation is presented in a calibration curve. The drying mass of microorganisms is expressed as MLSS value. The MLSS is an abbreviation of Mixed Liquor Suspended Solid, and it represents a weight of non-soluble fraction in liquid phase. The non-soluble fraction in this case is bacterial cells. The unit of MLSS is mg per liter.

- (2) About 3 g of sample material on which microorganisms absorbed is dipped to 10 ml of NaOH solution with the concentration of 0.1N. (Too high alkali concentration will have bad influence on the quantification of proteins by Lowry method.).
- (3) The sample material is left with room temperature for 2 days while stirring periodically followed by the protein measurement with Lowry method. It takes 2 days to get complete lysis of microbial cell under moderate conditions described above.
- (4) The absorbance from Lowry method is converted into MLSS value from a calibration curve.
- (5) After the sample material is washed with distilled water carefully and entirely, dried mass of the material is measured.
- (6) The accumulation quantity of microorganisms on a solid surface is obtained as a value mg-MLSS per g-material.

2.2 Accumulating tests of microorganisms to the powder magnetic samples

In order to know whether or not there are some differences in adsorption characteristics of microorganisms, we used two types of activated sludge microorganisms: no inoculation sludge (open to the air) and inoculated with soil one. Both sludges were cultivated with JIS-3364 medium at 30 °C in shaken cultures (120 rpm). When the MLSS value of the culture exceeds 2000 mg per liter, about 3g of sample magnetic material was put into the microbial suspension, and 5 days cultivation was performed. The medium was exchanged with 1/4 of whole volume every day. After 5 days, the magnetic material was collected from the microbial suspension, and was well washed with distilled water to remove non-adhering microorganisms and proteins in the medium. Then the quantity of the microbial accumulation on the magnetic material was measured with the method explained in the 2.1. Table I shows the data of the powder magnetic materials used in this study. In the manufacturing process of secondary particles from primary particles of magnetic material (strontium ferrite; Fe/Sr=5.9), the preparation conditions of the materials were varied as shown in Table I in order to change the physical characteristics of the material surface. The SEM photograph of sample ④ as an example is shown in Fig.1. Only the sample ④ was sintered with activated carbon added and porous surface configuration was clearly shown, but no apparent difference in the SEM photos was observed in the other samples of ①~③ (data not shown).

3. RESULTS AND DISCUSSION

The calibration curve representing the relation between the MLSS value of microorganisms and the absorbance by Lowry method is shown in Fig.2. Linear relation was obtained in the MLSS region of 5000 mg/l below. And it was confirmed that the magnetic material itself had no effect on the absorbance after well washed with distilled water.

Table I Magnetic materials used in this study

Sample	Base	Temp. * 1	Note
①- 1	Ferrite hollow particle	1000°C	* 2
①- 2	Ferrite hollow particle	1100°C	* 2
①- 3	Ferrite hollow particle	1200°C	* 2
②- 1	Ferrite particle	1000°C	* 3
②- 2	Ferrite particle	1000°C	* 3/* 4
③- 1	Ferrite particle	1000°C	* 5
③- 2	Ferrite particle	1000°C	* 5/* 4
④	Ferrite hollow particle	1000°C	* 6

* 1: Sintering temperature

* 2: produced by spraying

* 3: produced by chemical reaction

* 4: 20% of camphor added, molded into particles of about 2mm diameter

* 5: finely smashed particle

* 6: sintered with activated carbon added, porous

Fig.2 shows some examples of the measurement of microorganisms accumulation on the magnetic materials. The experiment was carried out several times for each sample to confirm the reproducibility of the measurement results. Almost samples tested in this study showed the microbial accumulation of 3 to 5 milligram per gram of material. Only sample ④, with porous configuration, had a high adsorption capacity of about 10 mg/g. It is considered that the porosity of the material increased by the addition of active carbon to the strontium ferrite, which leads to a relatively high affinity to the microorganisms.

In order to know the practical meaning of adsorption capacity of 10 mg/g (sample ④), we tried the same experiment using ceramic materials which were practically used as carriers in waste water treatment process. The data regarding the ceramic materials used is shown in Table II. The ceramic materials were used in a state of flakes and fine powder. Fig.4 shows the results of the accumulation tests. The adsorption capacity was 2~15 mg/g which is approximately near to the values of the magnetic materials tried in this study, meaning that there is a possibility to use these magnetic materials as microbial solid supports in practical processes.

From the results shown in Fig.4, several implications concerning the controlling factors of the microbial accumulation on the materials can be obtained. First, the accumulation showed almost the same results regardless of the state of the material (flakes or powder) or the sludge conditions (no inoculation or soil inoculated), which means that these factors may not be significant. Second, the ceramic ① showed little accumulation, while the ceramic ③ had a large capacity of 13~15 mg/g in any cases although hole volume is not so different. The difference of the two materials will depend on the surface area because the ceramic ① is spherical shape while the ceramic ③ is honeycomb shape with a hole of 1mm × 1mm square.

The relation between the microbial accumulation and volume densities of the magnetic materials used in this study is shown in Fig.5, which indicates the smaller the

volume density, the larger the microbial accumulation. The importance of the surface configuration of the material on the bacterial accumulation may be suggested from these results.

The effect of sintering temperature of the magnetic on the affinity to the microorganisms was investigated using samples ④. The results are shown in Fig.5. Although there is not a large difference, a weak trend can be observed that higher sintering temperature brings larger microbial accumulation. According to the SEM observation, the magnetic material with higher sintering temperature seems to have a firm fine porous structure on the surface, which may cause the larger microbial accumulation. These findings also will suggest the importance of physical properties and surface conditions of the magnetic materials on the accumulation quantity of microorganisms.

4.CONCLUSIONS

In this study we tried to develop a new type of magnetic material as a solid carrier for microorganisms. At first, a method to estimate the quantity of microbial accumulation to the material can be established by measuring proteins from bacterial body using Lowry method. Then we achieved the bacterial accumulation tests for magnetic materials (strontium ferrite) with various characteristics using activated sludge. It was found that a porous magnetic material had almost the same capacity of microbial accumulation as that of certain ceramic material practically used, meaning that there is a possibility to use these magnetic materials as microbial solid supports in practical processes. And it was suggested that physical properties and surface conditions of the magnetic materials would be the key factor to the accumulation quantity of microorganisms.

Table II Data of ceramic materials

Sample	Hole Volume (cc/g)	Chemical Component (%)		
		SiO ₂	Al ₂ O ₃	Fe ₂ O ₃
ceramic①	0.41	75	20	4
ceramic②	0.70	58	37	2
ceramic③	0.35	51	34	13

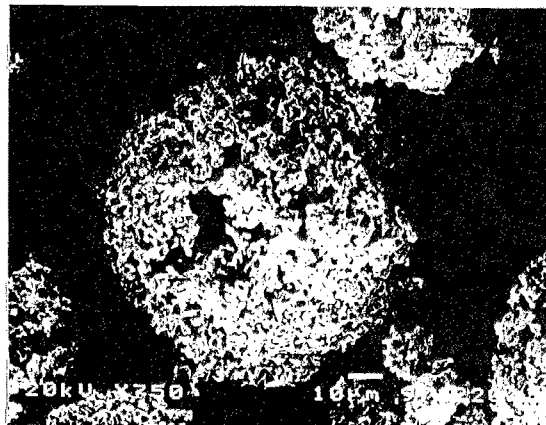


Fig.1 SEM photograph of Sample④

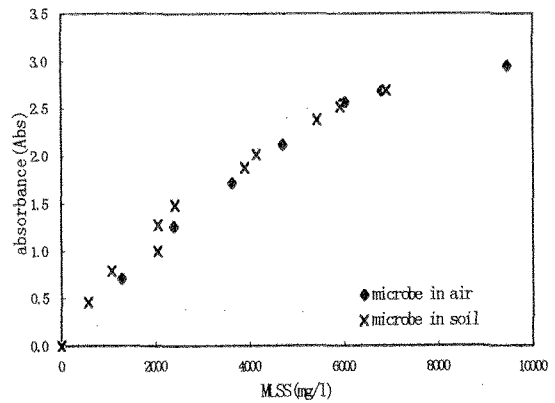


Fig.2 Relation between MLSS and absorbance

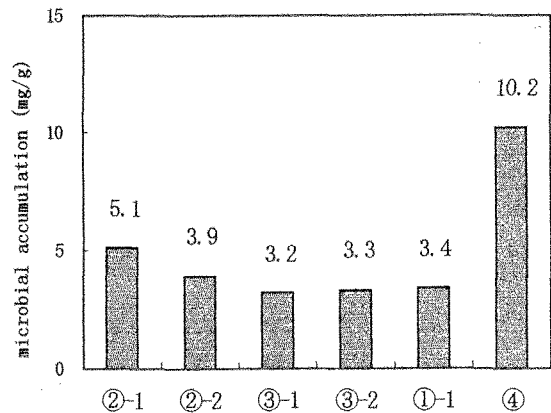


Fig.3 Microbial accumulation on each magnetic material

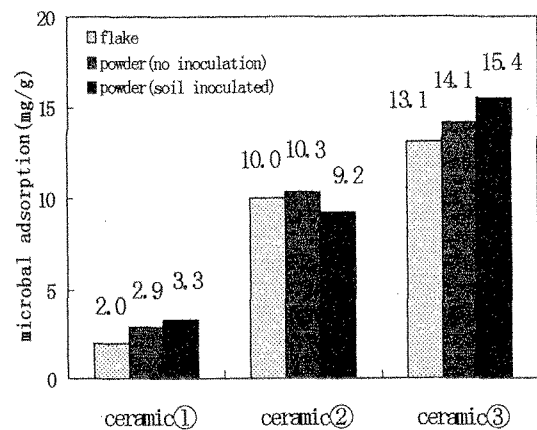


Fig.4 Microbial accumulation on ceramic material with is practically used

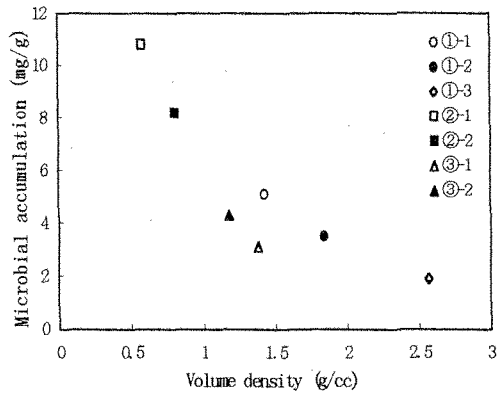


Fig.5 Relation between volume density and microbial accumulation

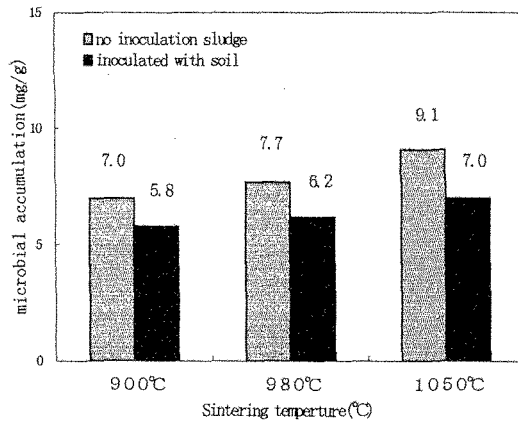


Fig.6 Microbial accumulation of the materials with various sintering temperature

5. REFERENCES

- 1) K.Okuno, K.Tuchiya, T.Ano and M.Shoda : J.Ferment. Bioeng., **75**, 103(1993)
- 2) M.Yanase et. al. :Jpn. J. Cancer Res., **89**, 463(1998)
- 3) A.Kondo : Chemical Engineering, **39**(1), 80(1994)
- 4)Lowry, O.H., Rowebrough, N.J., Farr, A.L. and Randall, R.J.:J.Biol.Chem.,193, 265(1951)

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