# Characteristics of Growth of Activated Sludge Cultivated with Cow's Milk

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#### **Abstract**

An activated sludge was cultivated with cow's milk to prepare a sample to study a metabolism of organic substrate by activated sludge, and to compare characters of the sludge cultivated with cow's milk with those cultivated with artificial sewage containing glucose, polypepton etc.. The characteristics of growth of the sludge were investigated as a preliminary test. It seems that our attempt was successful at least on finding the culture conditions enable to obtain a stable activated sludge by using cow's milk as a substrate. It is necessary for the growth of activated sludge that initial load of cow's milk per initial concentration of MLSS ( $F_{\rm o}/M_{\rm o}$ ) exceeds 0.8-0.9 mL cow's milk/gMLSS per day. When  $F_{\rm o}/M_{\rm o}$  exceeded about 1.5 mL cow's milk/gMLSS per day, SV<sub>30</sub> increased rapidly, filamentous microorganisms grew in the sludge, and SVI exceeded 200. An increase in MLSS with cultivation caused decrease of  $F_{\rm o}/M_{\rm i}$  (i: cultivation days), filamentous microorganisms disappeared and SVI decreased. The activated sludge is able to be utilized conveniently as a sample for the education of environmental science.

### Introduction

A biochemical treatment of wastewater by activated sludge is the main current of wastewater treatment and has been widely adopted treatment of domestic and many industrial organic wastewater. So far, a purpose of the wastewater treatment was removal of BOD (organic pollutants). Recently, removal of nitrogen and phosphorous has become important because the eutrophication in closed water such as lake is anxious. There are many studies on the removal of nitrogen and phosphorous by activated sludge<sup>1–3)</sup>. However, the properties of the sludge are not necessarily reproducible on removal of nitrogen and phosphorous. The cause seems that the sludge of each treatment plant shows different action dependent on the component of influent. Therefore, each plant has unavoidably adopted a treatment method fitted by trial and error.

To study wastewater treatment by activated sludge, it is necessary that the activated sludge used in laboratory is stable on the properties. Therefore, such sludge is

usually cultivated with artificial sewage<sup>4,5)</sup>. From the viewpoint of wastewater treatment, raw sewage is the best substrate for cultivation. However, it is difficult to treat it everyday. Therefore, a variety of artificial sewage utilized popularly have components similar to raw sewage. We considered to utilize cow's milk as the culture substrate. To get commercial cow's milk is easy and it is a low cost relatively. Aims of this report are firstly to prepare an activated sludge having constant properties to study a metabolism of organic substrate in wastewater, and secondly to compare characters such as the state of growth, increase and/or decrease of MLSS and SV, of the sludge cultivated with cow's milk with those cultivated with different substrate.

#### Materials and Method

Experiment (1). Activated sludge used to experiment was tabenout from aeration tank of a treatment plant. The mixed liquor of 5 L was filled in a plastic bucket (10 L) and aeration was started without substrate. After 24 hours, aeration was stopped and activated sludge was separated by centrifugation (2000 rpm, 10 min). The sludge was suspended in tap water filled in beaker (2 L) and the MLSS was measured.

The activated sludge was cultivated in 3 flasks (500 mL) shown Fig. 1. Water temperature in water bath was kept at about 25  $^{\circ}$ C. The concentration of MLSS in each flask was adjusted about 500 mg/L and the MLSS and SV<sub>30</sub> were measured exactly.

Commercial cow's milk of 1, 2 and 3 mL was added in the flask A, B and C, respectively and the mixed liquor was aerated. Aeration rate was 1 mL/min. When cow's milk of 1 mL was added to tap water of 500 mL, the TOC was 110-120 mg/L. That is, the load of TOC per the mixed liquor (500 mL) was 55-60 mg/day.

After 24 hours, aeration was stopped, and the mixed liquor was transferred to a measuring cylinder (500 mL). The MLSS and  $SV_{30}$  were measured and supernatant was discarded. The same volume of tap water and cow's milk were added and aeration was

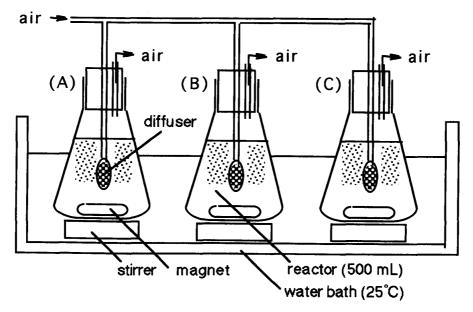


Fig. 1 Experimental equipment

started again. This operation was repeated during 10-20 days.

SVI (Sludge Volume Index) was estimated from values of MLSS and  $SV_{30}$  as follows;

$$SVI = SV_{30} \ (v/v\%) \ \times 10000 \div MLSS \ (mg/L).$$

Experiment (2). Experimental equipment, items of observation and experimental method were same to experiment (1) except that initial load of cow's milk was 0 mL for A, 2.5 mL for B and 4.5 mL for C and the initial concentration of MLSS was about 2000 mg/L in each flask.

#### Results

*Experiment* (1). It was observed that the difference of load of cow's milk supplied to activated sludge once a day influenced increase of MLSS (Fig. 2A). Initial rate of increase of MLSS became larger with an increase in the load, and the concentration of MLSS after 10 days was proportional to the load. The rate of increase of MLSS in flask A supplied cow's milk of 1 mL/day (initial load was about 2 mL/gMLSS/day) increased slowly with time, and the MLSS was about 1000 mg/L after 10 days and was twice of the initial MLSS. Then cow's milk of 2 mL/day was supplied in flask A (▼ in Fig. 2A). The load is corresponding to about 2 mL/gMLSS per day that is the same as the beginning of cultivation. Consequently, MLSS started increasing again.

Time course of  $SV_{30}$  was shown in Fig. 2B.  $SV_{30}$  of flask A was stable and was about 10%. Although cow's milk of 2 mL/day (about 2mL/gMLSS/day) was supplied in flask A ( $\nabla$  in Fig. 2B) after 10 days, the  $SV_{30}$  did not change.  $SV_{30}$  of flask B supplied cow's milk of 2 mL/day (initial load was about 4 mL/gMLSS/day) increased until 5 days and after that, decreased slowly.  $SV_{30}$  of flask C supplied cow's milk of 3 mL/day (initial load was about 6 mL/gMLSS/day) increased rapidly until 3-4 days and the sludge did not settle at 10 days. After that, it decreased slowly.

Time course of SVI was shown in Fig. 2C. SVI is the volume in milliliters occupied by 1 g of a suspension after 30 min settling. Therefore, it is an index of settling of sludge. SVI of flask A decreased gradually. SVI of flask B increased until 3 days and decreased after that. Although SVI of flask C increased rapidly until 3-4 days, it decreased slowly after that. Dependent on increase of initial load of substrate per gMLSS, SVI was larger and the value exceed 200 at the initial stage. It seems that bulking of activated sludge was caused by filamentous microorganisms (Photo. 1). Dependent on the growth of activated sludge, load of substrate per gMLSS decreased substantially in the late stage of time course. Then, settling of sludge was recovered gradually.

Experiment (2). Time course of MLSS in the flask (A) not given cow's milk was shown in Fig. 3. In the case, the concentration of MLSS decreased exponentially with time and it was one third of the initial value after 10 days. Time course of  $SV_{30}$  also decreased similarly. Therefore,  $SV_{30}$  was correlated strongly with MLSS (Fig. 4). The result suggests that bacteria in activated sludge die out gradually.

On the other hand, correlation between MLSS and SV<sub>30</sub> in flask (B) given cow's milk

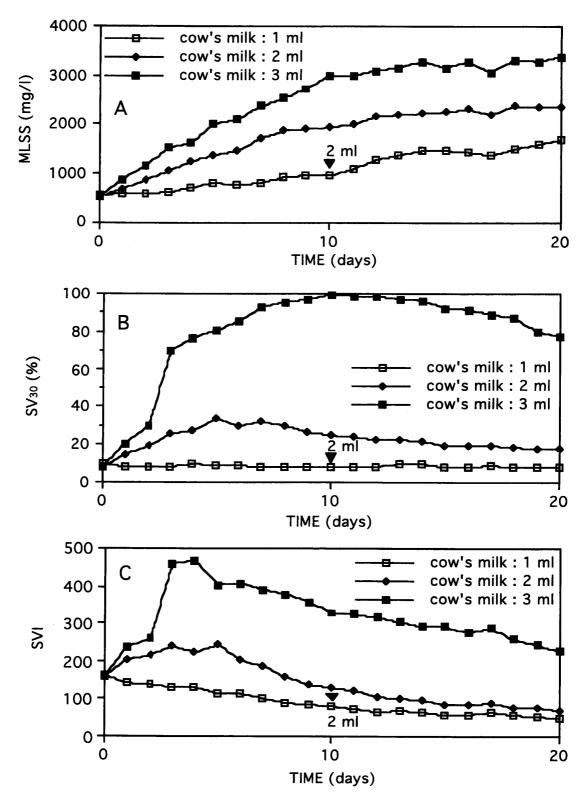


Fig. 2 Time course of MLSS, SV<sub>30</sub> and SVI

2.5~mL (initial load: about 1.5~mL/gMLSS) was shown in Fig. 5A. In the case, negative correlation was recognized because MLSS increased and  $SV_{30}$  decreased with time. The result suggests that floc-forming bacteria become to grow with cultivation. It seems that the growth of filamentous fungi does not occur in load of 1.5~mL cow's

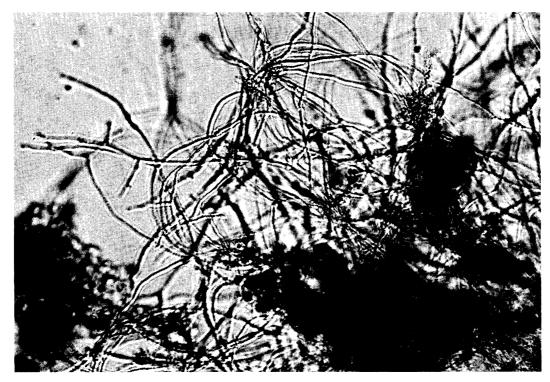


Photo. 1 Filamentous fungi in activated sludge cultivated with cow's milk

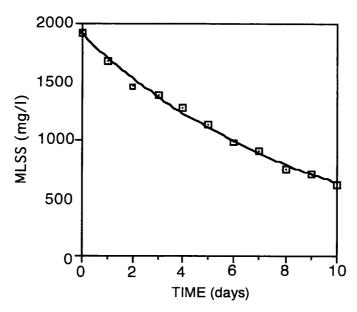


Fig. 3 Time course of MLSS of the sludge cultivated without cow's milk

milk/gMLSS. Relationship between MLSS and  $SV_{30}$  in flask (C) given cow's milk 4.5 mL (initial load: about 2.4 mL/gMLSS) was shown in Fig. 5B. In the flask, MLSS increased in the early stage of time course, and  $SV_{30}$  also increased. However, when the value of MLSS exceeded 3000 mg/L, namely the load became 1.5 mL cow's milk/gMLSS substantially,  $SV_{30}$  began to decrease slowly. In the case, it seems that filamentous microorganisms grow preferentially in the early stage of cultivation, growth of the filamentous microorganisms is suppressed after 4 days and floc-forming

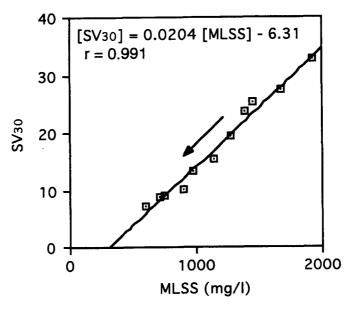


Fig. 4 Correlation of MLSS and  $SV_{30}$  of the sludge cultivated without cow's milk

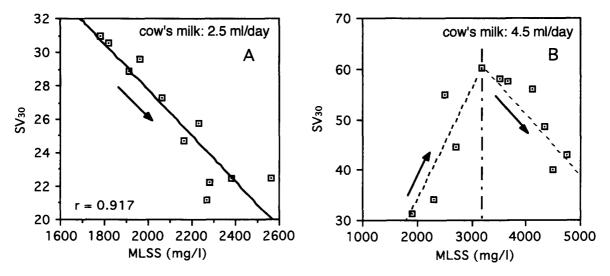


Fig. 5 Correlation of MLSS and SV<sub>30</sub> of the sludge cultivated with cow's milk

organisms become to grow gradually.

Load of cow's milk for growth of activated sludge. Fig. 6 shows relationship between growth rate per initial concentration of MLSS ( $M_i/M_o$ , i : cultivation days) and initial load of cow's milk per initial concentration of MLSS ( $F_o/M_o$ ) of activated sludge cultivated with different  $F_o/M_o$  during 10 days. It seems that MLSS increases when the value of  $F_o/M_o$  exceeds 0.8-0.9 mL cow's milk/gMLSS, and the growth rate becomes large with an increase in  $F_o/M_o$ . For growth of activated sludge, the load of 0.8-0.9 mL cow's milk (90-100 mgTOC)/gMLSS per day is necessary at least.

## Discussion

It was elucidated that activated sludge grew with cow's milk and the characteristics of growth depended to load of cow's milk. It is necessary for the growth of activated

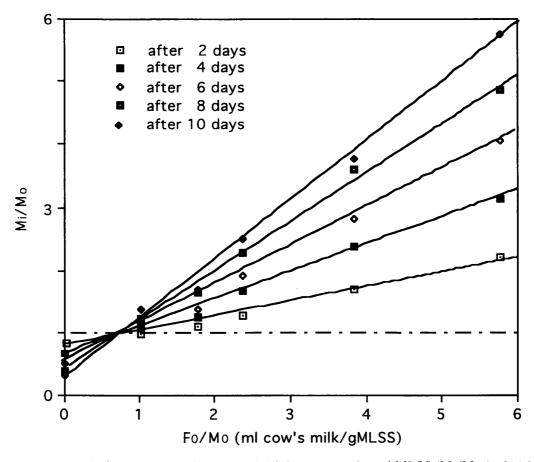


Fig. 6 Relationship between growth rate per initial concentration of MLSS ( $M_i/M_o$ ; i=2, 4.6, 8, 10) and initial amount of cow's milk per initial concentration of MLSS ( $F_o/M_o$ )

sludge that  $F_{\circ}/M_{\circ}$  exceeds 0.8-0.9 mL cow's milk/gMLSS per day. Activated sludge does not grow below the value and gradually decreases because it becomes starvation. On the other hand, large  $F_{\circ}/M_{\circ}$  brings about the growth of filamentous microorganisms and causes bulking in the initial stage and the bulking ceases with an increase in MLSS. These results are familiar with the case of a general activated sludge and are not new information. However, an aim of our studies was whether cow's milk was able to utilize instead of artificial sewage on the cultivation of activated sludge. From our experiment, it seems that our attempt was successful at least on finding the culture conditions enable to obtain a stable activated sludge with cow's milk as substrate. At present, we already found that the activated sludge cultivated long for days with cow's milk is not able to reduce nitrite in anoxic conditions. The characteristics of metabolism by the activated sludge have to be investigated more exactly and the results will be reported in next step.

#### Conclusion

An activated sludge was cultivated with cow's milk and the characteristics of growth were observed. It is concluded as follows;

- 1) The activated sludge grew sufficiently with cow's milk and the increase of MLSS depended on initial load of cow's milk.
- 2) When  $F_o/M_o$  exceeds about 1.5 mL cow's milk/gMLSS,  $SV_{30}$  becomes large rapidly, filamentous microorganisms grow in the sludge, and SVI exceeds 200. An increase in MLSS with cultivation causes decrease of  $F_o/M_i$ , filamentous fungi disappear and SVI drops.
- 3) It is necessary for the growth of activated sludge that  $F_o/M_o$  exceeds 0.8-0.9 mL cow's milk/gMLSS per day.
- 4) The activated sludge is able to be utilized conveniently as a sample for the education of environmental science.

#### References

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