

## Microbial Activity of Gravel Intertidal Zone for Purification of Polluted Near Shore Water

Young-Chae Song\* · Ja-Hwan Gu\*\* · In-Seok Park\*\*\* · Jong-Su Yoo\*\*\*\*

\*Division of Civil and Environmental System Engineering, Korea Maritime University, Busan 606-791, Korea

\*\*Department of Civil and Environmental Engineering in Graduate School, Korea Maritime University, Busan 606 791, Korea

\*\*\*Division of Ocean Science, Korea Maritime University, Busan 606-791, Korea

\*\*\*\*Research Institute of Marine Science and Technology, Korea Maritime University, Busan 606 791, Korea

**Abstract :** Microbial activity of biofilm formed on the surface of gravels from intertidal zone was estimated using an aerobic respirometer system, and compared with that of suspended marine microorganisms contained in a near shore water. The maximum oxygen uptake rate of the suspended marine microorganisms was 0.15mg O<sub>2</sub>/L/hr, indicating the potential of purification of polluted near shore water. For the gravels from the intertidal zone, the maximum uptake rate of oxygen was affected by the vertical positions, but their gross value was 0.77mg O<sub>2</sub>/L/hr, which was around 5.1 times higher than the purification potential of polluted near shore water by the microorganisms contained in the near shore water. The nitrogen removed by the gravels from the intertidal zone and the marine microorganisms was about 1/20~1/39 times of the total consumption of oxygen, which was similar to that of the phosphate. The gravel intertidal zone contained lots of particulate organics, over than that in the near shore water, and this was confirmed from the large difference between total oxygen consumption and the removed soluble COD in the microbial activity test. This indicates that the gravel intertidal zone plays an important role in controlling the non-point source pollutants from land, as well as self purification of polluted near shore water by trapping and degrading the particulate organics.

**Key words :** microbial activity, near shore water, gravel intertidal zone, pollution, purification

### 1. Introduction

The near shore sea with tiny waves has been developed for a fish breeding ground or a port for ship to be anchored, but is fragile to pollution due to inflow of lots of pollutants from land and low exchange rate of the water with clean offshore water (Laws et al, 1999; Zilan and Lixian, 1999). The major sources of the pollutants flowing into the near shore water are: firstly, point source pollutants from land such as municipal and industrial wastewater, secondly, non point source pollutants from land by run-off, and thirdly, marine origin pollutants such as spilled oil, pollutants from aquaculture aging or reclamation works (Laws et al, 1999; Hisano and Hayase, 1991; Mohammed, 2002; Song, 2002). Generally, type of intertidal zone and their biological activity to purify the pollutants have an influence on the quality of near shore water. It is well known that pollutants flowing into near shore water could be actively decontaminated in the intertidal mud flat as well as coastal marsh (Decho, 2000; Wang, 1997; Blanchard et al., 2000). However, in the case of steep rocky intertidal zone, the biological activity is not sufficient for the purification of the pollutants (Dyer et al., 2000; Ellis,

2003). One of the interesting natural shorelines is a gravel intertidal zone. It is considered that the gravel intertidal zone has much higher biological activity than that of the rocky intertidal zone, and may contribute to control the non-point source pollutants flowing into the near shore water, as well as in purification of contaminated near shore water (Dyer et al., 2000). However, the study to elucidate the role of gravel intertidal zone on the quality of near shore water was hardly performed. Therefore, in the study, the microbial activity of gravels from intertidal zone was estimated, and the role of gravel intertidal zone for the improvement of the quality of near shore water was studied.

### 2. Materials and Methods

#### 2.1 Near Shore Water and Gravels of Intertidal Zone

From the gravel intertidal zone, naturally formed at T amusement park in P metro city (Fig.1a), the three kinds of gravels were taken from the upper part (less than 5cm in depth from the surface), the middle part (between 10 and 20cm) and the bottom part (over 30cm), respectively. The

\* Corresponding Author: Young-Chae Song, soyce@mail.hhu.ac.kr

\*\* zangguny@hanmail.net

\*\*\* ispark@kmaritime.ac.kr

\*\*\*\* jsyoo@hanara.kmaritime.ac.kr

spherical equivalent mean diameters of the gravels from both the upper and the middle parts were 1.42cm. However, the gravels from the bottom part was a mixture of small size gravels and large size sands, and their mean size was smaller than the others. In order to prepare the medium for the microbial activity test, the sea water was taken from a shore near T amusement park, and glucose, potassium dihydrogenphosphate (KH<sub>2</sub>PO<sub>4</sub>) and sodium nitrate (NaNO<sub>3</sub>) were added into the sea water to adjust the initial quality of the medium, based on a severely polluted near shore water. The major characteristics of near shore water and the medium were presented at Table 1.

### 2.2 Microbial Activity Test

For the microbial activity test, 250mL of the medium was filled into three test bottles, and three kinds of gravels taken from the upper, the middle and the bottom parts of the intertidal zone were added into the bottles. The total volumes of medium and gravels in the test bottles were 500mL. The total surface areas of the gravels from the upper and the middle parts in the test bottles were around 1,140cm<sup>2</sup>, but the surface area of gravels from the bottom part was around 3.9 times wider than that of the upper or the middle part gravels.

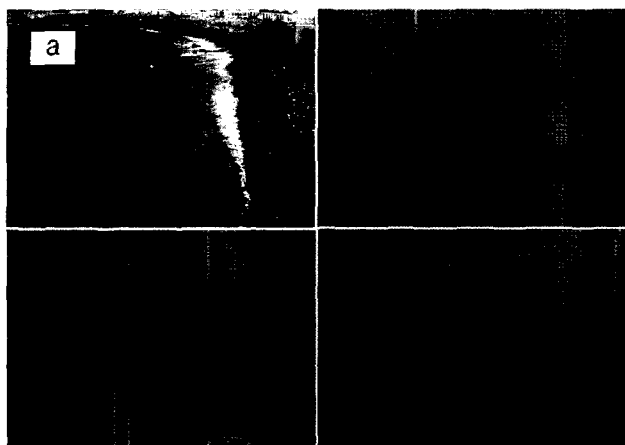


Fig. 1 Photographs of the gravel intertidal zone naturally formed at T amusement park(a), and the gravels, the upper (b), the middle (c) and the bottom part(d), from the intertidal zone used for microbial activity test

Table 1 Characteristics of medium used for the biological activity test for intertidal zone gravel

| Content          | pH  | COD  | NO <sub>3</sub> <sup>-</sup> -N | PO <sub>4</sub> <sup>-3</sup> -P |
|------------------|-----|------|---------------------------------|----------------------------------|
| Near shore water | 7.4 | 2.88 | 0.01                            | 0.029                            |
| Medium           | 7.4 | 5.88 | 1.01                            | 1.029                            |

The test bottles prepared for the microbial activity test were incubated in a darkened water bath of which temperature was maintained at 21°C. During the incubation, the consumption and uptake rate of oxygen were monitored with a real-time aerobic respirometer system (Challenger AER-200 4-CELL AEROBIC/ ANAEROBIC RESPIROMETER), as shown in Fig.2. For the activity of the suspended marine microorganisms contained in the near shore water, the test bottle containing the medium only was incubated at above condition. In order to find out the relationship between the microbial activity and the potential of near shore water purification, when the microbial activity test was complete, the concentrations in soluble COD (Chemical oxygen demand) were analyzed by the alkaline method for sea water(Choi and Yoo, 1998), and the analysis of NO<sub>3</sub><sup>-</sup>-N and PO<sub>4</sub><sup>-3</sup>-P were according to the Standard Methods(1995).

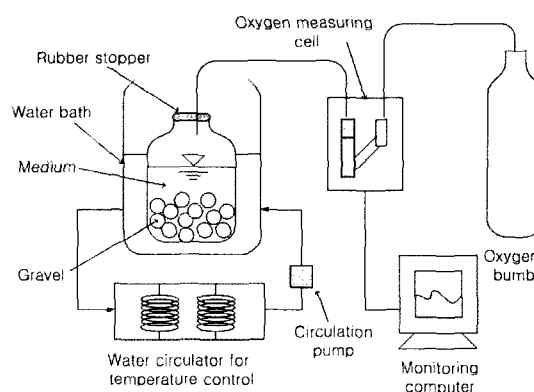


Fig. 2 Respirometer system used for the microbial activity test

## 3. Results and Discussion

### 3.1 Microbial Activity of the Near Shore Water

In aerobic biological system, the oxygen is generally used as an effective electron acceptor for energy production during the microbial oxidation process of organics (Ritmann and McCarty, 2001). This indicates that the microbial activity is closely related to the consumption of oxygen. Fig.3 shows the cumulative consumption and uptake rate of oxygen by the suspended marine microorganisms contained in near shore water. During about 90hrs of the microbial activity test, total consumption of oxygen was 5.01mg O<sub>2</sub>/L, and the maximum uptake rate of oxygen was 0.15mg O<sub>2</sub>/hr/L at around 30hrs. Here, the uptake rate of oxygen corresponds to the microbial activity and total consumption of oxygen, to the total degraded organics in

term of BOD (Biochemical oxygen demand). Therefore, these indicate that there are lots of active suspended marine microorganisms in the near shore water. However, the decreased soluble COD in the medium was only 0.76mg/L (Table 2), which was only 15% of the total consumption of oxygen. This means that lots of the biodegradable particulate organics was contained in the near shore water. For freshwater heterogeneous microorganisms, it is well known that the nitrogen required for cell synthesis is around 1/20 of the organic materials, while the inorganic phosphate requirement is 1/5 of this (Ritmann and McCarty, 2001). In the study, the removed nitrate nitrogen was 0.13mg NO<sub>3</sub><sup>-</sup>-N/L, which was not much different from that of ortho-phosphate. Therefore, the ratio of the removed total organics in BOD to the nitrate nitrogen was estimated to around 38.5, which was quite different from that of the freshwater heterogeneous microorganisms.

Table 2 Changes in SCOD, NO<sub>3</sub><sup>-</sup>-N, and PO<sub>4</sub><sup>3-</sup>-P in near shore water during the microbial activity test

| Content |           | pH  | SCOD (mg/L) | NO <sub>3</sub> <sup>-</sup> -N (mg/L) | PO <sub>4</sub> <sup>3-</sup> -P (mg/L) |
|---------|-----------|-----|-------------|--|---|
| Initial | Sea water | 7.4 | 5.88        | 1.01                                   | 1.03                                    |
| Final   | Sea water | 7.3 | 5.12        | 0.88                                   | 0.90                                    |

However, it is interesting that the ortho phosphate removed in the activity test was relatively higher, compared to the total degraded organics in terms of oxygen consumption. This might be due to the difference of the microbial community of the near shore water, probably containing microalgae, autotrophic microorganisms as well as heterotrophic microorganisms, with the freshwater heterogeneous microorganism. In the study, the microbial activity of near shore water indicates that the quality of the near shore water could be improved by self-purification. *In situ*, the self-purification potential of near shore water, however, is generally not as much as that in the laboratory condition. This is because the microbial activity depends on the environmental conditions including temperature, dissolved oxygen, and substrate and nutrients.

### 3.2 Microbial Activity of Gravels from Intertidal Zone

Fig.4 shows the cumulative consumption and uptake rate of oxygen for the three kinds of gravels from the intertidal zone. For the gravels from the middle part of intertidal zone, the maximum uptake rate of oxygen was 0.90mg O<sub>2</sub>/hr/L, which was about 6.0 times of that of the near shore water. This indicates that the biofilm was well formed on the gravel surface, and its microbial activity was much higher than the suspended microorganisms contained in near shore water. During the microbial activity test, the total consumption of oxygen was 25.5mg O<sub>2</sub>/L, which was about 8 times higher than 3.18mg/L of the removed SCOD (Table 3). This indicates that lots of particulate organics were trapped in gravels from the middle part of intertidal zone, and were removed by the biofilm. After the activity test, the removed phosphate was 0.83mg PO<sub>4</sub><sup>3-</sup>-P/L, which was similar to the removed nitrate nitrogen. This indicates that the requirement of inorganic phosphate in the degradation of organics was higher than that of the freshwater heterogeneous microorganism, probably due to the difference of the microbial population community in the biofilm on the surface of the gravels (Ritmann and McCarty, 2001).

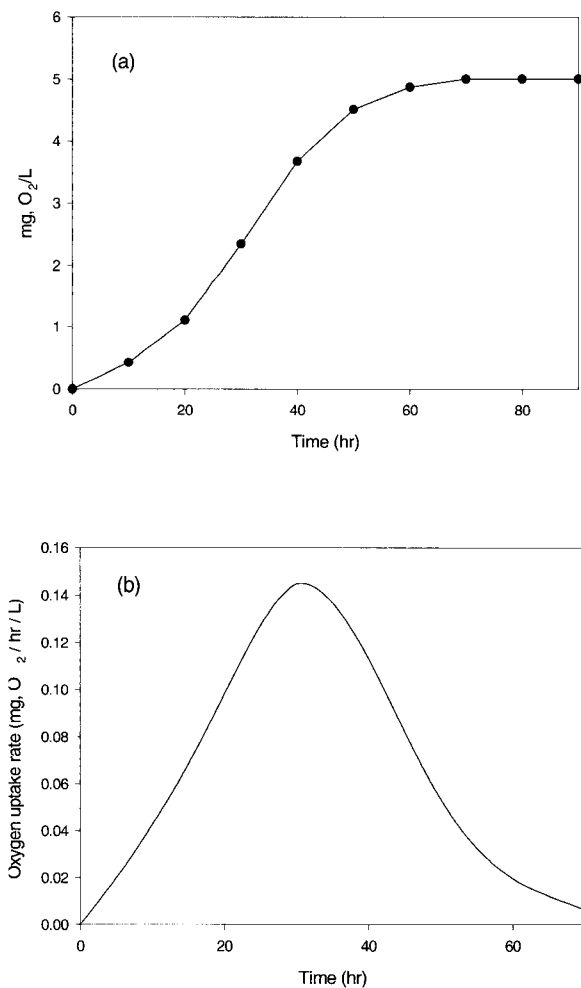


Fig. 3 Cummulative consumption (a) and uptake rate (b) of oxygen during the microbial activity test of the near shore water

For the upper part gravels of the intertidal zone, the maximum uptake rate of oxygen was  $0.52 \text{ mg O}_2/\text{L} \cdot \text{hr}$ , which were only 58% of that of the middle part gravels. This reflects the poor microbial community and their population existing on the surface of gravel, which was caused by some harsh environments such as exposure under sunlight, limited moisture and insufficient supply of nutrients. The total consumption of oxygen was amount to  $11.6 \text{ mg O}_2/\text{L}$ , and the ratios of total oxygen consumption to the removed nitrate nitrogen and phosphate were 18.4 and 15.9, respectively, which were a little different from those of the middle part gravels. For the gravels from the bottom part of intertidal zone, the oxygen consumption during the microbial activity test was  $28.8 \text{ mg O}_2/\text{L}$ , which was higher than that of the middle part gravels. This shows that the organics trapped in the gravels from the bottom part was more than the other part gravels. The surface area of the bottom part gravels was around 4 times higher than that in the upper or the middle part gravels, as presented Table 2. However, the maximum uptake rate of oxygen was  $0.68 \text{ mg O}_2/\text{hr/L}$ , which was 75.6% of that of the middle part gravels.

The bottom part gravels was a mixture of small size of gravels and large size of sands, and their porosity was relatively lower than those of other part gravels. Therefore, the less microbial activity of the bottom part gravels might result from the insufficient supply of nutrients and oxygen to the biofilm. The requirements of nitrogen and phosphate for the degradation of organics were similar to those of the middle part gravels, indicating that their microbial community might be similar to that of the middle part gravels.

Table 3 Changes in the soluble COD,  $\text{NO}_3^- \text{-N}$  and  $\text{PO}_4^{3-} \text{-P}$  for the intertidal gravels during the microbial activity test

| Content |        | pH  | SCOD<br>(mg/L) | $\text{NO}_3^- \text{-N}$<br>(mg/L) | $\text{PO}_4^{3-} \text{-P}$<br>(mg/L) |
|---------|--------|-----|----------------|-------------------------------------|--|
| Initial | Upper  | 7.4 | 6.4            | 1.148                               | 1.031                                  |
|         | Middle |     | 7.2            | 1.154                               | 1.035                                  |
|         | Bottom |     | 8.34           | 1.167                               | 1.037                                  |
| Final   | Upper  | 7.3 | 3.96           | 0.517                               | 0.306                                  |
|         | Middle |     | 4.02           | 0.344                               | 0.204                                  |
|         | Bottom |     | 5.38           | 0.236                               | 0.124                                  |

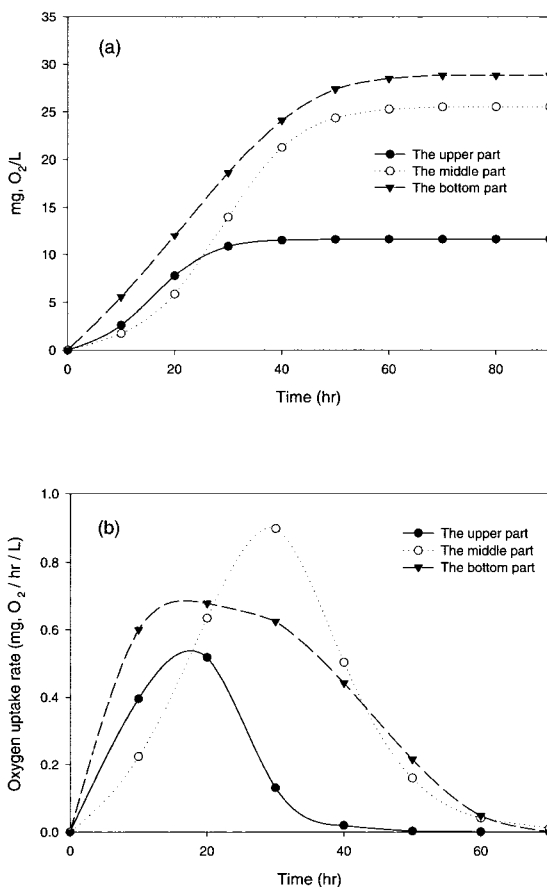


Fig. 4 Cummulative consumption (a) and uptake rate (b) of oxygen for the gravels from intertidal zone

### 3.3 Purification Potential of Gravel Intertidal Zone

In the study, the gross microbial activity of the gravel intertidal zone, estimated by the volume weighted method from the individual microbial activity (Fig.4b), was  $0.77 \text{ mg O}_2/\text{L/hr}$  of maximum oxygen uptake rate. Therefore, the purification potential of square meters of the gravel intertidal zone per day was about 18.48g of organics in terms of BOD, which was over 5 times greater than that in the near shore water. However, the estimated microbial activity of the intertidal gravels might be underestimated because the activity test was performed under a stationary state. This indicates that the biofilm on gravel intertidal zone, as well as the suspended marine microorganism contained in near shore water contribute for the self-purification of the polluted near shore water. Generally, higher microbial activity of the gravels appears to be reflected by the well formed biofilm on the surface (Decho, 2000). In the study, the microbial activity of the gravels was affected by their vertical positions in the gravel intertidal zone, and size of the gravel (Fig.3). The upper part gravels, which was less moisturized and exposed to the sunlight, showed less microbial activity than in the middle part gravels. In the case of the bottom part gravels with less porosity, the lower microbial activity appeared to

be due to the insufficient supply of substrate and nutrients into the biofilm. In the study, the gravels from the intertidal zone contained lots of the biodegradable particulate organics, and this could be explained by the difference between the total consumption of oxygen and the removed SCOD in the microbial activity test. Some of the particulate organics in the gravels might be trapped by the filtration during the inflow of pollutants from land. This indicates that the gravel intertidal zone may play an important role in controlling the non-point source pollutants from land, as well as the polluted near shore water.

#### 4. Conclusions

In order to elucidate the role of near shore water and gravels from intertidal zone on the purification of polluted near shore water, their microbial activities was estimated, and following conclusions were drawn. The microbial activity of near shore water was about 0.15mg O<sub>2</sub>/L/hr, indicating the higher self-purification potential of polluted near shore water. The microbial activity of the gravels from the middle part of the intertidal zone was 0.9g O<sub>2</sub>/L/hr, which was higher than those of the upper and the bottom part gravels. The average potential of the gravel intertidal zone for purifying organics was about 18.48g BOD/m<sup>3</sup>/d. For near shore water and three types of intertidal gravels, the requirements of inorganic phosphate for the biodegradation of organics were quite different from each other, reflecting the differences in their microbial population communities, but the phosphate requirements were similar to those of nitrogen. The gravel intertidal zone may play an important role in controlling the non-point source pollutants from land, as well as self-purification of polluted near shore water by the trapping the particulate organics.

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