Removal and Excretion of Dissolved Organic Matter by Periphyton Community Grown in Eutrophic River Water

Morihiro AIZAKI

Abstract

Removal and excretion activities of dissolved organic substances by periphyton communities were examined using artificial substrata submerged in the water of the eutrophic River Tamagawa and laboratory recirculating streams. These activities changed with the progress of biofilm development. High removal activity of dissolved organic substances was observed in the early stage of the biofilm development, while high excretion rate was found in the mature stage. A maximum exertion rate was 580 mgC·m⁻²·d⁻¹. Organic substances removed from the water were biologically labile compounds which can be determined by BOD, and excreted organic substances were regarded as microbiologically stable compounds. Decomposition products formed at the inside of periphyton biofilm were considered to be an important source as well as photosynthetic products by sessile algae for the excretion of organic substances from the periphyton community in the mature stage of biofilm development.

1. Introduction

To clarify the dynamics of organic substances in a river ecosystem, the balance of production, inflow and mineralization has to be clarified. It is difficult, however, to distinguish the inflow of organic substances from photosynthetic products, and to determine how the amount of each organic substances is utilized by the heterotrophic microbes in natural conditions (Haack and McFeter, 1982). In many urbanized rivers in which city pollutants are discharged, a large amount of periphyton grows on river beds and exerts a significant role in production and mineralization of organic substances (Aizaki, 1978; 1980). Wuhmann (1964, 1972) and Wuhmann et al. (1967) studied self-purification in large-scale outdoor artificial streams and found that the rate of mineralization of organic substances in streams decreased remarkably with an increase in the percentage of sessile algae in the attached microbial community. Tezuka et al. (1967) also reported a similar result in an artificial outdoor stream. These results suggest that the photosynthetic products by sessile algae repress the removal activity of dissolved organic substances in river water by periphytic heterotrophs.

In the present study, removal and excretion activities of dissolved organic substances by periphyton community were examined by using artificial substrata submerged in the water of the eutrophic River Tamagawa and laboratory recirculating streams. According to the use of these artificial substrata and laboratory streams, it was made clear that the removal and excretion activities of dissolved organic substances changed with the progress of the biofilm development of the periphyton community.

2. Materials and Methods

2-1. Experiment using artificial substrata

The Tamagawa River is a typical eutrophic river in Japan. In the midstream of the river algal blooms with maximum densities approaching ca. 500 mg·chl-a·m⁻² have occurred on the river bed from spring to autumn (Aizaki, 1978). Artificial substrata made by polyvinylchloride were submerged in this region of the river. About sixty polyvinylchloride plates (5 cm × 5 cm, the surface roughed by rasp) were fixed on an iron frame with wires, and submerged in the river water in April, 1974. Six plates were removed from the frame at 4–5 day intervals and brought to the laboratory in the wet state. Then the plates were used for the measurement of removal and excretion activities of organic substances and periphyton biomass. The
study site and method for measuring the periphyton biomass have been described in the previous study (AIZAKI, 1978).

Removal and excretion rates of organic substances by periphyton communities were measured by the following method. Two plates with the periphyton community were held vertically in a beaker (1 l), and 600 ml of the river water was poured into the beaker. Then the beaker was incubated at 20°C for about 24 h. The water in the beaker was stirred continuously with a magnetic stirrer. The removal and excretion rates of organic substances were estimated from the difference of water quality before and after incubation. In order to clarify the role of sessile algae in the removal and excretion rates, the rates were determined for four different combinations of microbial communities and incubation conditions as follows: 1) river water under light condition (3,000 lux), 2) heterotrophic periphyton community under dark condition, 3) periphyton community under dark condition, and 4) periphyton community under light condition (3,000 lux). The dark condition was created by covering with black vinyl sheet. The heterotrophic periphyton community used here was grown on the back surface of the plates where sessile algae scarcely grew (AIZAKI, 1979), while the periphyton community was grown on the top surface of the plate. The effect of the photosynthesis of sessile algae on the removal and excretion rates of organic substances was evaluated from the difference of removal rates of the periphyton community under light and dark incubation conditions. Concentrations of dissolved organic carbon (DOC) and biological oxygen demand (BOD) were analysed before and after incubations.

2-2. Experiment using laboratory streams

Two artificial recirculating streams were made in a laboratory. Each of the streams (Fig. 1) consisted of pair of parallel flumes made of polyvinylchloride. The size of the flume was 1.5 m long, 10 cm wide and 5 cm deep. Twenty liters of river water was collected from the midstream of the Tamagawa River and filtrated through a No. XX17 silk cloth net. Then the water was circulated in each artificial stream. Water was renewed at 3-5 day intervals. Current velocity, depth of water and water temperature in the streams were about 20 cm·sec⁻¹, about 3 cm deep and 17-22°C, respectively. Stream A was illuminated with fluorescent lamps for a period of 12 h per day. Light intensity at the surface of the stream was about 8,000 lux. Stream B was maintained under dark condition by covering it with a black vinyl sheet to prevent the growth of sessile algae.

The experiment was started on 9 March 1974, and water samples were collected almost every day and analysed for concentrations of DOC and BOD. At the start of the experiment, the periphyton collected from the midstream of the Tamagawa River was inoculated. About twenty polyvinylchloride plates (5 cm × 3 cm, the surface of which was roughed by rasp) were placed on the streams, and collected periodically to measure the periphyton biomass and activities of photosynthesis and respiration. Methods for measuring photosynthetic and respiration rates

![Fig. 1. Diagram of a laboratory stream. Water was circulated through a pump.](image-url)
have been described in the previous report (Aizaki, 1978). Water temperature and light intensity at the measurement of these activities were kept at the same conditions existing in the streams. On April 9, one month after the beginning of this experiment, glucose (400 mg C) and sodium acetate (400 mg C) were added to each artificial stream and their removal was determined.

2-3. Chemical analyses
Ammonium nitrogen (NH₄-N), nitrite nitrogen (NO₂-N), nitrate nitrogen (NO₃-N), inorganic phosphorus (PO₄-P) and DOC were analysed after filtering the water samples through a pre-combusted glass fiber filter (Whatman CF/C). Chemical analyses were made by the following methods: BOD by the standard methods (American Public Health Association, 1971); DOC by the method of Menzel and Vaccaro (1964); PO₄-P by the Strickland and Parsons method (1965); NH₄-N with Nessler reagent after distillation; NO₂-N by colorimetry with sulphanilamide and N-(1-naphthyl) ethylenediamine; NO₃-N by colorimetry after being reduced to nitrite (Strickland and Parsons, 1965); glucose by the anthrone method.

3. Results
3-1. Experiment using artificial substrata
3-1-a. Standing biomass
Figure 2 shows the changes in standing biomasses of periphyton communities and suspended solid in river waters during the experiment. The biomass of periphyton communities grown on the top surface of the plates increased rapidly after the 8th day due to the growth of sessile algae and reached a maximum on the 16th day. Maximum values of dry weight, carbon and chlorophyll-a contents in periphyton community using light incubation condition were 136 g dry wt. m⁻², 37.6 g C m⁻³ and 2,360 mg chl-a m⁻², respectively (Fig. 2-a). In the periphyton community using dark incubation condition these maximum values were 108 g dry wt. m⁻², 32.6 g C m⁻³ and 1,110 mg chl-a m⁻³, respectively (Fig. 2-b). The difference of the maximum value between the two conditions may be caused by the loss of periphyton at the mature stage of the biofilm development. The dominant species of sessile algae were diatoms in the early stage and Stigeoclonium sp. in the mature stage. The biomass of heterotrophic periphyton grown on the back surface of the plates increased gradually through the experiment. Maximum values of 163 g dry wt. m⁻² and 3.9 g C·m⁻² were observed at the end of the experiment (Fig. 2-c). Sphaerotilus sp. dominated in this community. The ranges of the biomass suspended in river waters in this experiment were 11-21 g dry wt. m⁻³, 1.9-3.6 g C·m⁻³ and 22-62 mg chl-a·m⁻³, respectively (Fig. 2-d).

Maximum numbers of aerobic heterotrophic bacteria in the periphyton communities using light and dark incubations were observed on the 25th day to be 3.9-7.0×10⁸ cells·cm⁻². That of the heterotrophic periphyton community was 3.3×10⁹ cells·cm⁻³ at the end of the experiment. In river waters the bacterial number fluctuated between 1.6-7.0×10⁹ cells·ml⁻¹.
3-1-b. Removal and excretion of dissolved organic material

The water qualities using incubations are shown in Table 1. Concentrations of organic materials determined by BOD and DOC ranged from 4.6-10.9 mg·l⁻¹ in the former to 5.8-8.8 mg·l⁻¹ in the latter. Nutrient concentrations were superfluous for the periphyton growth.

BOD removal by the periphyton community with progress of the biofilm development under light incubation condition is shown in Fig. 3. The removal rate decreased with the increase of the biomass. Even negative values were observed on the 12th and 16th days. The maximum removal rate per unit area of 880 mg·m⁻²·d⁻¹ was observed on the 20th day, just after the scour of a large portion of periphyton. Removal rate per unit carbon weight showed relatively high values in the early stage of biofilm development and decreased with the increase of the biomass. The rate ranged from -10 to 200 mg·gC⁻¹·d⁻¹.

Removal rates of BOD per unit area by the heterotrophic periphyton community increased

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<th>BOD (mg·l⁻¹)</th>
<th>DOC (mg·l⁻¹)</th>
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<th>NO₂-N (mg·l⁻¹)</th>
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Removal and Excretion of DOM by Periphyton

Ifi3 continuously with increase of biomass and showed a relatively high value of about 940 mg·m⁻²·d⁻¹ on the 31th day. On the other hand, the rates per unit carbon weight showed almost constant values of 270–330 mg·gC⁻¹·d⁻¹ during the experiment (Fig. 4).

The removal rates of BOD under four different incubation conditions were compared with each other in Fig. 5. It is evident that the highest BOD removal on average rate was shown by the heterotrophic periphyton community under dark incubation condition, followed by suspended microbes in the river water under light incubation condition, periphyton community under dark incubation condition and finally periphyton community under light incubation condition. The rate by the suspended microbes in river water was calculated by assuming that the water depth was 25 cm, which was estimated to be average water depth at the study site on the Tamagawa River.

When the biomass increased to a relatively high value, organic matters as indicated by BOD were released from the periphyton community into the water. This phenomenon was more evident when the amount of organic matter was determined as DOC, which is the total concentration of both microbiologically stable and unstable dissolved organic substances. Figure 6 shows the DOC removal in the same experiment as in Fig. 5. It is notable that the removal rate of DOC by the periphyton community grown on the top surface of plates showed almost a negative value, especially under light incubation condition. Thus, a considerable amount of organic matter was released into the water during the maximum stage of the periphyton growth.

3-2. Experiment using laboratory streams
3-2-a. Standing biomass

Main chemical properties of the waters which were collected from the midstream of the Tamagawa River and circulated in laboratory streams are shown in Table 2. The concentrations of organic substances and nutrient were extremely high. As a result, a large amount of sessile algae grew on the bed and walls in Stream A which illuminated with fluorescent lamps.

The biomass in Stream A rapidly began to increase from the 7th day, and reached a maximum of 54.5 g dry wt·m⁻², 18.0 g C·m⁻² and 1,345 mg chl-a·m⁻², respectively on the 24th day (Fig. 7). Achnanthes sp. appeared on the 7th day and dominated in the early stage of the community. The number of diatom ranged

![Fig. 5](image1.png)

Fig. 5. Comparison of removal rates of BOD under different combinations of periphyton and incubation condition. (○), periphyton community under light incubation condition; (△), periphyton community under dark incubation condition; (●), heterotrophic periphyton community under dark incubation condition; (●), suspended solid in river water under light incubation condition.

![Fig. 6](image2.png)

Fig. 6. Comparison of removal and excretion rates of DOC under different combinations of periphyton and incubation condition. (○), periphyton community under light incubation condition; (△), periphyton community under dark incubation condition; (●), heterotrophic periphyton community under dark incubation condition; (△), suspended solid in river water under light incubation condition.
from $2.8 \times 10^7$ to $5.2 \times 10^7$ cells·cm$^{-2}$ in the mature stage. *Stigeoclonium* sp. appeared on the 11th day and dominated in the latter stage. These genera also dominated in the midstream of the Tamagawa River during the experiment.

On the other hand, in Stream B which was maintained under a dark condition, the biomass increased very slowly to a maximum of 2.8 g dry wt·m$^{-2}$ and 0.5 g C·m$^{-2}$ on the 24th day (Fig. 7). These values were significantly lower than those of Stream A.

The total number of aerobic heterotrophic bacteria was also higher in Stream A than in Stream B, being $4.9 \times 10^7$ cells·cm$^{-2}$ in the former and $1.0 \times 10^7$ cells·cm$^{-2}$ in the latter on the 31st day. However, these values were relatively low as compared with those in the midstream of the Tamagawa River (AIZAKI, 1980).

**3-2-b. Photosynthesis and respiration**

Changes in the photosynthetic and respiration rates in Stream A and B during the course of the experiment are shown in Fig. 8. The photosynthetic rate in Stream A showed a relatively high value on the 7th day and increased further with an increase in the biomass to a maximum of 660 mg O$_2$·m$^{-2}$·h$^{-1}$ in gross production and 505 mg O$_2$·m$^{-2}$·h$^{-1}$ in net production on the 24th day. These values were comparable with those of the Tamagawa River (AIZAKI, 1978; 1980).

![Fig. 7. Changes in carbon weight and chlorophyll-α concentration in periphyton community grown in laboratory streams. (○), carbon weight in Stream A; (●), carbon weight in Stream B; (△), chlorophyll-α concentration in Stream A.](image)

![Fig. 8. Changes in photosynthetic and respiration rates of periphyton communities in laboratory streams. (○), gross production rate in Stream A; (●), respiration rate in Stream A; (△), respiration rate in Stream B.](image)
The respiration rate in Stream A increased rapidly until the 11th day and fluctuated slightly thereafter. A maximum of 150 mg O₂/m²·h⁻¹ was observed on the 24th day the same as with the biomass. In Stream B, this rate remained relatively low. A maximum of 105 mg O₂/m²·h⁻¹ was observed on the 11th day, and then it decreased gradually from this maximum to 30 mg O₂/m²·h⁻¹ on the 28th day.

3-2-c. Removal of organic substances

Changes in the concentration of DOC in the water of two laboratory streams which were circulated are shown in Fig. 9, and the removal rates of BOD and DOC are shown in Table 3. In the first four days of the experiment, the removal of dissolved organic carbon from the water was considered to be degraded by the suspended microbes in water, because few attached organisms grew on the bed and walls of the streams. In this period, 8–11% of DOC and 55–62% of BOD were removed from the water in both of the streams. Thereafter, the removal rates were different between Stream A and Stream B, especially for the removal of DOC.

In Stream B, the removal rate of DOC ranged from 30 to 55% during the first day after each renewal of the water. However, it did not increase further in the following days and remained approximately at the same value on the 3rd–5th days after each renewal of the water. In Stream A, the removal rate of DOC showed relatively low values of 9–31% on the first day. Subsequently, DOC increased owing to the release of organic matter from the community into the water, especially in the latter period of the experiment. Therefore, the removal rates ranged from −34 to 34% on the 3rd–5th days after each renewal of water. The most abundant release of DOC was observed during the period from the 24th to the 28th day, when the biomass, photosynthetic and respiration rates maxima were observed. DOC concentration increased by about 34% during this period.

BOD removal, however, showed almost no difference between either stream. The removal rate of BOD ranged from 57 to 97% in Stream A and from 68 to 93% in Stream B on the 3rd–5th days after each renewal of water (Table 3). These data suggested that the rapid

![Fig. 9. Changes in DOC concentration during the circulation of the water in the laboratory streams after each renewal of water. (○), Stream A; (●), Stream B.](image)

Table 3. Removal rates of DOC and BOD in two laboratory streams during 4 days (*3 days, **5 days) after each renewal of water.

<table>
<thead>
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<th>Date of water exchange</th>
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<th>Stream B (dark)</th>
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<tr>
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decrease of DOC during the first day was due to the decomposition of the microbiologically labile organic compounds. On the other hand, since 43–66% of DOC always remained in the water without being decomposed in Stream B, the microbiologically stable organic compounds might occupy about 50% of the total dissolved organic substances in the water in the midstream of the Tamagawa River. Organic substances excreted from the periphyton community seemed to be one of the important sources of such microbiologically stable organic substances, because in Stream A, BOD was removed from water at relatively high rates in spite of the increase in the DOC concentration. The organic substances released from the periphyton community in Stream A were regarded as microbiologically stable compounds.

On the last day of the experiment, glucose and sodium acetate were added to both laboratory streams, and their removal was determined. Figure 10 shows the glucose removal and Fig. 11 indicates the DOC removal in both streams. The concentrations of glucose and DOC decreased rapidly in Stream A as compared with Stream B in the first 7 hours. Thereafter, these concentrations were virtually unchanged in Stream A, whereas they decreased continuously in Stream B. Finally, these concentrations were lower in Stream B than in Stream A at the 24th hour after the addition of glucose and acetate. This result suggested that many algae in a stream promoted the removal activities of the microbiologically labile organic substances in high concentration after loading, but that the photosynthetic products released from the algal community apparently repressed the removal activities of dissolved organic substances in low concentration.

4. Discussion

Seasonal changes in concentrations of DOC and BOD in the midstream of the Tamagawa River are shown in Fig. 12. BOD concentration showed frequently very high values of over 15 mg l⁻¹ in the winter period, and a maximum value of 32 mg l⁻¹. That in the summer period ranged from 5 to 10 mg l⁻¹. DOC concentration was also high in the winter period and ranged from 10 to 15 mg l⁻¹, against 5 to 10 mg l⁻¹ in summer. The ratio of BOD to DOC, which was calculated by conversion of BOD concentration into carbon concentration assuming an RQ ratio of 1.0, was about 40% in the summer period and about to 60–70% in the winter period.

Although the main sources of DOC in the midstream of the Tamagawa River have been considered to be the discharge of city waste, the details on these sources have not been clarified (Ogura et al., 1975). Since in this region of the river abundant periphyton with maximum densities approaching ca. 500 mg chl-a m⁻² had grown on the river bed (Aizaki, 1978), the periphyton community may well become one of the important DOC sources in this river. Many investigators have studied the excretion of organic substances from living algae and confirmed that the excretion from algae is one of the important DOC sources in rivers and lakes.

Fig. 10. Changes in glucose concentration during the circulation of the water in the laboratory streams after the addition of glucose and sodium acetate. (○), Stream A; (●), Stream B.

Fig. 11. Changes in DOC concentration during the circulation of the water in the laboratory streams after the addition of glucose and sodium acetate. (○), Stream A; (●), Stream B.
Removal and Excretion of DOM by Periphyton

(STORCH and SAUNDERS, 1978; KAPLAN and BOTT, 1982).

Dissolved organic matter excreted by algae contained many kinds of organic compounds from low to high molecular weight (HELLEBUSCH, 1965; WATANABE, 1980). KAPLAN and BOTT (1982) reported that low molecular weight compounds excreted by algae are selectively uptaken by coexistent heterotrophs; as a result, high molecular weight compounds remain in the water. In this study using laboratory streams, biologically labile organic substances determined by BOD were rapidly consumed in the first day after each water renewal. Therefore the dissolved organic substances accumulating in the laboratory stream water are regarded as microbiologically stable compounds of probably high molecular weight. In the experiment using artificial substrata, biologically labile organic substances determined by BOD were also consumed rapidly, and biologically stable compounds determined by DOC were excreted into water. OGURA et al. (1975) measured the component of dissolved organic substances in the water collected at the midstream of the Tamagawa River and reported that high molecular weight compounds of over 10,000 molecular weight accounted for about 60–70% of total organic substances. This value was almost the same as the residual DOC percentage after the 3–5 day incubation from each renewal of water in laboratory Stream B. These results suggest that biologically stable organic substances made up a large portion of the total dissolved organic substances in the water of the Tamagawa River, and that excreted organic matter from the periphyton community become one of the important sources of these substances.

In this study, removal and excretion activities of organic substances were investigated in particular in relation to biofilm development of the periphyton community. In a eutrophic stream, the biomass of periphyton always fluctuated owing to growth and loss, and homeostatic conditions rarely exist (AIZAKI, 1978). Therefore activities of the periphyton community in eutrophic streams must be investigated in relation to biofilm development. However, there are few studies conducted with this purpose (AIZAKI, 1980).

From the results of the present study, a high removal rate of dissolved organic substances by the periphyton community is expected in the early stage of biofilm development, while a high excretion rate is observed in the mature stage. The maximum excretion rate observed in this study was 580 mg C·m⁻²·d⁻¹ in the light incubation condition of the artificial substrata experiment. An excretion rate of 400 mg C·m⁻²·d⁻¹ was also observed in the dark incubation condition. The organic substances excreted in the dark condition are considered to derive from decomposition of accumulated particulate organic substances on the inside of the biofilm. The different amount of 180 mg C·m⁻²·d⁻¹ between light and dark incubation condition probably originated from photosynthetic product by living sessile algae. This value changed also with the progress of biofilm development of the periphyton community, and a maximum of 280 mg C·m⁻²·d⁻¹ was observed just after the scour of a large portion of periphyton (see Fig. 6). These excretion rates observed in the present study are somewhat lower than that reported by KAPLAN and BOTT (1982) in a piedmont stream, but relatively higher than reported by WATANABE (1980) in the small eutrophic Lake Nakanuma.

Acknowledgement

The author is deeply indebted to Drs. Y. TEZUKA, S. TAKII and H. HAYASHI, and to the other scientists of the Laboratory of Microbiological Chemistry, Tokyo Metropolitan University, for their valuable suggestions and stimulating discussions related to this work.
摘　要
河川付着微生物群集による溶存有機物の除去および溶出速度を、富栄養河川である多摩川流域に浸漬した人工基盤および室内に作った人工河川を用いて測定をした。人工河川には多摩川流域の河川水を循環させ、3～5日間で水の交換を行い、その結果以下のことことが判明した。
1. 溶存有機物の除去および溶出速度は、付着微生物群集の膜形成の発達段階によって大きな違いを示した。すなわち、溶存有機物の高い除去活性は膜の発達初期に観察され、厚い膜が形成された後には、逆に付着微生物群集からの溶存有機物の溶出が観測された。
2. 付着微生物群集により除去される溶存有機物は、BODで測定可能なことから微生物により分解されやすい有機物と考えられ、逆に付着微生物膜より溶出してくれる有機物は微生物の分解をうけにくい化合物であると推測された。
3. 厚い付着微生物膜の形成後に付着微生物膜から溶出する有機物の量を測定すると、付着基盤の光合成産物を含む、付着膜内部における蓄積した固形有機物の分解産物が重要であることがわかった。

References


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Received: 7 December 1984
Accepted: 13 February 1985