

The Accumulation of Dissolved Organic Substances in Closed Recirculation Culture Systems

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(Received September 1985; revised version received 3 July 1986; accepted September 1987)

ABSTRACT

Dissolved organic carbon in culture water and the light absorption due to the dissolved organic substances in a closed recirculation aquarium increase with the cumulative amount of food, especially in a linear correlation within the relatively low accumulation. The absorption was extremely high at the far-ultraviolet region and the spectrum exhibited no peak. The salt concentration of the culture water showed no effect on the light absorption. Aquarium water used for long cultivation was highly fluorescent. Ultrafiltration of the culture water revealed that more than half of the total dissolved organic substances had a molecular weight of the order of 10^4 .

The elements C, N and P in the organic substances accumulated in a culture water accounted for only 3.2% of the amount added to the aquarium as food even in the most extreme case. Four experimental cultures with different intervals of washing filter sands revealed that the growth of fish and the accumulation level of nitrite were arranged in the order of frequency of washing, while the organic substances were in the reverse order. Accumulation of organic substances may be a cause which leads to the suppression of growth of fish cultured in closed recirculation systems.

INTRODUCTION

The changes in the inorganic composition of aquarium water in closed recirculation systems have been studied by several investigators (Saeki, 1958, 1963; Kawai *et al.*, 1964; Hirayama, 1970, 1974; Tiews, 1981). They found that nitrate and phosphate accumulate and that this accumulation is associated with the acidification of the water. Although it is well

known that the water in closed recirculation systems becomes yellowish-brown after long use (Otte *et al.*, 1977; Rosenthal *et al.*, 1978), the accumulation process and properties of the organic substances, including the component causing water discoloration, have still to be studied. The review of Ogura (1980) on ultraviolet absorbance as an index of organic pollution indicated that the content of organic carbon in natural waters can usually be monitored by measuring the UV absorption of the water (Foster and Morris, 1971; Foster and Foster, 1977). There have been some attempts to use the UV absorption of pond water as an index of the organic pollution in intensive fish culture (Doi and Kabasawa, 1978; Toi and Satomi, 1978; Onari and Yamagata, 1980; Wickins, 1985). These studies suggested a close correlation between content of dissolved organic carbon and UV absorption and the presence of proteinaceous materials, based on the absorption spectra patterns and gel filtration of the culture water.

In the present study, the culture water in experimental aquariums operated as closed recirculation systems was investigated spectrophotometrically and fluorimetrically for the accumulation of organic substances. Aquarium water used for a long period of cultivation was filtered through several types of ultrafiltration membrane to separate the organic substances into fractions with different molecular sizes. Also, we examined the final fate of the elements C, N and P in formulated food introduced in aquariums which had different intervals of sand filter washing.

MATERIALS AND METHODS

The experimental aquariums used in this study were the same type as in the experiment of Hirayama (1970) (Fig. 1). The tanks, which had a total water volume of about 50 litres, were made of polyvinyl chloride plates. The filter bed, which had a volume of about 9 litres, was packed with river beach sand (particle diameter 2–5 mm), and the flow of water was kept at more than 3 litres min⁻¹ by means of an airlift system. Water temperature was maintained at 20–25°C. Acidification of the culture water was neutralized by adding sodium bicarbonate at appropriate intervals.

Culture 1

One experimental culture was conducted to study the accumulation process and properties of organic substances in the culture water. Each

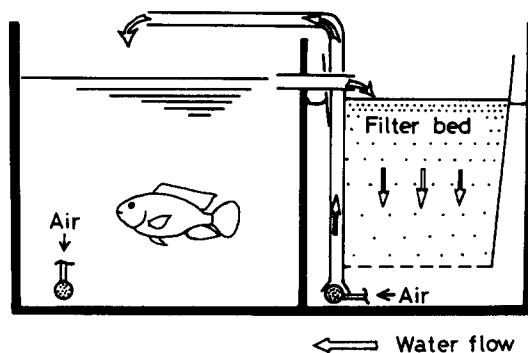


Fig. 1. Diagram of experimental aquarium.

group of five individuals of tilapia, *Sarotherodon niloticus* (average body weight about 65 g) was cultured in a seawater aquarium or in a freshwater aquarium, provided with previously well conditioned filter sands. In the seawater aquarium at the start of culturing, diluted seawater of half strength was used. However, 60 days after culture with tilapia, the salt concentration was gradually increased to that of normal seawater, through evaporation and supplementation of seawater after sampling. The average weight of pelleted commercial food for tilapia culture supplied during a culture period of 60 days was 1.4 g day^{-1} in the seawater aquarium, and for 102 days 1.5 g day^{-1} in the freshwater aquarium.

Culture 2

Another experiment was conducted to learn the effect of washing the filter sands on the accumulation of organic substances and the final fate of C, N and P introduced into aquariums as components of the food. We prepared four aquariums with the filter bed in which two kinds of unconditioned sand and previously well conditioned sand had been packed in equal volume. After 10 days of circulation of tap water without fish, we started the culture with fresh tap water and introduced five individuals of *Sarotherodon niloticus* (average body weight about 60.6 g) into each tank. For 16 days after the start of culture, a commercial diet was supplied every day into every culture tank at 2 or 3 g day^{-1} ; for 26 days after that, at 6 g day^{-1} ; then for 8 days, at 8 g day^{-1} ; for the next 57 days, at 18 g day^{-1} . The feeding rate in culture 2 was much greater than in culture 1. The filter bed in three of four aquariums was washed at intervals of 4, 8 and 16 days, respectively. One aquarium was kept without washing as control. To wash the filter bed, a half volume of the filter

sands was taken out of an aquarium, put into a container with 10 litres of tap water and then stirred vigorously. After washing, the sands were screened with a 1.5 mm mesh and put back into the filter bed. At the end of culture, all filter sands and solids on the bottom and walls of the aquarium were thoroughly washed in the tap water, in almost the same manner as described above. A part of the water used for washing was kept at -20°C for analysis.

Analysis

A water sample taken at intervals of several days was filtered through a Whatman GF/C filter previously burned at 450°C for 2 h.

Analytical methods for the filtered culture water were as follows. Phosphate, the colorimetric method of Strickland and Parsons (1972); nitrite, the colorimetric method using sulphanilamide and *N*-(1-naphthyl)-ethylenedihydrochloride (Bendschneider and Robinson, 1952); nitrate, the reduction method to nitrite by passage through the copperized-cadmium column of Wood *et al.* (1967); dissolved organic carbon (DOC), the wet oxidation method of Menzel and Vaccaro (1964) with a Model 524 C Analyzer of Jasco International Co., Ltd; absorption spectra, with a Hitachi 102 spectrophotometer in a 1 cm quartz cell using distilled water as a blank; fluorescence, with a Hitachi Fluorescence Spectrophotometer Model 512 in a 1 cm quartz cell.

Aquarium water without filtration and the water used for washing were analyzed as follows: TOC, wet oxidation method the same as that for DOC; TOP, the colorimetric method after decomposition by autoclaving with potassium peroxodisulfate; TON, the Kjeldahl digestion method on the sample dried by evaporation.

Components of carbon and nitrogen in food and the fish body were analyzed using a CHN coder of Yanagimoto (MT-500). The phosphorus component was analyzed with almost the same method as that for the wash water.

The inorganic substances present in natural waters seldom absorb light of wavelengths longer than 240 nm, unless nitrate and bromide are present in high concentration (Ogura and Hanya, 1967). Of typical changes in composition of inorganic components in the circulating aquarium water, the accumulation of nitrite and nitrate may be considered as the only inorganic substances that influence the light absorption of the water, because the effects of ammonia and phosphate are negligible. In order to determine the light absorption due to the organic substances accumulated in the water, the portions absorbed by nitrite

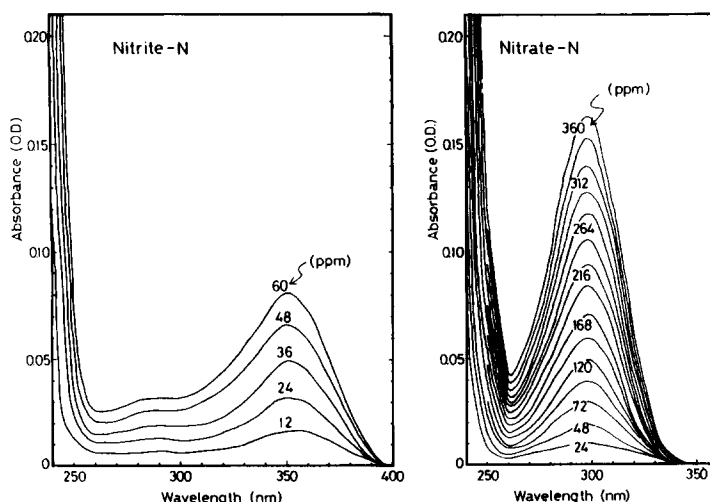


Fig. 2. Absorption spectra of nitrite and nitrate at various concentrations.

and nitrate were subtracted from the total apparent absorption of the aquarium water. The absorption spectra of nitrite and nitrate dissolved at various concentrations in distilled water are shown in Fig. 2.

Ultrafiltration

The water samples of freshwater and seawater in two aquaria were filtered through a Milipore filter (GS; pore size, $0.22 \mu\text{m}$), before ultrafiltration. Both Milipore filter and ultrafiltration membranes were thoroughly washed with organic free water before use. One hundred millilitres of the water sample was placed in an ultrafiltration cell (Amicon Model 202; membrane diameter, 64 mm) and filtered through Diaflo UM-05 or UM-10 in a nitrogen atmosphere at 4 kg cm^{-2} or through Diaflo XM-50 or XM-100A at 2 kg cm^{-2} . Ultrafiltration was stopped when 50 ml of the filtrate had been obtained. Then, the retention (R) of DOC and of light absorbing materials at various wavelengths was calculated with the following equation:

$$R = (1 - a/A) \times 100$$

where A and a represent either the concentrations of DOC or the optical densities due to the organic substances in the sample water before ultrafiltration and in the ultrafiltrate fraction, respectively. Retention of fluorescent materials was determined in the same manner with the water sampled from the freshwater aquarium.

RESULTS

Culture 1

Accumulation of organic substances

Figure 3 shows the absorption spectra of the dissolved organic substances that accumulated in the water of the two aquaria. Both systems showed substantially the same spectral pattern, which exhibits neither a maximum nor a minimum and decreases with increase in wavelength. This result suggests that the spectral pattern of absorption does not depend on the salt concentration of the culture water.

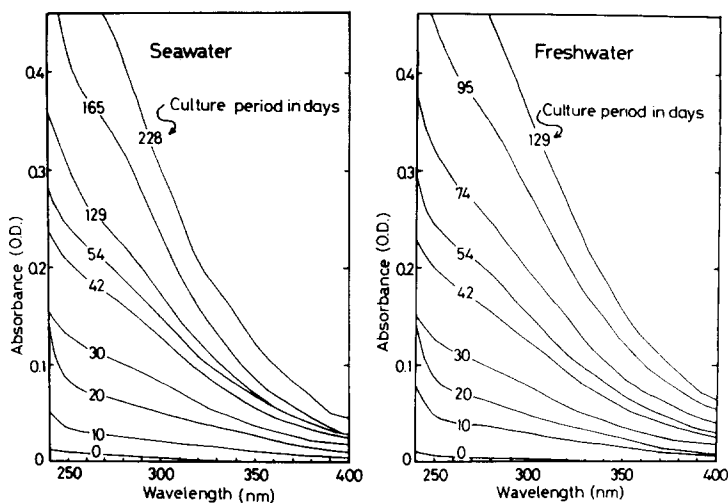


Fig. 3. Absorption spectra of the organic substances that accumulated in the seawater and freshwater aquaria.

In Fig. 4 are shown the absorbance of the accumulated organic substances at 260 nm and the dissolved organic carbon (DOC) as functions of the cumulative amount of food supplied in either the seawater or freshwater aquarium, as well as the length of the corresponding culture period.

Ultrafiltration

Freshwater and seawater samples were taken at the 72nd day and the 42nd day of culturing (the former, 0.289 in optical density at 260 nm in light absorption and 18.5 mg C litre⁻¹ in DOC; the latter, 0.189 in optical density and 11 mg C litre⁻¹ in DOC). The retention of DOC and light

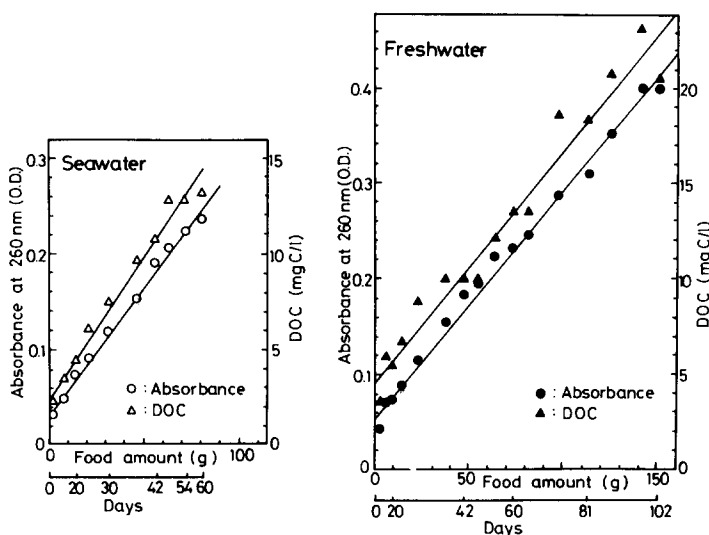


Fig. 4. The relationships of the absorbance at 260 nm and the DOC (mg C litre^{-1}) to the cumulative amount of food supplied in the seawater and freshwater aquaria.

absorbing substances at various wavelengths present in culture water was measured on several types of ultrafiltration membrane. Nominal molecular weights separated by the membranes of UM-05, UM-10, XM-50 and XM-100A, are 500 , 1×10^4 , 5×10^4 and 1×10^5 , respectively. The retention of DOC in seawater and freshwater was 68.6 and 82.5% for UM-10, and 2.3 and 22.5% for XM-100A, respectively (Fig. 5). The retention of light absorbing substances increased slightly at the longer wavelengths. The average retention of substances in seawater and freshwater, in the range of the wavelengths examined, was 76% and 78% for UM-10 and 5% and 10% for XM-100A, respectively. The retention of fluorescent substances in the culture water of the freshwater aquarium on the 138th day of culturing was measured by the fluorescence at the peak wavelength (353 nm) of the excitation spectrum and for the full range of the emission wavelength. It was 69.4% for UM-05 and 64.7% for UM-10. None of the DOC, light absorbing and fluorescent substances showed large differences between the retention values for UM-05 and for UM-10. As judged from the properties of the ultrafiltration membranes, almost all of the organic substances participate in the absorption and fluorescence of the water, and a component of the order of 10^4 in molecular weight accounts for a large proportion of the dissolved organic substances while a component from 500 to 1×10^4 is present as a very small amount.

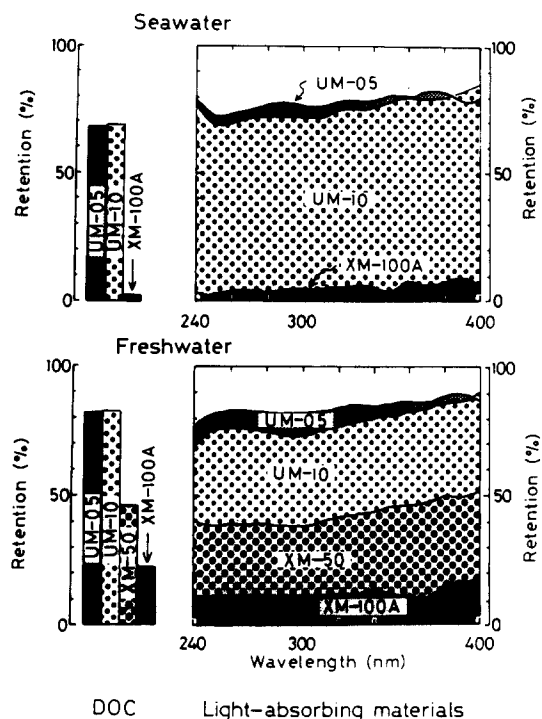


Fig. 5. The retention of DOC and light absorbing substances at various wavelengths by ultrafiltration of the culture water with several types of membrane.

Culture 2

Accumulation of the organic substances and of nitrate

The rates of increase of the three parameters of absorption, DOC and fluorescence intensity, concerned with the accumulation of organic substances, were much faster in Culture 2 than those in Culture 1, because of greater rates of feeding. The relationships of the absorbance, the DOC and fluorescence intensity (excitation, 350 nm; emission, 430 nm) to the cumulative amount of food in the control aquarium with no washing of the filter bed are shown in Fig. 6. All three parameters increased with the cumulative amount of food, as long as the amount of food remained under 450 g. Those facts, and the same relationships obtained in Culture 1, imply a close correlation between the absorption due to organic substances, DOC content accumulated and the fluorescence intensity in the aquarium water. In the other aquaria, with the filter washed, those parameters concerned with the organic substances gradually increased in the culture period, though accumulation rates were lower than those in the

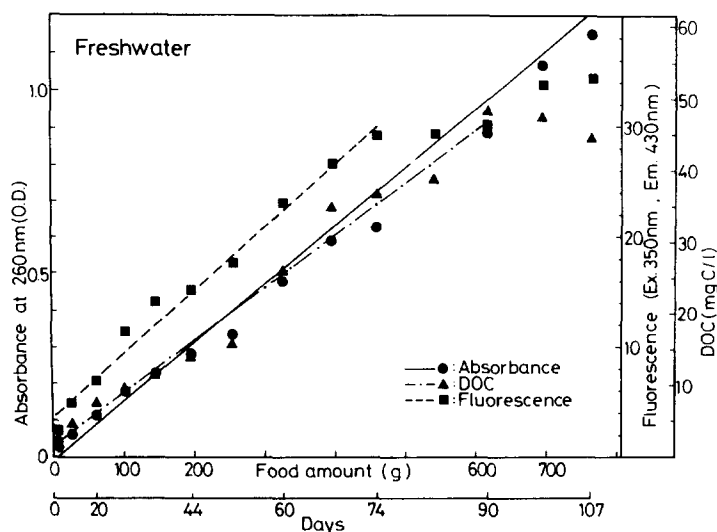


Fig. 6. Relationships of the absorbance at 260 nm, the DOC (mg C litre^{-1}) and the fluorescence intensity (excitation, 350 nm; emission, 430 nm) to the cumulative amount of food supplied in the control aquarium with the filter but without washing.

control aquarium. In the waters of four aquaria with different intervals of filter bed washing, relationships of the DOC value and the fluorescence intensity to amount of light absorption (Fig. 7) indicated that the organic substances accumulated in the culture water lost the proportional relations among three parameters after the accumulation exceeded a certain level. Nitrate in the control aquarium decreased after vigorously stirring the filter sands inside the filter bed on the 52nd day of culture (Fig. 8). On the 74th day, the nitrate reached the lowest value and then gradually increased again, in contrast with the accumulation process in the other aquaria. Three parameters of organic substances and nitrate concentration on the final day of culture and the culture records of fish in the four aquaria are summarized in Table 1. In four aquaria, gain in body weight, food efficiency and concentration of nitrate can be arranged in the order of frequency of washing the filter, in contrast to the reverse order of three parameters concerning the accumulated organic substances.

Final fate of the elements N, C and P

Each of the elements C, N and P constituting the food introduced into the aquarium during the culture period was transferred into the components of fish body, of dissolved matter in the culture water and of solid matter accumulated in an aquarium. Total weight of food introduced into an aquarium was 764 g. C, N and P in the food were analyzed as 41.0,

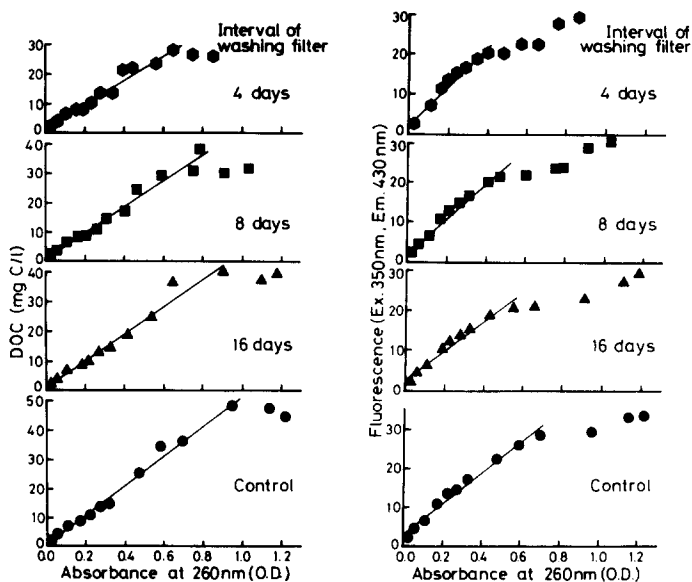


Fig. 7. Relationships of the DOC (mg C litre^{-1}) and the fluorescence intensity to the absorbance at 260 nm in four aquaria in which the filters were washed at different intervals.

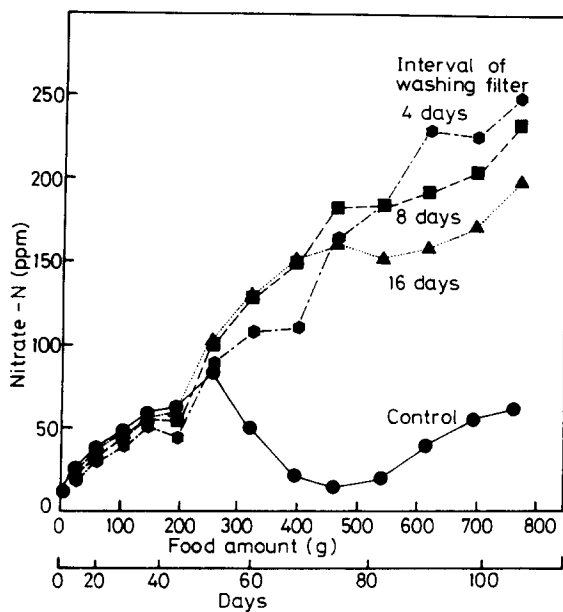


Fig. 8. Accumulation process of nitrate in four aquaria.

TABLE 1
Three Parameters Concerning Organic Substances, Nitrate Accumulated and Culture Records on the Final Day of Culturing in Four Aquaria

<i>Interval of washing filter</i>	<i>Absorbance at 260 nm (OD)</i>	<i>DOC (mg C litre⁻¹)</i>	<i>Fluorescence^a</i>	<i>Nitrate-N (ppm)</i>	<i>Gain of body weight</i>	<i>Food efficiency (%)</i>
Control ^b	1.224	44.6	34.3	62	309	40
16 days	1.182	39.4	30.0	198	449	59
8 days	1.033	31.4	30.9	232	430	56
4 days	0.858	26.2	29.5	248	498	64

^aExcitation, 350 nm; emission, 430 nm.

^bNo washing.

5.1 and 1.3% in weight, respectively. Therefore, the weights of C, N and P introduced into an aquarium were estimated as 313.2, 39.0 and 9.9 g, respectively. C, N and P in the tilapia's body account for 13.4, 2.8 and 0.7% in weight, respectively. Therefore, from the weight gain of fish (Table 1), C, N and P transferred into the fish body can be calculated and the percentage in various components into which each element was transferred is shown in Fig. 9. A percentage of the portion which was obtained by subtracting the sum of the contents in the gain of fish body and in the dissolved and solid matter from the content in the food added, is shown as the residue, in Fig. 9.

Dissolved organic carbon accounted for less than 1% in every aquarium. Dissolved inorganic carbon was not analyzed. However, as shown in Fig. 9, the residue accounts for about 70% in every aquarium. Most of the residue may be inorganic carbon which is dissolved in the water and escaped into the air.

Dissolved organic nitrogen accounts for only about 1% at almost the same level as DOC. Most of the dissolved inorganic nitrogen is nitrate and other forms of inorganic nitrogen were negligible. In the aquarium with less frequent intervals of filter washing, the residue accounted for the greater proportion. A large part of the residue may be occupied by the portion that escaped into the air through denitrification.

Compared with other elements, dissolved organic phosphorus occupied a relatively large part, but only accounts for 3.2% even in the most extreme case. The residue accounts for 14% in the most extreme case. The formation of the compounds of phosphate and Ca or Mg which attached firmly on the wall may form a large part of the residue.

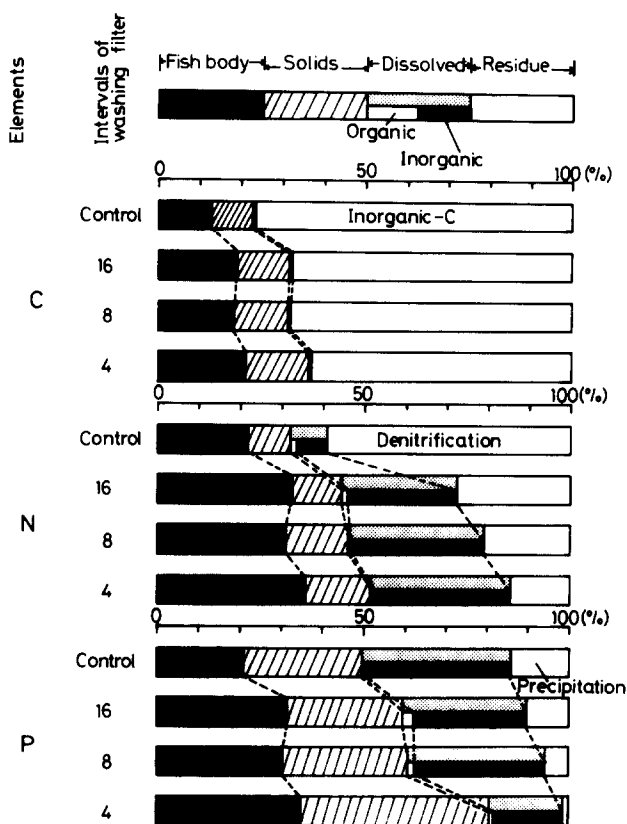


Fig. 9. Percentage of each element (C, N and P) transferred into various components at the final day of culturing, against the amount of the element as the component in the food supplied into the aquarium during cultivation.

DISCUSSION AND CONCLUSION

Aquarium water used in a closed culture system for a long time exhibited high absorption at short wavelengths and high fluorescence due to the accumulation of organic substances. The absorption due to the accumulation of organic substances was extremely high in the far-ultraviolet region and the spectrum exhibited no peak over the wide range of wavelengths tested. The salt concentration of the water showed no effect on the light absorption.

Ultrafiltration of the culture water revealed that more than a half of the total organic substances existed as organic carbon with a molecular weight of the order of 10^4 . The relative amount of each fraction of different molecular weights, obtained by ultrafiltration, was at almost the same

level, regardless of the parameter used for estimation of the organic substances. This fact indicates that almost all the organic substances participated in producing the absorption and fluorescence.

Kalle (1966) showed that Gelbstoff or humic materials (the so-called yellow substance) exhibit an absorbance that decreased with an increase in wavelength with no peak and Skopintsev (1959) suggested that the so-called water humus, a stable form of organic matter, was re-synthesized from the breakdown products of dead organisms, as Ogura (1977) summarized in his review. The fractionation of dissolved organic matter in coastal seawater by ultrafiltration has shown that the fraction with a molecular weight of an order of 10^4 usually accounts for the largest proportion of the total DOC (Ogura, 1974). These facts imply a close connection between two kinds of dissolved organic substances in natural and culture waters.

Three parameters representing the chemical properties of the organic substances which accumulated in culture water increased in proportion to the cumulative amount of food supplied. However, the proportional relations of each parameter of DOC and fluorescence intensity to the cumulative amount of food could be established only within the relatively low accumulation. Light absorption was the only parameter which increased linearly after the other parameters lost the linear increase (Fig. 6). Also, proportional relations among three parameters could be established only within the relatively low accumulation (Fig. 7). These facts indicate that the properties of the organic substances tend to change after a large accumulation.

In closed recirculation culture systems, specific dissolved organic substances accumulate. However, the portion of the elements (C, N and P) constituting the substances accounts for only 3.2% of the amount of the element added as the component of the supplied food into an aquarium, even in the largest case of phosphorus (Fig. 9).

The growth of fish and the food efficiency were greater in the aquarium where the filter bed was washed more frequently. The accumulation level of nitrate in the four aquaria was arranged in the order of the frequency of washing, while the organic substances were in the reverse order (Table 1). The reports of nitrate toxicity against freshwater fishes by several researchers (Colt and Tchobanoglous, 1976; Poxton and Allouse, 1982) and the review on the water quality requirement for intensive aquaculture by Wickins (1981) suggest that nitrate has no harmful effect on tilapia culture at the accumulation level which occurred under the conditions of the present cultures. The accumulation of organic substances might be one of the causes which lead to the suppression of growth of fish cultured in closed recirculation systems.

ACKNOWLEDGMENTS

We wish to express our sincere thanks to Dr J. W. Atz, American Museum of Natural History, and Dr John Mark Dean, Professor of the University of South Carolina, for their kind reading of our manuscript. We are also very grateful to Dr T. Muramatsu, Faculty of Fisheries, Nagasaki University, and to Dr N. Ogura, Faculty of Agriculture, Tokyo University of Agriculture and Technology, for giving valuable advice.

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