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Microbial Immobilization of Phosphorus as a Potential Means of Reducing Phosphorus Pollution of Water

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Aquatic weed control is a major problem for authorities responsible for water management. It is the largest item of annual expenditure for Internal Drainage Boards in the Fens and similar areas of intensive agriculture. A recent survey by the Weed Research Organization indicated that, in the last decade, the occurrence and relative importance of submerged weeds and filamentous algae (blanket weed, Cott) has increased more than that of any other weed group. A factor contributing to this is an increase in the plant nutrient status of the water, that is eutrophication (Greene et al 1975). Phosphorus is the principal cause of excessive algal growth in most waters and algal blooms rarely occur at phosphorus levels below 10 $\mu g\ l^{-1}$ (Pitcairn and Hawkes 1973).

Excessive algal growth can result in blooms uniformly distributed through the water column or in formation of dense scums or surface mats. These may produce disagreeable odours or tastes in water, affect dissolved oxygen concentration, impede water flow and drainage or seriously hinder recreational use of the water body. In rare instances they may produce toxins in sufficient concentration to kill fish, water fowl or livestock. At present these problems are controlled by the physical removal of the algae, treatment with herbicides such as terbutryne or diquat, or manipulation of the environment, for example by planting trees to shade the water. All these methods are limited by factors such as cost, short duration of effect and environmental side-effects and also treat only the symptoms rather than the cause of the problem. One type of environmental manipulation that has received attention is the removal of phosphorus from the water to reduce algal growth by nutrient limitation. Thus, ferric alum has been used to adsorb dissolved phosphorus and prevent growth of toxin-producing blue-green algae (May 1981). Investigations (Street 1979) into the stimulation of invertebrate productivity by the addition of straw to the water surface of a gravel pit have also shown that this treatment can substantially reduce algal growth. (Street M. pers comm. 1983). In addition the claim by a lake construction consultant (Hatt W.J. pers. comm. 1980) that hay introduced accidentally into the feeder stream of a small lake, and its subsequent deliberate introduction to other lakes, had also resulted in marked reduction of algal growth suggested an

alternative approach to phosphorus removal from water.

Microorganisms responsible for decomposition of organic detritus in water assimilate large amounts of the essential nutrients (Fenchel and Harrison 1976). Fenchel and Jorgensen (1977) showed that the increase in microbial biomass which occurs during detritus decomposition is quantitatively associated with the depletion of dissolved phosphorus in the surrounding water. The phosphorus accumulated in the microbial biomass is not quickly returned to the water (Johannes 1964) and thus the microorganisms are serious competitors with algae for this essential nutrient (Lund 1965). This paper describes laboratory experiments that investigate phosphorus immobilization by the microflora developing on submerged straw, and the effects of such factors as straw mass, temperature, dissolved phosphorus concentration and water flow rate. Fresh straw is used in the experiments as its phosphorus content is lower than that of hay.

MATERIALS AND METHODS

Five experiments to investigate phosphorus immobilization by the microflora developing on barley straw were done using several laboratory systems.

Barley straw obtained from the farm at the Weed Research Organization (WRO) was cut into 1 to 20 cm lengths, according to the experiment. The cut straw was flushed with flowing tap water for at least 72 h to remove soluble phosphorus and then placed in spring water for at least 48 h to allow colonization by microorganisms before use. All experiments used water (1.1 - 7.6 $\mu g\ P\ l^{-1}$; 5.3-20.2 mg NO_3^--N l^{-1}; hardness 385-404 mg $CaCO_3\ l^{-1}$; alkalinity 199-215 mg $CaCO_3\ l^{-1}$; conductivity at 20°C 649-718 $\mu S\ cm^{-1}$; pH 7.6 - 8.4) freshly collected from a spring at WRO. In the static systems, water removed for phosphate analysis was replaced each day with spring water. Phosphorus adsorption onto the equipment used in the experiments was prevented by an overnight soaking of glassware in 50% H_2SO_4 and of polyethylene bottles in 10% H_2SO_4. All tubing (Tygon and polyvinyl) was iodized (Standing Committee of Analysts 1980). Analar anhydrous potassium dihydrogen orthophosphate (0.4394 g) dissolved in distilled water (1 l.) was used as stock solution (1 ml=100 μg P) in all experiments. The method of phosphorus analysis used was based on that of Murphy and Riley (Standing Committee of Analysts 1980) and had a limit of detection of 0.5 - 0.7 $\mu g\ P\ l^{-1}$. Where necessary samples were filtered through GF/A filters (Whatman, England) to remove any particles that might have affected spectrophotometric reading.

The effect of straw biomass on the rate of phosphorus immobilization was determined using four fermenter vessels (500 series, LH Fermentation, Stoke Poges, Bucks, UK) each containing 2000 ml spring water. Open ports on the top of the equipment allowed contact between water and atmosphere. The vessel contents were maintained at 15±1°C and stirred at 200 rpm. After 24 h, 0, 5.0, 8.75 and 13.0 g $\overline{(}$d.m.) of straw, cut into 1.0 - 1.5 cm lengths excluding nodes and leaf material, was placed in the vessels. After 50 h, and thereafter every 24 h, 2 ml of stock

KH_2PO_4 solution were added to each vessel. This produced a concentration of approximately 100 µg phosphorus l^{-1} at the start of the experiment, and subsequently each day raised the residual phosphorus concentration by 100 µg P l^{-1}. Water samples for phosphorus analysis were taken immediately after addition and after 0.5, 1.5, 3.0 and 24 h.

The effect of temperature on the rate of phosphorus immobilization was determined using four 30 l fibreglass tanks lined with polyethylene sheet and containing 25 l of spring water. The observation referred to above (Street M. pers. comm. 1983) showed that an application of 1 kg straw/m^2 to the water resulted in a marked reduction in algal growth. Thus, two tanks (water surface area 640 cm^2) each received 64 g (d.m.) of straw cut into 4-6 cm lengths. Two control tanks with no straw were also set up. A treated and a control tank were incubated at $4^{\circ}C \pm 2$ and a similar pair were maintained at $19^{\circ}C \pm 2$. At the start of the experiment, and subsequently each day, 25 ml of stock KH_2PO_4 solution were added, with gentle stirring to the tanks. This produced a concentration of approximately 100 µg phosphorus l^{-1} at the start of the experiment and subsequently each day raised the residual phosphorus concentration by 100 µg l^{-1}. Samples for phosphorus analysis were taken as in the first experiment.

The effect of dissolved phosphorus concentration on the rate of immobilization was determined in tanks kept in a glasshouse at ambient temperature (min. 14.2-17.8°C, max. 21.3-28.7°C. Three tanks containing 25 l spring water each received 64 g (d.m.) of straw cut into 4-6 cm lengths and a fourth received no straw (control). Stock solution of KH_2PO_4 (12.5, 25 and 50 ml) was added to the tanks, giving concentrations of 50, 100 and 200 µg P l^{-1} respectively at the start of the experiment. Subsequently, similar additions were made daily. Each day 25 ml of stock KH_2PO_4 solution were added to the control tank. Experimental details are as in the first and second experiments.

The effect of water flow and phosphorus concentration on the rate of immobilization was determined using glass columns containing straw. This experiment was done while observations were being made on phosphorus concentrations in Gludy Lake (Brecon, S. Wales) and its feeder streams. Thus an attempt was made to simulate in the laboratory the water flow rate and phosphorus concentration of one of the feeder streams. (Av. values:- flow 57600 l h^{-1}, concentration 190 µg Pl^{-1}). Previous laboratory experiments indicated that an average phosphorus immobilization rate by the straw microflora was 3 µg P h^{-1} g^{-1} (d.m.) straw. Thus at a convenient laboratory flow rate of 72.6 ml h^{-1} it was calculated that at a concentration of 190 µg P l^{-1}, 4.6 g (d.m.) straw were theoretically required to immobilize completely the phosphorus in solution. Experience has shown that the walls of containers of phosphorus solutions, particularly solutions in water from natural sources, are rapidly colonized by microorganisms to such an extent that marked reductions in phosphorus concentration occur. Thus to minimize this a sterile concentrated solution of KH_2PO_4 (2.34 mg P ml^{-1}) and spring water were pumped from separate reservoirs into a mixing chamber stirred at 200 rpm and maintained at $3 \pm 1^{\circ}C$. The

resulting dilute solution (190 μg P ml^{-1}) passed by gravity at a rate of 72.6 ml h^{-1} to the top of a straw-filled glass column. The column (200 x 28 mm i.d.; approx. working volume 115 ml) contained 4.6 g (d.m.) of straw cut into 1.0 to 1.5 cm lengths, excluding nodes and leaf material. A second column was exposed to a solution containing 950 μg P l^{-1}. A control (no straw) was not set up as previous work had shown that the amount of phosphorus removed by the apparatus used in this type of experiment was negligible. Samples for phosphorus analysis were taken both from the top and the bottom of the columns.

To determine the effect of water flow rate on phosphorus immobilization, 4.8 g (d.m.) of straw cut into 15 - 20 cm lengths was placed in a 100 ml burette connected to a reservoir via a constant head device. An empty burette was connected similarly as a control. Spring water containing added phosphorus equivalent to 100 μg P l^{-1} was run through the burettes at flow rates of 15, 30, 60 and 120 ml min^{-1}. Flow was from bottom to top of the burette to minimize channelling and compaction of the straw. Samples for phosphorus analysis were taken initially and then at 30 minute intervals over 3 h after which the flow was stopped and the burettes left full of phosphorus solution for 21 h. A sample was then taken for phosphorus analysis and the burettes flushed with fresh phosphorus solution (100 μg P l^{-1}) and flow recommenced for a further 3 hours. The columns were covered with silver foil to reduce algal growth and sited in a glasshouse at ambient temperature (min. 16.4-19.6°C, max. 26.6-33.4°C).

RESULTS AND DISCUSSION

For reasons of space economy only selected results which represent the changing immobilization pattern throughout the first experiment (29 days) are shown in Fig.1. Increasing the straw mass always resulted in an increase in the rate of phosphorus immobilization. Immobilization was rapid for the first few hours following phosphorus additions and then gradually slowed. All three levels of straw had removed almost all of the added phosphorus from the system after 24 h. This effect continued until about day 21. Subsequently, especially at the lowest straw mass, only part of the phosphorus added daily was immobilized and thus there was an overall gradual increase in phosphorus concentration. The daily accumulation of phosphorus in the control was almost constant until about day 20 after which it progressively increased. At the end of the experiment the control phosphorus concentration was 1.5 mg P l^{-1} compared with the theoretically possible value of 2.1 mg P l^{-1}.

Immobilization was, as expected, greatest at the higher temperature (Table 1) and almost all the phosphorus added to the system was removed. Only part of the phosphorus added daily was immobilized at 4°C and, thus, the phosphorus concentration in the tank gradually increased.

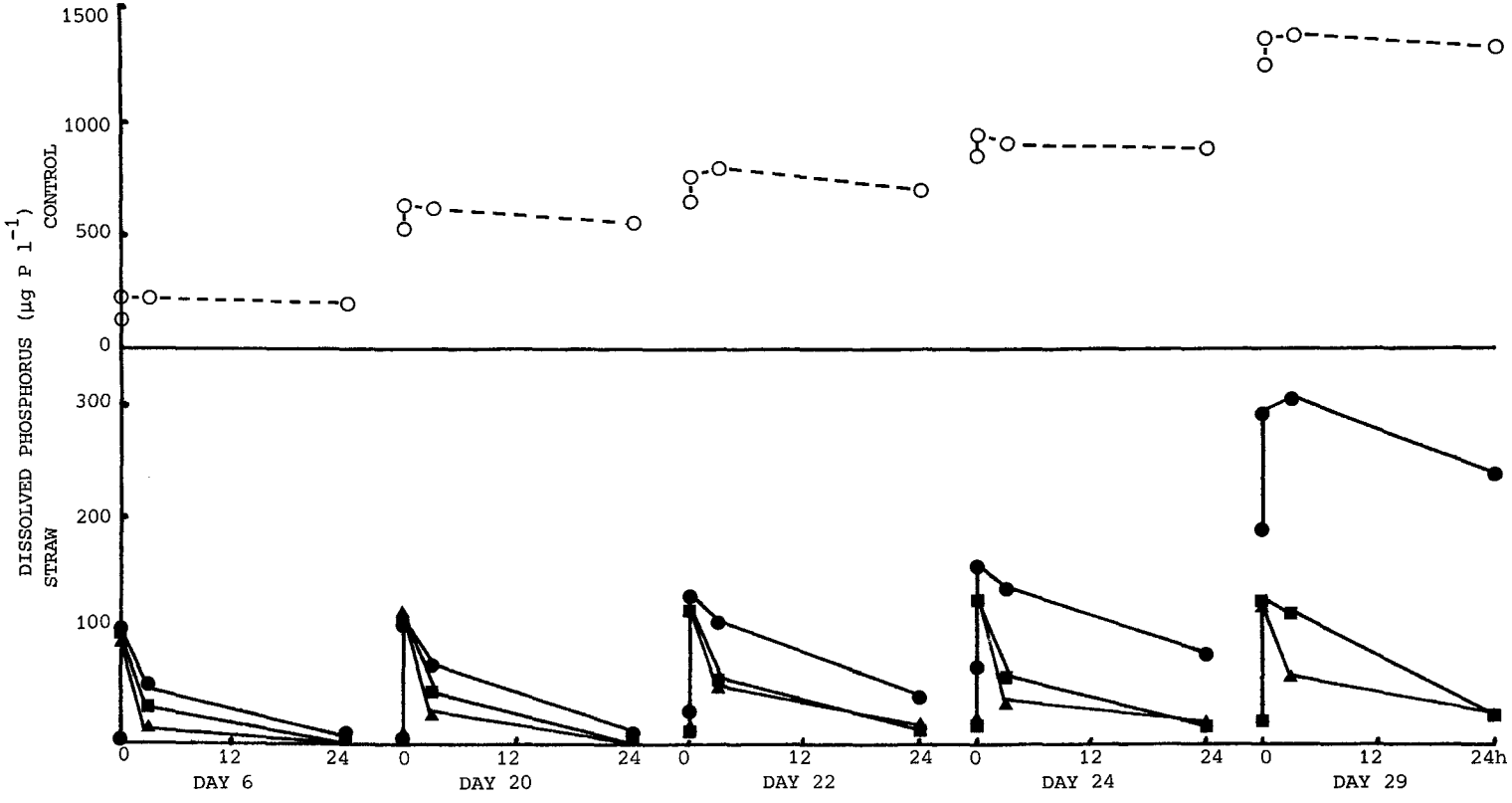

Fig.1. The effect of straw mass on phosphorus immobilization.
○ - - - - ○ control, ● —— ● 5.0 g (d.m.) straw, ■ —— ■ 8.75 g (d.m.) straw, ▲ —— ▲ 13.0 g (d.m.) straw.

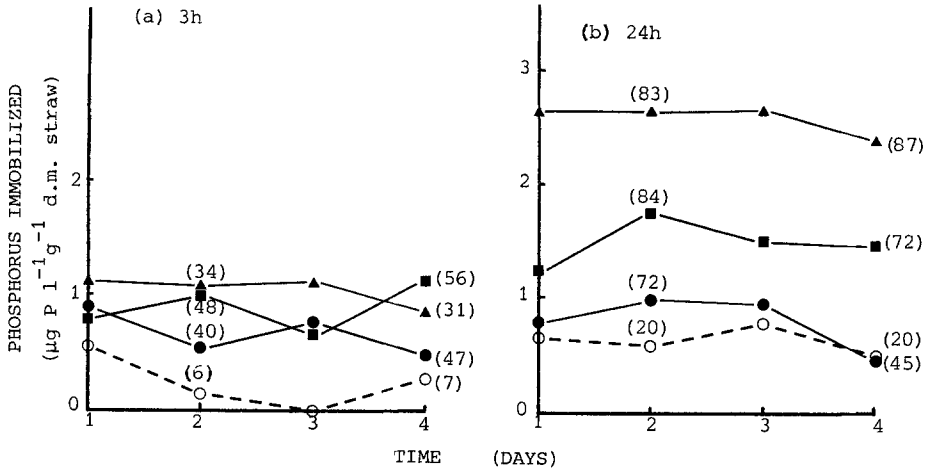

Fig.2. The effect of phosphorus concentration on phosphorus immobilization in a static system. Each point represents the amount of phosphorus immobilized during 3h (a) and 24h (b) periods after daily phosphorus additions. Figures in parentheses show these amounts expressed as % of the phosphorus concentration present immediately after addition.

0 - - - - 0 control, ● —— ● 50, ■ —— ■ 100, ▲ —— ▲ 200 µg P l^{-1}.

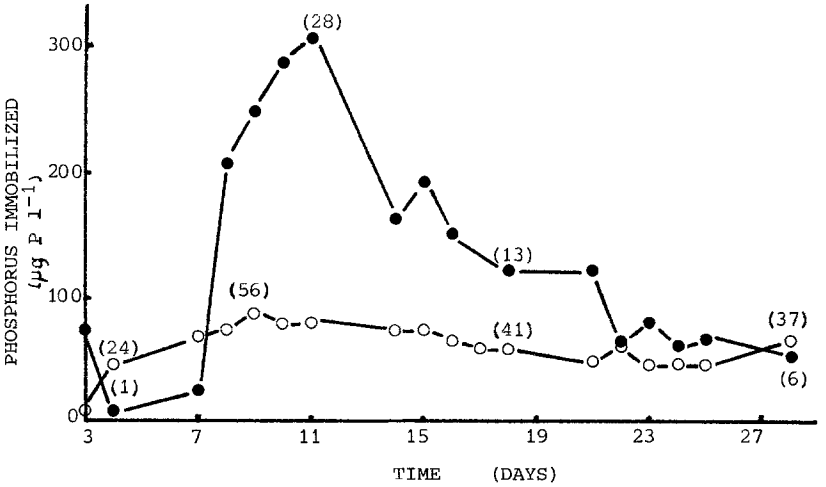

Fig.3. The effect of phosphorus concentration on phosphorus immobilization in a flowing system. Each point represents the difference in phosphorus concentration between the influent and effluent solutions. Figures in parentheses show the difference expressed as a % of the concentration in the influent solution.

Influent concentrations: O —— O 190 µg P l^{-1}, ● —— ● 950 µg P l^{-1}

Table 1. The effect of temperature on phosphorus immobilization in a static system. Figures are dissolved phosphorus concentrations ($\mu g\ P\ l^{-1}$) 24h after daily 100 $\mu g\ P\ l^{-1}$ additions.

TIME (Hours)	CONTROL	STRAW	CONTROL	STRAW
	20°C		4°C	
24	69.4	28.0	75.6	60.5
48	181.3	32.5	160.0	115.6
72	260.7	14.3	229.5	126.8
96	303.1	12.5	285.0	187.5

The amount of phosphorus immobilized during the three hour periods (Fig.2a) following each daily phosphorus addition was similar at all concentrations. During the 24 h periods immobilization was greatest at 200 $\mu g\ P\ l^{-1}$ and lowest at 50 $\mu g\ P\ l^{-1}$ (Fig.2b). When the amount of phosphorus immobilized is expressed as a percentage of that added, immobilization after 3 hours, was lowest at 200 $\mu g\ P\ l^{-1}$ while there was little difference between the other concentrations. After 24 hours the percentage of phosphorus immobilized increased at all three concentrations compared with the 3 hour levels but, in contrast, was greatest at 200 $\mu g\ P\ l^{-1}$ and lowest at 50 $\mu g\ P\ l^{-1}$.

Phosphorus immobilization on straw exposed to 950 $\mu g\ Pl^{-1}$ was slow until day 7 (Fig.3), then rapidly increased until day 11 and subsequently declined slowly. After a slow increase until day 9 immobilization of phosphorus on straw exposed to 190 $\mu g\ Pl^{-1}$ remained almost constant for the rest of the experiment. The amount of phosphorus immobilized was very similar at both concentrations from day 22 onwards. Although more phosphorus was immobilized from the higher concentration between days 8 and 25, when results are expressed as the percentage of the influent phosphorus immobilized, the proportion of phosphorus immobilized throughout the experiment was greater at the lower concentration. The percent phosphorus immobilized from the lower concentration rapidly rose from day 3 reaching a peak at day 9 whereas at 950 $\mu g\ Pl^{-1}$ a lag in immobilization occurred until day 8 and a peak at day 11. Thereafter immobilization declined in a similar way at both concentrations, except that 20-25% more phosphorus was immobilized at the lower concentration.

The phosphorus concentrations in the column effluents at each of the flow rates were almost constant during the three hour flow periods. Thus mean values of the seven samples taken during each of these periods are used in the calculations of data (Table 2).

Table 2. The effect of flow rate on phosphorus immobilization

Flow rate ($ml\ min^{-1}$)	Phosphorus immobilized ($\mu g\ P\ h^{-1} g^{-1}$ d.m. straw)	(% influent P)
120	5.1	4.2
60	5.5	7.9
30	5.2	16.8
15	4.4	31.2

Absolute amounts of phosphorus immobilized by the straw are calculated by subtracting control effluent phosphorus values from effluent values of the columns containing straw. Because hourly uptake is similar at the 120, 60 and 30 mls min^{-1} flow rates the percentage of the incoming phosphate immobilized by the straw increases as the flow rate decreases.

Excessive growth of algae, particularly of filamentous species, often starts early in the spring when water temperatures are too low to permit growth of macrophytes. Thus, there is relatively little competition for dissolved phosphorus and so algal growth is not limited. The results in this paper show that immobilization of phosphorus by the microflora on submerged straw still occurs, albeit at a reduced rate, at a temperature as low as $4^{o}C$. Therefore, it offers some potential to limit algal growth by imposing competition for this essential nutrient, both early in the spring and during the warmer part of the season.

Phosphorus loadings in water bodies arise from different sources. Obviously, inputs into the water from external sources such as sewage discharges can be very important. In such circumstances it is necessary to determine if immobilization of dissolved phosphorus by straw microflora still occurs at a range of water flow rates. Our experiments used a range of flow rates which, relative to the amount of straw used, simulated the flow conditions encountered at different times during the year in feeder streams of a small eutrophic lake near Brecon in S. Wales. The extent of immobilization of phosphorus appeared to be relatively unaffected by the flow rates used. However, with the amount of straw used in these experiments, it is clear that the proportion of phosphorus removed from the water falls dramatically at flow rates above 15 ml min^{-1}. Thus, the potential to reduce algal growth by limiting phosphorus in the receiving water body, as a result of treating feeder streams with straw, is probably low except at slow flow rate or by significant increases in the amount of straw used. Such low flow conditions may pertain in drainage ditches. Some water movement is probably necessary to maintain efficient contact between straw and dissolved phosphorus as the phosphorus immobilization rates were greater in the experiments with flow than those with no flow.

Dissolved phosphorus in lakes can arise in considerable quantities from mineralization of organic forms in the sediments. Bjork and Andersson (1979) have shown that in a shallow lake (Lake Trummen, Sweden) the net internal input of total phosphorus to the lake water during July was 10 mg P m^{-2} d^{-1}. Assuming an immobilization rate of 5 μg P g^{-1} dry straw h^{-1}, the maximum achieved in our experiments, it would require approximately 85 g m^{-2} dry straw to immobilize this input. Even at the low rate of 0.5 μg P g^{-1} dry straw h^{-1} only 850 g m^{-2} dry straw would be required.

If the potential to control excessive algal growth by microbial immobilization of phosphorus is to be realized, the period during which immobilization occurs is critical. In those situations where growth of macrophytes can be used as a significant competitor against algae it may be sufficient to reduce the

dissolved phosphorus for a relatively short period until the growth of macrophytes is established. On the other hand, in water where macrophytes are sparse, microbial immobilization might have to be encouraged throughout the growing season. It is known that microorganisms have a limited capacity to immobilize phosphorus and once that capacity is reached, they will mineralize cell reserves and return inorganic phosphorus to the environment (Alexander 1977). However, if the microbial population is maintained in a state of rapid division and high metabolic activity mineralization may be prevented. Barsdale et al. (1974) showed that phosphorus turnover is nearly 12 times higher in grazed compared to pure bacterial cultures. At the same time immobilized phosphorus is transferred to a higher level in a food chain. Thus, if the grazing protozoa are themselves preyed upon by invertebrates which in turn form the food for fish or wild fowl, phosphorus can be effectively transferred to long-lived animals and thus immobilized for long periods or removed from the water in the form of animal flesh. Preliminary laboratory experiments have already shown that considerable populations of protozoa, ostracoda, Gammarus pulex and Lymnaea sp. develop on submerged straw (Lucas J.R. pers. comm. 1982). Field observations (unpublished results) confirmed this and further demonstrated that submerged straw, and its high population of invertebrates, attract large numbers of fish.

The onset of mineralization is dependant upon the amount of phosphorus immobilized and, thus, on the concentration of dissolved phosphorus in the system. In the first experiment which received daily additions of phosphorus, such that the initial concentration of about 100 $\mu g\ P\ l^{-1}$ was reduced to almost zero each day, mineralization, as evidenced by an overall increase in dissolved phosphorus, was not detected until after 20 days and then only in the smallest mass of straw used. At higher straw masses, there was relatively little evidence of mineralization even at day 29, the end of the experiment. Similarly, in a preliminary experiment which included invertebrates and had a constant input of 25 $\mu g\ P\ l^{-1}$, mineralization was not detected even after 35 days (Sampson K.L. pers. comm. 1982). In contrast, the experiment using 190 and 950 $\mu g\ P\ l^{-1}$ in a flowing system, gave evidence of mineralization at 11 and 9 days respectively, for the two concentrations. It is also possible that lack of other essential nutrients may have initiated mineralization.

The control phosphorus concentration in the first experiment was at no stage during the experimental period as high as that theoretically possible. This was probably due to phosphorus immobilization, principally on the surfaces of the apparatus, by microorganisms introduced with the spring water. Towards the end of the experiment, however, the proportion of each daily phosphorus addition that was immobilized became increasingly smaller. Phosphorus mineralization, depletion of essential nutrients and lack of grazing may have been important in this change.

The results obtained in this work offer one possible explanation of the verbal reports (Hatt W J, Street M.) that addition of hay

or straw to a water body can result in reduction in algal growth. It is clear, however, that the complexities of the process are such that microbial immobilization of phosphorus is unlikely to be effective in reducing algal growth in all situations. Its greatest usefulness is most likely to occur in small eutrophic lakes and reservoirs, particularly those where the major inputs of phosphorus are from sediments, and also in slow flowing drainage ditches. However, problems of excessive algal growth are frequent and often severe in these situations and this method thus seems to offer great potential as an additional tool in their control.

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