Water Research, Pergamon Press 1967. Vol. 1, pp. 523–541. Printed in Great Britain.

ECOLOGY OF *SPHAEROTILUS* IN AN EXPERIMENTAL OUTDOOR CHANNEL

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(*Received* 6 *March* 1967)

Abstract—This paper describes ecological investigations performed in an outdoor experimental channel receiving Huron River water and beet sugar or crude molasses during two summers of operation (1965 and 1966).

The channel was assembled in two lengths, 645 ft (1965) and 704 ft (1966), with sampling stations located one near the beginning, one in the middle and one near the end. At each station, three strands of knitting yarn were suspended and allowed to remain for 24 hr. After this time, the strands were removed, together with attached growth, and fresh strings replaced. The replicate strings were cut into 10 cm sections, with one-half of these sections used for dry weight determinations.

The attached organisms were removed by violently shaking the strings suspended in a measured quantity of water. One drop was then removed and direct microscopic counts and identification made immediately. Dry weights were obtained by drying the strings in aluminum weighing pans overnight at 90° C.

A total of 12 experiments was run, the duration of each being in most cases five days.

In the first series of experiments flow through the channel was at 100 g/min. at an essentially uniform velocity of 1 ft/sec. Nutrient concentration was varied from 1 mg/l as sucrose.

In the last series of experiments, nutrient concentration was held at 5 mg/l and velocity varied at the three stations by altering the height of an overflow weir between 0.0 and 0.6 ft, and changing the flow between 25 and 200 g/min. This provided a range of velocities at the station from 0.09 to 1.49 ft/sec.

The results of attached organism determinations are presented following each experiment and their response to nutrient concentration and velocity summarized in the Discussion.

Sphaerotilus-dominated biological flocs were stimulated to bloom proportions within 30 hr after the addition of as little as 1 mg/l of sucrose. Maximum growth was obtained at a concentration of 5 mg/l at velocities from 0.58 to 1.49 ft/sec in the temperature range of 20–28° C after around 72 hr of feeding. About this time, detaching and floating material was equivalent to new material being formed, and the term "saturation population" was proposed to describe this condition.

The biological floc community was composed chiefly of *Sphaerotilus natans*, *Melosira varians*, *M. granulata*, *Nitzschia* sp., *Navicula* sp., *Cosmarium* sp., *Euglena* sp., and the protozoans, *Bodo* sp., *Tetrahymena pyriformis*, *Colipidium colpoda*, and *Amoeba* sp., tendipedid and simuliid larval forms also apparently found a mutualistic association within the flocs.

Saturation population and classical *Sphaerotilus* flocs could not be obtained at temperatures below 17° C. The typical form was replaced in dominance at low temperatures by an unidentified filamentous bacillus, the total mass of which never approached that of the classical growth.

INTRODUCTION

THERE have been many laboratory investigations into nutrition and biochemistry of *Sphaerotilus* since the first successful isolation of the organism in pure culture by BÜSGEN (1894). Unfortunately, the organism has not been so extensively studied in its natural environment.

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The ecology of "sewage fungus" was first studied by BUTCHER (1932) who included, in addition to *Sphaerotilus, Beggiatoa alba, Thiotrix nivea,* several true fungi, the protozoan, *Carchesium,* and the uncertain organism, "*Zooglea ramigera*".

LIEBMANN (1951) observed the same associations within the biological floc and asserted that he could determine the age of the floc by the relative abundance of the ciliates, *Colpidium colpoda* and *Glaucoma pyriformis* (= *Tetrahymena pyriformis*). NAUMANN (1933*a*, *b*) attributed these associations to decomposition of the floc.

Perhaps the general consensus at that time was expressed by HARRISON and HEUKELEKIAN (1958) when they stated, referring to the associated organisms in the biological floc, "There is no experimental evidence, however, that any of these accompanying organisms play a significant role in limiting or stimulating the development of the community."

Recently, the biological floc community was studied under simulated stream conditions by ZIMMERMAN (1961) who employed three channels about 243 ft long, 7.9 in. wide, and 5.9 in. deep to study the effects of velocity in biocoenosis. His data suggested that current is more important than pollutional loadings in the composition of the biota in a flowing stream. JAAG and AMBÜHL (1963) tend to support this observation as a result of their research into the effects of current on the macroinvertebrates indigenous to flowing waters.

WUHRMANN (1964) fed sewage in concentration of 2, 4 and 10 per cent in outdoor channels to study the effects of *Sphaerotilus* in selfpurification. The channels employed in his experiments were about 11.7 in. wide, 7.9 in. deep, 690 ft long with a slope of 0.15 per cent. This provided a discharge of about 126 g/min. His results indicated that the amount and extent of *Sphaerotilus* growth was proportional to the concentration of sewage being fed.

HARRISON and HUEUKELEKIAN (1958) reviewed the methods for quantitating slime growth used by various investigators pointing out the difficulties involved in sampling. These techniques involved the use of nets (POTONIE and WUNDSCH, 1933), glass slides (BUTCHER, 1946), strings (MCKEOWN, 1962), and underwater photography (LIEBMANN, 1953).

The purpose of the present work was first to develop procedures which allowed reliable quantitation of growth of *Sphaerotilus* and associated organisms under simulated stream conditions; then to determine the effects of nutrient concentration, runoff and velocity on the amount and character of the growth; and lastly to evaluate the role of associated organisms in stimulating or inhibiting the development of a *Sphaerotilus* bloom.

MATERIALS AND METHODS

The outdoor experimental channel (FIG. 1) described elsewhere in detail (GANNON *et al.*, 1966) was used in this investigation. In brief, the channel is composed of 4-ft repeating aluminum sections 18 in. wide, and made water-tight with a polyvinyl plastic liner. In the first series of experiments, during the summer of 1965, 645 ft were used. During the spring–summer of 1966, 704 ft were utilized.

In the first series, sampling stations were installed at three points, No. 1 at 12 ft, No. 2 at 314 ft, and No. 3 at 625 ft, (see FIG. 2 for location). In the second series, four stations were maintained. The locations were: Station No. 1 at 12 ft, No. 2 at 240 ft, No. 3 at 460 ft, and No. 4 at 685 ft.

FIG. 1. Aerial view of experimental channel.

(*Facing p.* 524)

FIG. 3a. Strings at original installation.

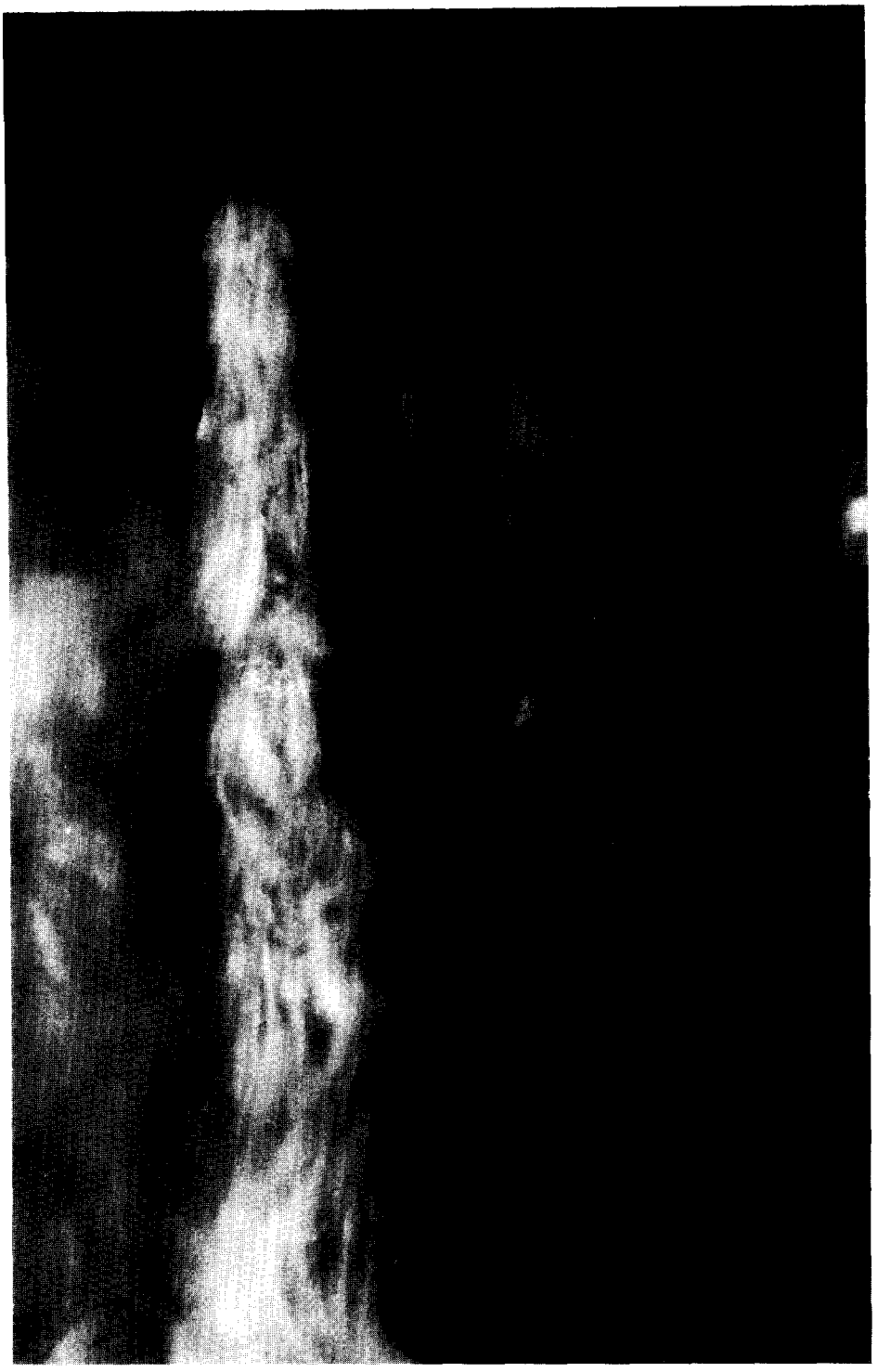

FIG. 3b. Strings during slime building.

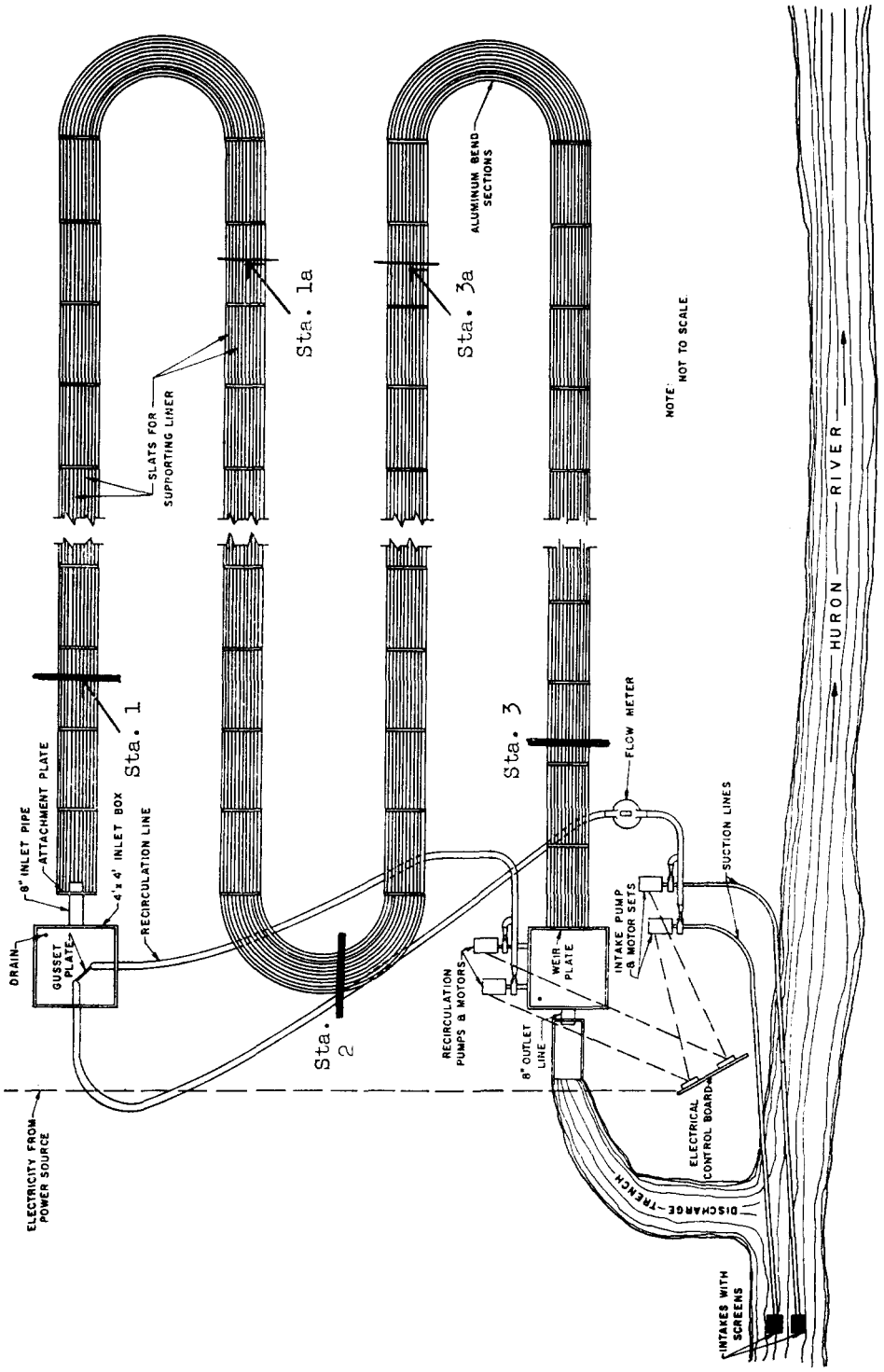

FIG. 2. Schematic layout of experimental channel at Ann Arbor treatment plant, summer 1965.

Three strands of Bear Brand Super Spun Nylon 3-ply knitting yarn (Lot No. 3401) were suspended by means of screw hooks from each station and weighted to the plastic liner by heavy hexagonal nuts to a wetted length of about 20 cm. FIGURE 2 illustrates the completed installation.

After the physical and hydraulic characteristics of the channel were defined, one string was removed from each station every 24 hr and replaced with a fresh string. In this manner it was possible to obtain (a) 24-hr strings throughout each experiment and (b) accumulation representing 24-, 48-, and 72-hr growth. However, the 24-hr strings proved to be more satisfactory so all three strings were harvested from each station every 24 hr during the summer of 1966. This practice offered the additional advantage of providing replicate samples for both dry weights and microscopic determinations.

At the beginning of the experiment, plankton samples were taken daily from an influent sampling valve on the pump and at the overflow weir near the outlet structure. Later, it was found that the plankton apparently did not change significantly in the short time of passage through the channel, so only influent plankton was collected with occasional checks of the effluent. The procedure consisted of removing 3–5 l. of water and concentrating to 30 ml by means of a No. 20 plankton net. Further concentration and enumeration of organisms was performed following LACKEY's (1938) drop sedimentation technique. Early in the research, samples were preserved in formalin to a final concentration of 4 per cent. This later discontinued and samples were examined within 30 min of collection.

From the strings, two 10 cm sections were measured and cut. One string with attached growth was resuspended in approximately 4 ml of river water and violently shaken. After discarding the string, the exact number of drops in the sample was counted. One drop was removed with a small bore pipette, placed on a clean glass slide and covered with a No. 1 cover slip. Thus by counting the organisms in one drop and multiplying by the number of drops in the concentrate, it was possible to determine the number of organisms per 10 cm of string.

The remaining 10 cm sections of string were placed into weighed aluminum pans, dried overnight in a drying oven at 90° C and the dry weight determined. Then by substracting out the known dry weight of unused 10 cm sections of strings (which was remarkably uniform) the dry weight of the growth *per se* was obtained.

pH was continuously monitored in the first series and checked periodically during the second series of experiments.

Temperature was also measured twice daily at each station using a standard mercury thermometer.

Nitrate, nitrite and total nitrogen, along with orthophosphate determinations, were made daily on the influent and effluent channel water in accordance with STANDARD METHODS (1965).

Velocities were varied by changing the height of the overflow weir, and velocity determination made using a current meter after each alteration in weir height. Times of passage were measured using a Turner Fluorometer and Rhodamin B dye.

Several sets of hydraulic characteristics were used in this research. In the first series of experiments, flow was maintained at 100 g/min free flow on a straight pass through basis giving essentially a uniform velocity of 1 ft/sec. In the second series, flow was from 25 to 200 g/min with the overflow weir varied to secure velocities from 1.48 to 0.09 ft/sec at the stations.

The first series consisted of five experiments with sucrose concentration as the only variable (except temperature, of course). These concentrations were 1, 5, 10, 20 and 40 mg/l sucrose in the form of crude molasses or refined beet sugar.

In the second series, 5 mg/l sucrose was used and the hydraulic characteristics changed as outlined above.

A uniform procedure of operation was adopted which consisted of cleaning the channel thoroughly, admitting river water only at the desired flow for 2 days, then feeding sucrose to give the desired concentration for 4.5 days.

RESULTS

The Huron River, at the channel site, was rather low in plankton concentration, averaging around 360 per ml. Total dry weight of suspended solids averaged 0.6 mg/l during the study. The population is far from diverse, partly because the stretch studied, although free-flowing, lies in the backwater of an impoundment.

Because of the relatively short time of passage through the channel, the effluent plankton was essentially the same as the influent, except for a reduction in numbers. This reduction was probably due to the attachment of some organisms to the plastic liner.

Channel water pH was almost constant at 8.4 during this research, although it varied at times from 8.2 to 8.6.

Dissolved oxygen (DO) content of influent river water followed a typical diurnal curve, the maximum being attained around 3 p.m. with the minimum occurring around 3 a.m. During the first two to three days of each experiment there was an increase in DO through the channel, the magnitude of which was proportional to the deficit of the incoming water. In some experiments, there was an increase of up to 2.2 mg/l in 704 ft. After this time, when slime growth reached a maximum, a depression occurred, varying from about 0.4 mg/l to 4.0 mg/l.

Influent channel water varied in total nitrogen from 0.28 to 1.68 mg/l, with a mean value of 0.85 ± 0.32 mg/l. Nitrate nitrogen ranged from zero to 0.11 mg/l with a mean of 0.007 mg/l. Nitrite nitrogen varied from zero to 0.16 mg/l, with a mean of 0.016 mg/l.

Influent ortho-phosphate ranged from 0.001 to 0.01 mg/l.

In most cases there was a reduction in phosphate concentration through the channel in the order of 0.003 to 0.006 mg/l, but there was no discernible pattern to the behaviour of nitrogen through the channel.

Attached organisms

In the first set of experiments, flow was essentially uniform at 1 ft/sec throughout the channel, so the attached population at station 2 was considered typical of the entire channel. Preliminary findings indicated this to be a valid assumption.

Although individual organisms were counted, they are expressed in terms of percentages of the total string population; the most frequent genera occurring each day are reported.

Experiment 1 (1 mg/l at 1.0 ft/sec)

Temperature in the channel during this experiment was 15–18° C.

Initially the attached growth was *Melosira* (33 per cent), *Nitzschia* (6 per cent), *Navicula* (3.8 per cent), *Ceratium* (9 per cent), *Aphanizomenon* (5 per cent) and *Chlamydomonas* with a total of 21 genera present.

Sphaerotilus was evident microscopically at the end of 24 hr, while the population was otherwise practically unchanged.

After 48 hr, *Sphaerotilus* was grossly evident, but in a concentration of less than 1 per cent. There was an increase in the green flagellates—*Euglena*, *Phacus* and *Phacotus*. *Ceratium* increased to 7.5 per cent and *Fragilaria*.

FIGURE 4 is a presentation of the dry weight increase during the experiment.

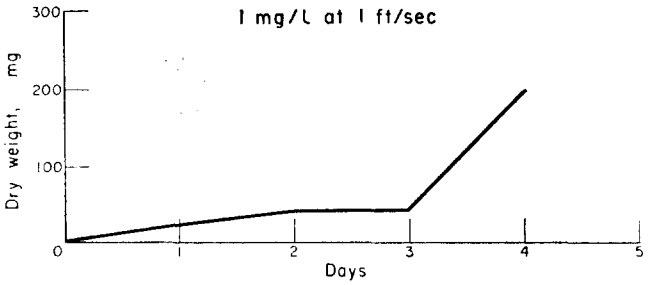

FIG. 4. Dry weight of attached growth.

Experiment 2 (5 mg/l at 1.0 ft/sec)

Water temperatures were in the 20–26° C range during this experiment. At the time nutrient feed was initiated the attached population consisted chiefly of *Navicula* (27 per cent) and *Cocconeis* (23.3 per cent) together with large numbers of *Gyrosigma* and *Stauroneis*. The macroscopic organisms were simuliid larva and pupae, two species of hydropsychids, *Culicoides* sp., *Tendipes* spp. and nematodes. In all there were 27 genera.

Twenty-four hours later, strings indicated a population shift from a diverse population, chiefly of diatoms to one predominantly *Sphaerotilus* (85 per cent) and *Melosira* (3.5 per cent) with some *Anabaena*, *Ankistrodesmus*, *Diatoma*, *Pediastrum* and *Scenedesmus*. There was an increase in simuliid and hydropsychid larvae while the other macroinvertebrate population remained relatively unchanged. There were 14 genera present.

Forty-eight hours after the feed began, *Sphaerotilus* accounted for about 90 per cent of the population, with *Tetrahymena*, *Nitzschia* and *Cosmarium* increasing. The macroinvertebrates remained in about the same relative numbers. There were 11 genera present.

Attached growth at the end of 72 hr was chiefly *Sphaerotilus* (90 per cent), *Nitzschia*, *Cosmarium* and the protozoan, *Tetrahymena*, with no significant change in the macroinvertebrates.

At the end of four days, *Sphaerotilus* represented about 99 per cent of the total biomass of the strings with the remaining 2 per cent consisting of the bacteria-feeding protozoans, *Lacrymaria*, *Tetrahymena* and *Vorticella*; the algae, *Nitzschia*, *Gyrosigma*, *Mallomonas*, *Synura* and *Cosmarium*; *Tendipes* increased about sixfold and planarians made their first appearance. *Daphnia*, simuliid and hydrosychid larvae were all dead within the flocs when collected.

By the fifth day of feed, *Sphaerotilus* had increased to about 99 per cent of the total string biota. There was a tremendous amount of detached and floating *Sphaerotilus* in the channel and the growth on the strings and channel was anaerobic in the interior. Apparently an equilibrium had been established, such that the amount of material sloughing was about equivalent to the amount of new material formed. The term, "saturation population" was used to describe this condition.

On the sixth day, anaerobic conditions prevailed throughout the attached growth and few other organisms were present other than the protozoans, *Tendipes* and nematodes. Dry weights are presented in FIG. 5.

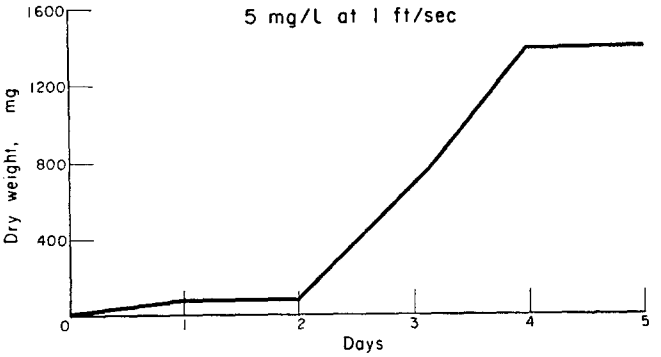

FIG. 5. Dry weight of attached growth.

Experiment 3 (10 mg/l at 1.0 ft/sec)

Water temperature in the channel was 27–28° C during this experiment.

Before nutrient was added, the populations consisted of *Stephanodiscus* (26 per cent), *Cosmarium* (21 per cent), *Melosira granulata* (18 per cent), *Nitzschia* (10 per cent), *Navicula* (8 per cent), *Melosira varians* (6 per cent) with the euglenoids—*Euglena pisciformis* (3 per cent) and *E. fusca* (2 per cent), and *Phacotus lenticularis* (1 per cent). *Sphaerotilus* appeared to the extent of 5 per cent; *Vorticella* appeared at 1 per cent of the population; *Melosira varians* increased to 25 per cent while *M. granulata* decreased to 5 per cent, *Cymbella* increased to 4 per cent and *Cosmarium* to 22 per cent.

On the second day, *Sphaerotilus* increased to 80 per cent of the total string population, with a decrease in frequency of the other forms. The number of genera decreased to 13.

After 3 days, *Sphaerotilus* dominated the population to the extent of 95 per cent. There was a large amount of floating growths indicating that saturation population had occurred. The other organisms remaining were *Euglena*, *Nitzschia* and *Stephandoiscus*, all at 1 per cent along with countless tendipedid larvae. There were 11 genera in all.

On the fourth day of the experiment, *Sphaerotilus* made up over 95 per cent of the population with the organisms present on the third day still to be found, but considerably reduced in relative numbers. Only 10 genera remained at this time.

Dry weight increase for this experiment is shown in FIG. 6.

Experiment 4 (20 mg/l at 1.0 ft/sec)

Channel water temperature was 22–23° C during this experiment.

When nutrient feed was started, the population was made up of *Melosira varians*, *Ceratium hirundinella*, *Aphanizomenon* and *Fragilaria* in that order of magnitude,

along with *Cocconeis, Cymbella, Diatoma, Gomphonema, Navicula, Nitzchia, Pediastrum, Scenedesmus* and a macroinvertebrate population of simulii dlarvae, hydropsychids and *Tendipes* sp. 21 genera were present on the strings.

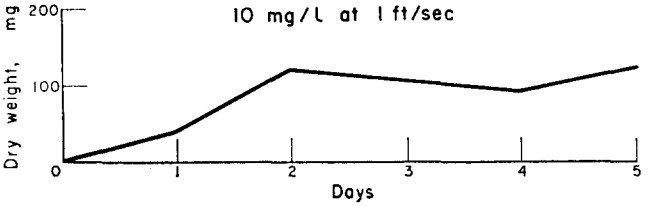

FIG. 6. Dry weight of attached growth.

Twenty-four hours after the introduction of nutrient, string populations consisted of *Melosira varians* (19.5 per cent), *Aphanizomenon* (10.4 per cent), *Fragilaria* (9.7 per cent), *Gomphonema, Ceratium hirundinella* and *Cymbella* (8.4 per cent each) with *Sphaerotilus* following closely behind (5.0 per cent). The macroinvertebrates showed no change except for the appearance of nematodes. The number of genera increased to 31.

Forty-eight hours post-feed, *Sphaerotilus* had achieved dominance (33.0 per cent) over *Melosira granulata* (15 per cent), *Aphanizomenon* (10.4 per cent), *Navicula* (7 per cent) and *Ceratium hirundinella* (5 per cent). This growth comprised 22 genera.

After 72 hr of feeding, *Sphaerotilus* accounted for 70 per cent of the attached population, *Melosira varians* 9 per cent, *Navicula* 4 per cent, and *Aphanizomenon* 3.4 per cent; *Tetrahymena, Tabellaria, Scenedesmus, Cymbella* and *Cosmarium* were also present. The population consisted of 20 genera.

Four days after the feed was started the population was chiefly *Sphaerotilus* (90 per cent), *Melosira* and *Navicula.* No other genera were represented by significant numbers.

Dry weights for this experiment appear in FIG. 7.

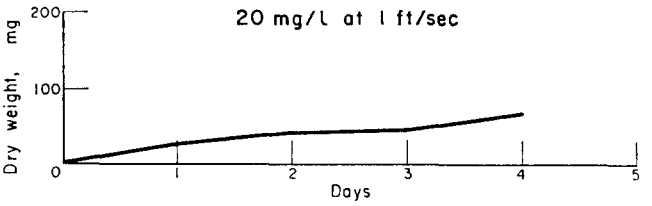

FIG. 7. Dry weight of attached growth.

Experiment 5 (40 mg/l at 1.0 ft/sec)

Channel water temperature was 21–24° C during this experiment.

When nutrient feed began the string population was composed chiefly of *Ceratium hirundinella* (46.8 per cent), *Melosira granulata* (18 per cent), *Phacus* (9 per cent), and the diatoms, *Navicula, Surirella, Synedra* and *Diatoma.* Simuliid larvae were the chief macroinvertebrates. A total of 22 genera was present.

At the end of 24 hr, the population still consisted chiefly of *Ceratium* and *Melosira* with a reduction in other diatoms and the appearance of the myxophyceans, *Anabaena circinalis, Aphanizomenon* and the rotifers *Keratella* and *Polyarthra.* The macroinvertebrates were simuliid and tendipedid larvae. Only 10 genera remained at this time.

After 48 hr of feeding nutrient, *Sphaerotilus* was sharing dominance with *Ceratium* and *Melosira*. Simuliids and tendipedids increased and two species of hydropsychid larvae appeared. The number of genera was unchanged.

Seventy-two hours after the feed was started, *Sphaerotilus* was dominant with little change in the associated organisms other than a reduction in numbers.

Four days after the introduction of nutrient, apparently saturation population had been reached, although the magnitude did not approach that obtained with 5 mg/l sucrose. *Sphaerotilus* was the dominant organism followed by *Ceratium hirundinella* and *Melosira granulata*.

FIGURE 8 indicates dry weight increase during this experiment.

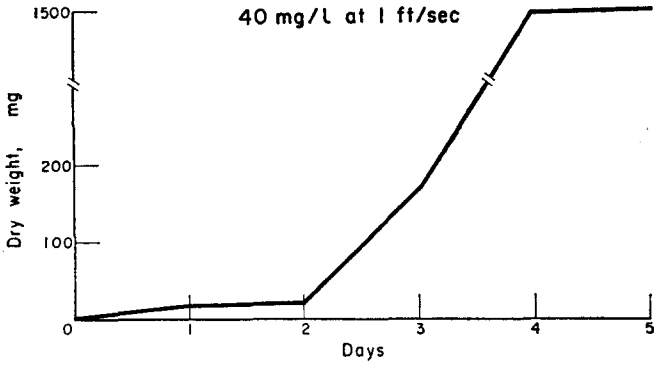

FIG. 8. Dry weight of attached growth.

The second series of experiments was designed to determine the effect of velocity, so sucrose concentration was held constant at 5 mg/l.

Experiment 6 (5 mg/l at 0.09 ft/sec)

Temperature was 26–28° C during this experiment. The initial population was *Stephanodiscus* (30 per cent), *Amoeba* (8 per cent) and *Melosira granulata* (3 per cent) along with *Bodo*, *Pedistrum* and *Pandorina*. There were 16 genera present.

After receiving nutrient for 24 hr, *Sphaerotilus* was present and *Stephanodiscus* decreased to 10 per cent. Other organisms were reduced in numbers while *Chlorella*, *Difflugia*, *Fragilaria*, *Scenedesmus* and *Staurastrum* disappeared. Only 8 genera remained.

On the second day, *Sphaerotilus* was 10 per cent of the attached population; *Stephanodiscus* and *Euglena* were 2 per cent each; *Oscillatoria* reappeared and *Sphaerocystis* was no longer present. Seven genera remained.

By the third day *Sphaerotilus* had increased to 25 per cent of the population, which consisted of *Oscillatoria*, *Euglena*, *Bodo*, *Tetrahymena* with the bacteria *Beggiatoa* and *Spirillum* sp. *Tendipes*, *Nais* and adult hydrophilids were the macroinvertebrates.

The population was unchanged on the fourth and final day of the experiment. Black granular sludge with an odor of hydrogen sulfide was present throughout the channel at the conclusion of this experiment. The dry weights of attached growth appear in FIG. 9.

Experiment 7 (5 mg/l at 0.29 ft/sec)

Channel water temperature was also 26–28° C during this experiment.

The initial population consisted of *Stephanodiscus* (20 per cent), *Melosira granulata*

(15 per cent), *Navicula* (10 per cent) with *Amoeba, Boda, Chilomonas* and *Cosmarium. Tendipes* and hydrophilids were the macroinvertebrates. In all, 18 genera were present.

After 24 hr, *Sphaerotilus* was noted with a reduction in numbers of other organisms. *Melosira varians, Chlorella, Coelastrum, Coleps* and *Cosmarium* were no longer present. The number of genera decreased to 12.

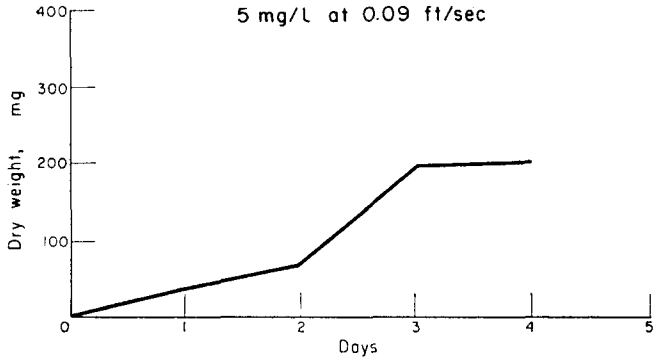

FIG. 9. Dry weight of attached growth.

On the third day of feeding, *Sphaerotilus* had increased to 20 per cent of the population with *Stephanodiscus* (5 per cent) the only organism remaining in quantity.

Four days after the feed was started, *Sphaerotilus* amounted to 80 per cent of the population, followed in dominance by *Tetrahymena* (1 per cent). *Euglena* and *Oscillatoria* were observed in quantity along with *Beggiatoa* and *Spirillum. Nais, Tendipes* and the hydrophilids were the only macroinvertebrates.

FIGURE 10 is a graphical presentation of the results of dry weight determinations.

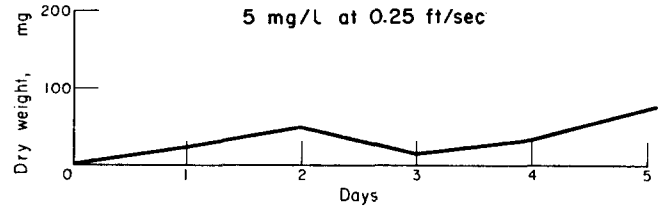

FIG. 10. Dry weight of attached growth.

Experiment 8 (5 mg/l at 0.39 ft/sec)

Channel water temperature during this experiment was 26–27° C.

Before adding nutrient the string population was *Melosira varians* and *M. granulata* at 25 and 20 per cent respectively, along with *Stephanodiscus* (15 per cent), *Cosmarium* (6 per cent), *Oscillatoria* (5 per cent) and *Schroederia* (2 per cent). Twenty-one genera were present in the string population.

After 24 hr of receiving nutrient, strings showed *Sphaerotilus* at 2 per cent of the population. *Stephanodiscus* increased to 30 per cent, *Cosmarium* to 20 per cent, *Nitzschia* to 5 per cent. Other organisms generally decreased. One hydrophilid beetle was seen and some midge eggs appeared on the strings. Twenty genera were present.

At the end of 48 hr *Sphaerotilus* accounted for 70 per cent of the population on the strings with *Stephanodiscus* second in dominance at 10 per cent. Still present in large

numbers were *Cosmarium* (8 per cent) *Nitzschia* (5 per cent), *Navicula* (3 per cent) and *Vorticella* (3 per cent). Again large quantities of midge eggs were present with one scavenger beetle noted. The number of genera dropped to 17.

Three days after nutrient feed began, *Sphaerotilus* reached 90 per cent of the total attached organisms at this station. Only *Stephanodiscus* (5 per cent), *Cosmarium* (3 per cent and *Oscillatoria* (1 per cent) remained in quantity. Neither *Melosira granulata* nor *M. varians* were observed at this time. Other than their disappearance, along with that of *Ulothrix*, there was merely a reduction in numbers from that observed on the second day. The number of genera present dropped to 13.

On the fourth and final day of nutrient feed, *Spaerotilus* was 98 per cent of all attached organisms. Although the organisms present were the same as the third day, none amounted to 1 per cent of the population, and the number of genera remaining dropped to 11.

Dry weights for this experiment appear in FIG. 11.

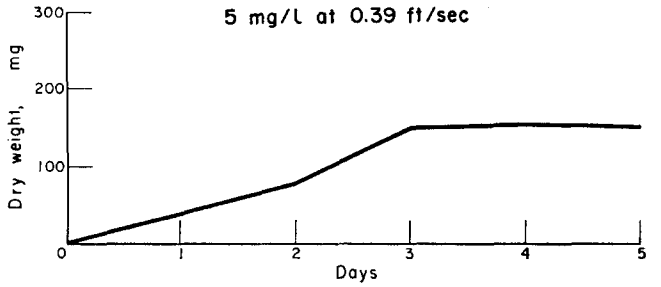

FIG. 11. Dry weight of attached growth.

Experiment 9 (5 mg/l at 0.50 ft/sec)

The temperature of the channel water during this experiment was also 26–27° C.

The initial population before nutrient was introduced consisted of *Melosira varians*, *M. granulata* and *Stephanodiscus* at 27, 22 and 18 per cent respectively, along with *Cosmarium*, *Navicula* and *Oscillatoria* at about 5 per cent each. This population comprised 22 genera.

Twenty-four hours later *Sphaerotilus* was present but less than 1 per cent of the total population; *Cosmarium* increased to 18 per cent; *Stephanodiscus* to 28 per cent; *Nitzschia* to 10 per cent and *Navicula* to 8 per cent, while *Melosira varians* decreased to less than 1 per cent. *Euglena fusca* appeared at 5 per cent. *Coelastrum*, *Tetrahymena* and *Colpidium* made their appearance. The number of genera remained at 22.

After 48 hr of feeding, *Sphaerotilus* had increased to 60 per cent of the string population, with *Stephanodiscus* at 20 per cent and *Cosmarium* at 10 per cent. Next in dominance were *Melosira granulata* (3 per cent), *Euglena fusca* (2 per cent) and *Melosira varians* (1 per cent). A small number of *Euplotes eurystomas* were present within the floc. Midge cases were noted attached to all strings at all stations. The number of genera decreased to 16.

On the third day of feeding, *Sphaerotilus* increased to 85 per cent of the attached population followed by *Stephanodiscus* (8 per cent), *Cosmarium* (5 per cent), and *Phacus* at 3 per cent. *Closterium* appeared in small numbers, while amoebas were no longer found. Only 13 genera remained.

At the end of four days, *Sphaerotilus* amounted to 98 per cent of the population with only *Stephanodiscus* (1 per cent) at one per cent or more of the total. The other organisms remained, but were reduced in numbers. The number of genera present remained 13.

FIGURE 12 is a presentation of dry weights for this experiment.

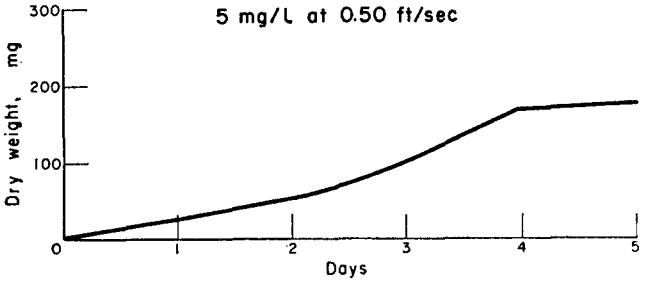

FIG. 12. Dry weight of attached growth.

Experiment 10 (5 mg/l at 0.58 ft/sec)

Channel water temperature was 26–27° C during this experiment.

Before the feed was started, the attached organisms were *Melosira varians*, (30 per cent), *M. granulata* (20 per cent) and *Stephanodiscus* (15 per cent). *Cosmarium*, *Nitzschia* and *Oscillatoria* were present at 5 per cent each.

After nutrient was added for 24 hr, *Sphaerotilus* appeared in quantity (5 per cent), *Colpidium* increased to 15 per cent, *Stephanodiscus* to 30 per cent and *Nitzschia* to 10 per cent while the other organisms showed little change. The number of genera present dropped to 14.

At the end of 48 hr of feeding, *Sphaerotilus* had reached bloom proportions (70 per cent) with *Stephanodiscus* and *Melosira varians* at 10 per cent each. *Cosmarium* (5 per cent, and *Oscillatoria* (2 per cent) were still present in numbers and *Ulothrix* appeared at 2 per cent. The number of genera had decreased from 28 before feed began to 10.

Three days after nutrient feed was started *Sphaerotilus* dominated the population to the extent of 90 per cent, followed by *Stephanodiscus* (5 per cent) *Cosmarium* (3 per cent), and *Oscillatoria* (1 per cent). Representatives of only 8 genera remained.

On the fourth day of feeding, *Sphaerotilus* reached saturation population amounting to 98 per cent of the attached population. The population was essentially the same as the third day except for a reduction in numbers. The attached population consisted of 8 genera.

FIGURE 13 shows the dry weight increase for this experiment.

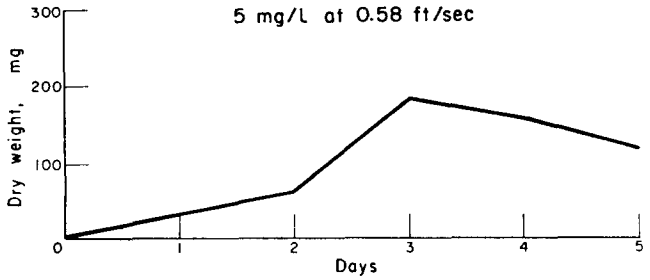

FIG. 13. Dry weight of attached growth.

Experiment 11 (5 mg/l at 0.72 ft/sec)

Temperature during this experiment averaged 26–28° C.

The initial population was composed of *Melosira varians* (30 per cent), *M. granulata* (25 per cent), *Stephanodiscus* (20 per cent), *Cosmarium* (15 per cent), *Euglena fusca* (5 per cent) and *Ulothrix* (2.5 per cent). Twelve genera were present in the population.

After 24 hr of feeding, *Sphaerotilus* appeared at 10 per cent. *Ulothrix* increased to 5 per cent; *Oscillatoria* increased to 3 per cent and *Euglena* was no longer found. The total number of genera remained unchanged at 12.

Forty-eight hours of nutrient feed produced a bloom of *Sphaerotilus* (90 per cent), with only *Melosira varians* (5 per cent), *Cosmarium* (2 per cent), *Stephanodiscus* (1 per cent) and *Ulothrix* (1 per cent) remaining in numbers. The attached population consisted of 9 genera.

After 3 days of receiving nutrient *Sphaerotilus* was 98 per cent of the attached population. No other organism was present at 1 per cent of the total. Only 5 genera made up the population.

On the fourth day *Sphaerotilus* was dominant to the extent of 99 per cent with the organisms present on the third day still persisting but in relative numbers. The number of genera remained at 5.

Dry weight increase for this experiment is presented in FIG. 14.

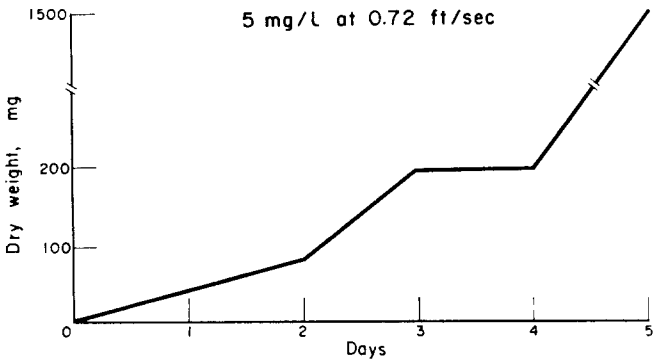

FIG. 14. Dry weight of attached growth.

Experiment 12 (5 mg/l at 1.48 ft/sec)

Temperature of the channel water was somewhat variable during this experiment ranging from 23–26° C.

Before nutrient feed was initiated, the population consisted of 14 genera. The predominant organisms were *Melosira varians* (40 per cent), *Melosira granulata* (30 per cent), *Cosmarium* (10 per cent), *Oscillatoria* (5 per cent), *Navicula* (3 per cent) and *Nitzschia* (2 per cent).

After being fed for 24 hr, *Sphaerotilus* was present at 55 per cent of the population. *Oedogonium* appeared at 3 per cent, *Ulothrix* at 5 per cent, and *Stigeoclonium tenue* at 15 per cent. Thirteen genera were present.

On the second day, *Sphaerotilus* increased to 80 per cent, sharing dominance with *Stigeoclonium* (15 per cent), *Ulothrix*, *Oedogonium*, *Melosira granulata* and *Nitzschia* were no longer observed in the string population. *Tendipes* were abundant on the strings and sides of the liner. Genera present decreased to 8 in number.

Three days after feed began, *Sphaerotilus* dominated the population to the extent of 99 per cent. *Stigeoclonium* (1 per cent) was the only organism remaining in quantity, although the total number of genera remained at 8.

On the fourth and final day, the attached population was 99 per cent *Sphaerotilus* with salml amounts of *Stigeoclonium*, *Nitzschia*, *Cosmarium*, the ciliates—*Colpidium* and *Tetrahymena*, and of course the tendipedid larvae. Growths were decomposed and the number of genera in the population dropped to 5.

Detailed results of this experiment appear in FIG. 15.

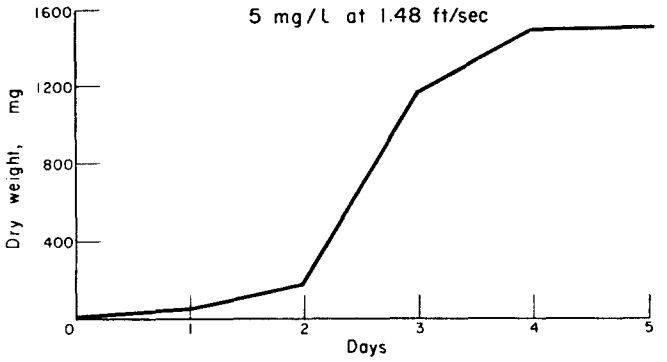

FIG. 15

DISCUSSION

The string method appears to be a reasonably reliable method of making quantitative measurements of growth and enumerating the organisms associated with slime buildup. This possesses the advantages of the submerged slide technique but does not appreciably alter the hydraulic characteristics of the channel as does the latter. Of all materials tested in a prototype flume (glass rods, slides, sticks, nets, etc.), this method gave as reproducible results as any. The reproducibility obtained between duplicate dry weights in the channel was of the order of ± 5 per cent for samples in the range of 25–100 mg; about 10 per cent in the range 100–300 mg; becoming ± 30 per cent in the range 300–1000 mg. Dry weights greater than 1000 mg varied as much as 50–60 per cent between replicates.

Reproducibility as far as enumeration of the attached organisms is concerned is, of course, dependent on several additional factors and would be difficult to assess directly.

The string idea is not new; it has been used by many investigators, but the use of nylon knitting yarn seems to be a novel approach. When viewed microscopically, each of the strands is made up of thousands of individual fibers radiating in all directions, each presenting a potential attachment site. This fine structure contributes the rough feel to the yarn, and because of this structure, the surface area of each 10 cm section is tremendous. Due to the large surface area, the yarn is able to support much more massive growths than a smooth string.

It is felt that the technique of expressing results as percentage of attached growth per 10 cm of yarn is a valid and useful one. Since the strings afford an equal opportunity for attachment to the incoming biota for 24 hr, their frequency is governed only by current, tolerance and generation time.

Counting becomes very difficult when dealing with massive quantities of *Sphaerotilus*. It is often necessary to dilute the material several-fold to free the associated organisms of their entanglement in the filaments.

The chief response of the attached organisms to nutrient addition and/or *Sphaerotilus* growth was a reduction in genera. Following is a list of frequent planktonic and channel forms and their response to nutrient and velocity:

Anabaena circinalis: Common river plankter in August; lacking in July, September and October. Fair channel growth at 10 mg/l and a velocity of 0.68 ft/sec; slight growth at 5 mg/l and a velocity of 1 ft/sec.

Ankistrodesmus sp.: Relatively abundant in early August, present throughout July, August, September and October in river plankton. Excellent channel growth at 10 mg/l and 0.68 ft/sec; slight growth at 5 mg/l and velocities from 0.29 to 0.58 ft/sec.

Aphanizomenon sp.: Common in river plankton in late August through September. Excellent channel growth at 20 mg/l and a velocity of 1 ft/sec and at 10 mg/l and a velocity of 0.25 ft/sec.

Asterionella formosa: Present in river plankton in early October. Excellent channel growth at 10 mg/l and 0.71 ft/sec.

Ceratium hirundinella: Most common river plankter in August through October. Best channel growth at 40 mg/l and a velocity of 1 ft/sec.

Chlorella sp.: Present in river plankton in August and September. Good channel growth at a velocity of 1 ft/sec and a sucrose concentration of 1, 5, 10 and 20 mg/l.

Chlamydomonas sp.: Common river plankter in July, August and September. Fair growth at 1 ft/sec with concentrations of 5, 10 and 20 mg/l.

Closterium sp.: Noted in river plankton in mid-August. Fair channel growth at 20 mg/l and a velocity of 1 ft/sec. Slight growth at all velocities tested at 5 and 10 mg/l.

Cocconeis sp.: Present in river plankton in mid-August. Fair channel growth at 20 mg/l sucrose and 1 ft/sec velocity, and 5 mg/l at 0.76 and 0.5 ft/sec.

Cryptomonas ovata: Present in river plankton throughout August. Fair channel growth at 20 mg/l and 1 ft/sec velocity.

Cyclotella sp.: Not frequently observed in river plankton. Slight channel growth at 5 ml/l sucrose and 1 ft/sec and 10 mg/l with velocities of 0.68 and 1 ft/sec.

Cymbella sp.: Observed in river plankton in August. Good channel growth at 5, 10 and 20 mg/l and 1 ft/sec and at 5 mg/l and 0.25 to 0.58 ft/sec.

Cymatopleura sp.: Present in river plankton in August. Good channel growth at 5 mg/l sucrose and velocities of 0.56 and 0.68 ft/sec. Fair growth at 5 and 10 mg/l at 0.25 and 0.71 ft/sec respectively.

Diatoma vulgare: Present in river plankton in August and September. Good channel growth at 5, 10 and 20 mg/l and 1 ft/sec and at 5 mg/l at 0.25 ft/sec.

Difflugia sp.: Present in river plankton in July, August and September. Fair channel growth at 10 mg/l sucrose and 0.71 ft/sec.

Dinobryon sertularia: Present in river plankton in August and October. Fair channel growth at 10 mg/l sucrose and 0.71, or 1 ft/sec.

Desmidium sp.: Present in river plankton in late August. Slight channel growth at 20 and 40 mg/l sucrose and 1 ft/sec and at 5 mg/l at 0.58 ft/sec.

Euglena sp.: Present in river plankton in August and September. Slight channel growth at all concentrations and velocities tested; best growth at 5 mg/l and 0.76 ft/sec.

Fragilaria crotonensis: Abundant in river plankton in September and October. Excellent channel growth at 1 mg/l and 1 ft/sec and at 5 mg/l and 0.58 ft/sec.

Gomphosphaeria aponina: In plankton during early and mid-October. Slight growth in channel at 20 and 1 mg/l sucrose and 1 ft/sec; at 10 mg/l sucrose at 0.71 and 0.25 ft/sec at 5 mg/l and 0.58 ft/sec.

Keratella sp.: Occasionally in river plankton in July through September. Slight channel growth at 40 mg/l sucrose at 1 ft/sec.

Lyngbya sp.: Present in river plankton during October. Good channel growth at 10 mg/l sucrose at 0.68 ft/sec.

Melosira granulata: Dominant river plankter in July through September. Excellent channel growth at 1 mg/l and 5 mg/l at 1 ft/sec and at 10 mg/l sucrose at 0.71 and 0.25 ft/sec.

Melosira varians: Common plankter in August through late October. Excellent channel growth at 5, 10 and 20 mg/l at all velocities except 0.09 ft/sec.

Microspora sp.: Not observed in river plankton. Excellent channel growth at 5 mg/l and 1 ft/sec.

Navicula sp.: Common river plankter in July, August and September. Good channel growth at 1, 5 and 10 mg/l sucrose with velocities from 0.29 to 1 ft/sec. Best growth at 10 mg/l and 1 ft/sec.

Nitzschia sp.: Common river plankter in August and September. Excellent channel growth at 10 mg/l sucrose and 0.71, and 1 ft/sec; slight growth down to 0.29 ft/sec at 5 mg/l.

Oscillatoria sp.: Present in river plankton in October. Excellent channel growth at 5 mg/l with velocities from 0.56 to 0.71 ft/sec. Good growth at all velocities with concentrations of 1 and 10 mg/l.

Pediastrum sp.: Present in river plankton in July through October. Slight channel growth at 10 mg/l sucrose with velocities of 0.71, 0.68 and 0.25 ft/sec and at 5, 20, and 40 mg/l at 1 ft/sec.

Phacus sp.: Present in river plankton in July through September. Good channel growth at 1 mg/l sucrose and 1 ft/sec velocity.

Pleurosigma sp.: Late October river plankter. Good growth at 5 mg/l sucrose with velocities of 0.71 and 0.56 ft/sec.

Rotifers, Unidentified: Found in river plankton in July through October. Good channel growth at 5 mg/l sucrose and 0.56 ft/sec velocity.

Scenedesmus sp.: Present in river plankton in August through October. Fair channel growth at 5, 10 and 20 mg/l sucrose at all velocities tested.

Stauroneis sp.: Rare in river plankton. Good channel growth with 5 mg/l sucrose with all velocities tested.

Stephanodiscus sp.: River plankter during July, August, and early September. Good channel growth at 5 mg/l sucrose with all velocities tested.

Stigeoclonium tenue: Not observed in river plankton. Excellent channel growth at 5 and 10 mg/l at 1 ft/sec.

Surirella sp.: Rare in river plankton. Fair channel growth at 5 mg/l sucrose and 0.71 ft/sec velocity.

Synedra capitata: Rare in river plankton. Fair channel growth at 10 mg/l and 0.68 ft/sec.

Synedra acus: Rare in river plankton. Good channel growth at 5 mg/l sucrose and 0.71 ft/sec velocity.

Tabellaria fenestrata: Present in river plankton intermittently during early August and throughout September. Excellent channel growth at 10 mg/l sucrose and 0.25 ft/sec velocity.

Ulothrix zonata: Not observed in river plankton. Growth noted in channel at 5 and 10 mg/l at 1 ft/sec.

Vorticella sp.: Very rare in river plankton. Growth noted in channel during heavy *Sphaerotilus* growth.

It is likely that some individuals noted were chance associations, but the regularity of appearance of forms such as *Melosira granulata*, *M. varians*, *Navicula*, *Nitzschia*, *Cosmarium* and *Euglena* in the biological floc seems to indicate a closer relationship. The exact nature of this relationship would have to be determined through extensive laboratory investigation beyond the scope of present work. There is a definite symbiotic relationship between the floc and simuliid and tendipedid larvae and pupae, in that early growths of *Sphaerotilus* were noted to attach first to the mucous and silken strands of these forms, incorporating them as central "stems" in the feathery growth. These macroinvertebrates in turn, were furnished shelter and food by the flocs. The hydrophyllid beetles also took advantage of the shelter and food value of the flocs, but it is doubtful that they contributed anything in return.

The exact manner in which *Sphaerotilus* is able to take over and dominate a niche from an established population is still obscure. One observation worthy of note is that in the early hours of nutrient feed, *Sphaerotilus* became attached to growths of the green alga *Spirogyra*. Soon, gas bubbles, presumably photosynthetic oxygen, formed between the alga and flocs, but after the alga was blandeted enough to exclude light, gas bubbles formed between the liner and the alga, breaking the latter loose from its attachment and leaving a clean niche, which *Sphaerotilus* immediately moved in to occupy.

Generally, saturation population occurred in the channel around 72 hr after feed was started. At temperatures of 20–28° C heaviest growths were obtained with 5 mg/l molasses, their magnitude being proportional to velocity, in the range of 1.49 to 0.58 ft/sec.

At temperatures below 20° C, saturation population was not attained even after 12 days accumulation. In fact, at temperatures from 9–17° C, *Sphaerotilus* was replaced in dominance by a small filamentous bacillus. It is the belief of the writer that a channel of some ten times greater length would be required to obtain saturation population at temperatures of 10–15° C. This is based in part on the writer's observation of growths in the Altamaha (river) in Georgia over a three-year period. With a relatively constant load of modified Kraft process wastes, growths of *Sphaerotilus* during the winter months extended over some forty miles of river, reaching a maximum around 5 miles below the outfall. At summer temperatures (28–30° C) however, growth receded to within one mile or less of the outfall, the maximum occurring just below that structure.

CONCLUSIONS

Sphaerotilus natans was stimulated to grow into bloom proportions by addition of 1 mg/l of sucrose to a natural water containing as little as 0.5 mg/l of total nitrogen and 0.001 mg/l of orthophosphate as phosphorus.

The optimum concentration of sucrose for heavy growth of *Sphaerotilus*-dominated flocs in the channel was 5 mg/l at velocities of 0.58 to 1.49 ft/sec in the temperature range of 20–28° C. Generally, growth was proportional to both temperature and velocity, and classical flocs did not occur in this system below 17° C.

The biological floc community consisted of *Sphaerotilus natans*, the diatoms, *Melosira granulata, M. varians, Navicula* sp., *Nitzschia* sp.: the desmid, *Cosmarium* sp.; the green euglenoids, *Euglena* sp.; and usually the bacteria-feeding protozoans, *Tetrahymena pyriformis, Colpidium colpoda*; occasional amoebae; and often *Bodo (caudatum?)* sp. Both tendipedid and siluliid larval forms found a favorable habitat of shelter and food within the biological growths, and the mucous and silken threads of these invertebrates served as formation "nuclei" for *Spaherotilus* growth.

After receiving nutrient for 72–96 hr, growth in the channel reached a type of equilibrium, at which time, the amount of old growth detaching and floating away just equalled the amount of new growth being formed. The term "saturation population" was proposed to describe this condition. Just after saturation population, the growths were blackened with an odor of hydrogen sulfide, indicating a critical thickness of the floc at which time it was no longer permeable to diffusion of oxygen and/or other gases.

Acknowledgements—Major financial support for this investigation was provided as research grant WP. 00091 from the Division of Water Supply and Pollution Control, U.S. Public Health Service.

The authors wish to express their sincere appreciation to C. PRESTON WITCHER and the staff of the Ann Arbor Waste Treatment Plant and to the City of Ann Arbor for generously providing the use of space and equipment.

This paper is taken, in part, from a dissertation submitted by the senior author to the Graduate School of The University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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