# **Removal of Sewage Nutrients by Algae**

R. H. BOGAN, Sc.D.

**E**XCESSIVE enrichment, or eutrophication, of receiving waters by nutrient-rich wastes is emerging as a major water pollution problem. For some time it has been recognized that ordinary domestic sewage is a rich source of the nutrients required by phytoplankton. Inorganic nitrogen and phosphorus are the most important of these nutrients, and it is on the supply of these that the degree of eutrophication depends.

From time to time, interest has developed in finding some method of waste treatment which would remove the offending fertilizing elements from sewage before it is discharged into the receiving water. Limnological investigations indicate that removal of phosphorus offers a practical and effective way of controlling algal growths in most natural waters (1). Phosphorus may be removed by biological or chemical means. Both approaches aim at the conversion of soluble inorganic phosphorus into recoverable insoluble matter. Of the two, the chemical method has received greater attention, and several promising but costly means of chemical coagulation have been proposed. Little has been done with biological treatment.

Dr. Bogan is associate professor in the civil engineering department of the University of Washington at Seattle. This article is based on a paper presented at a seminar on Algae and Metropolitan Wastes held at the Sanitary Engineering Center, Public Health Service, Cincinnati, Ohio, April 27-29, 1960.

Initial phases of the work, including the laboratory pilot-plant studies, were supported by the University of Washington Engineering Experiment Station. The City of Seattle provided funds for construction of the field-plant pilot plant. The usefulness of algae as a means of removing nutrients from domestic sewage is examined in this paper. This aspect of algal activity appears to have received relatively little attention; hence, much of what follows is based on research carried out at the University of Washington from 1956 to 1959. Wherever possible, pertinent data reported by others have been drawn upon.

## **Theoretical Concepts**

The concept which concerns the removal of nutrients from wastes by biological means can hardly be considered new. In any actively growing biological system nutrient materials are continually extracted from the environment through conversion to cell mass. The rate of nutrient removal, other factors being equal, is based on the rate of cell synthesis, while the amount of nutrient reduction is determined by cell mass composition and the mineral content of the medium.

Many excellent reports have appeared in the literature dealing with algal behavior in oxidation ponds and raw sewage lagoons (2-5) but, unfortunately, very few data have been reported regarding nutrient reductions achieved in such processes. Attention to the role of algae in sewage treatment has centered largely on the use of photosynthetic oxygen production. Available information indicates phosphorus reductions ranging from 10 to 90 percent or more, with performance erratic and unpredictable. There has been considerable difficulty in harvesting algal cell tissue, which, coupled with the slow rate of growth, probably accounts for some of the wide fluctuations noted in the mineral composition of lagoon effluents.

Rate of phosphorus removal in a biological treatment process is dependent upon the growth rate of the biological system being employed. The amount of phosphorus removed is determined largely by the composition of the microorganisms and by the composition of the waste. Cell tissue composition for activated sludge and for common fresh-water algae is shown in table 1. Growth rates commonly reported for algae, protozoa, and bacteria are shown below. In this comparison of growth rates, k (days<sup>-1</sup>) is based on  $N_t = N_o e^{kt} (t=1 \text{ day})$ .

Organism	Growth rate
Algae	0.20 to 2.0
Protozoa	1.0 to 4.0
Bacteria	2.0 to 60

Examination of the data presented in table 1 indicates that assimilation of 1 mg. per liter of phosphorus would be accompanied by a metabolism of 25 to 50 mg. or more per liter of carbon and 2 to 12 mg. per liter of nitrogen. Theoretical values of daily phosphate removal are shown in figure 1 for several concentrations of cell tissue having growth rates comparable to those commonly reported for algae.

Ordinary domestic sewage does not provide a balanced diet; both carbon and nitrogen are deficient in relation to the amount of phosphorus normally present. In activated sludge, organic carbon is usually limiting. Agricultural grade ammonia nitrogen could be made to serve as an economical source of supplemental nitrogen, but in most situations the cost of supplying adequate organic carbon would be prohibitive.

Algae do not suffer from the same dietary restrictions as bacterial complexes such as acti-

Table 1. Percent dry weight of components of activated sludge and common fresh-water algae

	Biological system				
Waste	Activated sludge <sup>1</sup>	Algae <sup>2</sup>			
Carbon Nitrogen Phosphorus	$\begin{array}{r} 41-53\\ 8-12\\ 0.\ 7-2.\ 2\end{array}$	$\begin{array}{r} 49-70\\ 1.\ 4-11\\ 0.\ 7-2.\ 0\end{array}$			

<sup>1</sup> See references 7 and 8.

<sup>2</sup> See reference 2, chapter 8.

vated sludge. Adequate amounts of carbon are normally available in the form of alkalinity and atmospheric  $CO_2$ . While the inorganic nitrogen supply may still be limiting, there is evidence that atmospheric nitrogen fixation might be made to serve as a significant source of nitrogen in large-scale algal cultures (1). In terms of nutritional requirements, algae appear to offer the most easily exploited biological system for extracting phosphorus from domestic sewage.

Significant amounts of phosphorus should of course be removed during conventional biological treatment of sewage. Based on a carbon to phosphorus ratio of 100:1 and a biochemical

Figure 1. Theoretical relationship between celltissue concentration, growth rate, and metabolic conversion of phosphorus by algae



NOTE: Light intensity determines cell-tissue concentration: k is regulated by temperature, diet, and species. In the Seattle area algal concentration ranged from 25 to 50 mg. per liter in 4-foot deep lagoons. The mean k was 0.30 days.



Figure 2. Photomicrographs of Stigeoclonium stagnatile: left, floclike colonies developed in aerated cultures, 185 imes; right, individual organisms, 445 imes

oxygen demand of settled raw sewage in the range of 100 to 200 mg. per liter, it becomes obvious that phosphorus reductions during the course of biological sewage treatment would on the average be limited to about 1 to 2 mg. per liter. Owen (6), in an investigation of sewage treatment plants in Minnesota, found that the average phosphorus removal ranged from 2 percent for primary treatment plants to 23 percent for plants employing biological treatment; this range was equivalent to approximately 1 to 2 mg. per liter of phosphorus. Analysis of effluents of biological sewage treatment plants in the Seattle area disclosed a reduction ranging from 15 to 40 percent, equivalent to 0.8 to 2.0 mg. per liter of phosphorus.

The most direct method of using algae for recovering nutrients from sewage would be to add the algae to the sewage in oxidation ponds. With such an arrangement, sewage would be continually mixed with actively growing algae and the nutrients would gradually be converted to new cell tissue. Algae could be removed for re-use or treated as waste, according to need. Experience indicates that the principal problem would be harvesting the algae.

A number of methods of harvesting algae from oxidation ponds have been investigated. All have been found wanting, usually in efficiency, often in cost. From the standpoint of all-around performance and economy, the most promising operation for separation of algae from sewage would appear to be some type of screening.

Screening results obviously would be related to the nature of the algal culture. However, if a readily recovered algal species could be established, then, through the simple mechanism of recovery and re-use, this species might be made to predominate. Other things being equal, the process would tend to contain the most readily utilized algae.

The ideal organism appears to be a large, rapidly growing filamentous alga. There is little evidence that desirable filamentous species normally grow in oxidation ponds or sewage lagoons, but this does not preclude the possibility of their being cultivated under such conditions. Failure of filamentous species to constitute a significant part of the algae population of most oxidation ponds may be due to the fact that they are relatively slow growers and tend to be overgrown by rapidly multiplying species, such as *Chlorella* and *Scenedesmus*.

## Laboratory and Pilot Plant Results

Early experimental efforts at the University of Washington to remove excessive amounts of nutrients from sewage were influenced by the principle of establishing and utilizing unique algal species. A large part of this investigation was concerned with the mechanisms by which algae removed phosphorus from solu-

Table 2. Effect of temperature and culture media on growth rate of Stigeoclonium stagnatile <sup>1</sup>

Tempera-		Growth rate <sup>2</sup>	
ture	Synthetic	Synthetic	Secondary
(degrees	sewage	sewage	sewage treat-
centi-	employing	employing	ment plant
grade)	NO <sub>3</sub> -N	NH <sub>3</sub> -N	effluent
10	0. 165	0. 140	0. 170
15	. 188	. 179	. 215
20	. 252	. 131	<sup>3</sup> . 131

<sup>1</sup> pH varied from 8.3 to 9.5; illumination was at 400 foot-candles.

<sup>2</sup> k (days<sup>-1</sup>); k computed from  $N_i = N_o e^{kt}$ .

<sup>3</sup> Scenedesmus appeared and began to predominate after approximately 15 days.

Table 3. Phosphorus and nitrogen content of Stigeoclonium stagnatile harvested from various culture media <sup>1</sup>

Constituent	Synt sew	hetic rage	Secondary sewage	½ sewage treatment plant
	NO3-N	N <b>H</b> 3-N	treatment plant effluent	effluent ½ NO <sub>3</sub> -N synthetic sewage
N P N/P	5. 71 2. 16 2. 64	6.59 1.81 3.63	$\begin{array}{c} 6. \ 52 \\ 1. \ 89 \\ 3. \ 44 \end{array}$	6. 00 3. 07 2. 89

<sup>1</sup> Expressed as percent dry-cell weight. Values reported represent an average of several determinations. tion. Attention subsequently centered upon the development and evaluation of a process of high-rate phosphorus removal. The following is a brief summary of some of the more significant findings of this research on available algae, the mechanism of phosphorus removal, the physical-chemical behavior of orthophosphates  $(PO_4 \equiv)$ , photosynthetic pH adjustment, and cell tissue recovery.

Several common fresh-water algae were grown in the laboratory on mixtures of lake water and raw and treated sewage. However, except for *Chlorella* and *Scenedesmus*, most species gradually died out after a brief period of active growth. A large filamentous alga, subsequently identified as *Stigeoclonium stagnatile*, was recovered from the rock of a biological filter in the area and successfully cultured. This alga, when grown under aeration, developed into settleable floc particles resembling activated sludge (fig. 2). The alga's growth characteristics and nitrogen and phosphorus content are shown in tables 2 and 3.

It was expected that phosphorus removal would follow a pattern predicted by the celltissue composition and growth rate (fig. 1). Contrary to expectations, orthophosphate residuals in batch-fed cultures decreased at a rate considerably in excess of that predicted by biological uptake. Response to repeated heavy phosphate doses was most interesting; in general 80 to 90 percent of the phosphate added was removed from solution within 2 hours. Obviously, more than metabolic uptake was involved. Examination of culture characteristics disclosed that phosphorus removal was related to pH. It thus appeared that coagulation and adsorption may have been significant.

At moderately high pH levels, generally in the range of 9.5 to 10.0, large amounts of phosphorus were extracted rapidly from solution without the use or need of auxiliary chemicals. At lower pH levels, the rate of phosphorus removal should, of course, be determined largely by algal growth rate. This hypothesis was subsequently tested in the laboratory and in the field. At pH levels of 9.5 and above, field cultures exhibited phosphate reductions comparable to those observed in laboratorybatch and pilot-plant studies.

Under suitable conditions, orthophosphate

may combine with a number of substances commonly present in sewage to form relatively insoluble complexes. Calcium ion concentration and pH were found to be the principal controlling factors in determining  $PO_4 \equiv$  solubility (fig. 3). Ammonia, iron, and magnesium in the amounts generally encountered in domestic sewage were not found to exercise any discernible effect on phosphate solubility.

In a study of the role of algae in adjusting pH, such factors as temperature, cell-tissue concentration, composition of culture media, and the degree of alkalinity were expected to influence the rate of photosynthetic pH adjustment. Light intensity was found to be the principal rate-controlling factor. Photosynthetic pH response under carefully controlled

Figure 3. Influence of pH and calcium concentration orthophosphate solubility



NOTE: Calcium ion concentration and pH adjusted by means of CaCl<sub>2</sub>, Ca(OH)<sub>2</sub>, and NaOH.  $PO_4 \equiv$ residuals for synthetic and treated sewages based on filtered supernatant.

Vol. 76, No. 4, April 1961 585760-61-3 laboratory conditions and under field conditions is shown in figure 4 and table 4. Where light was not limiting, pH response followed a pattern such as that shown in figure 4. Under field conditions, light intensity became limiting and the rate of change of pH was markedly reduced.

Judged solely on the basis of rate of pH adjustment, minimum light-intensity requirements appear to lie in the vicinity of 100 to 200 foot-candles. Where such light intensities are possible, the pH of raw and treated sewage may be photosynthetically increased to 9.0 and above in 4 to 12 hours.

When it was found that *S. stagnatile* could be effectively recovered by sedimentation, employment of a screen for cell-tissue recovery was abandoned. Repeated use of a *Stigeoclonium* culture during successive batch feedings or during cell-tissue recycling in pilot-plant operation resulted in marked improvement in settling characteristics. It thus appears that adsorption of insoluble calcium phosphate salts by the algae caused coagulation of cell tissue, thereby increasing its rate of subsidence.

During pilot-plant studies, the Stigeoclonium culture became contaminated with Chlorella Scenedesmus. At temperatures above and 20° C. Chlorella and Scenedesmus tended to predominate; Stigeoclonium was simply over-This gave rise to some serious grown. misgivings until it was observed that culture settling characteristics varied little with species because of the coagulation effect of the insoluble phosphate salts produced at high pH levels. This is most interesting, for it implies that the algae in most oxidation ponds might be effectively recovered by sedimentation if they are first permitted to increase pH levels above approximately 9.5 before recovery is attempted.

#### Discussion

Algae are capable of removing phosphorus from solution both by metabolic uptake and by chemical coagulation and adsorption. Adsorption and coagulation appear to play the major role in rapid removal of large concentrations of phosphorus. The relative significance of biological uptake depends, of course, on the algal growth rate and environmental conditions and on the time available for growth. In either



Figure 4. Influence of HCO<sub>3</sub> concentration on photosynthetic pH shift

Note: At light intensities above 200 foot-candles, Scenedesmus, Chlorella, and Stigeoclonium caused a rapid increase in the pH of raw and treated sewages. Alkalinity in amounts generally encountered in most sewages had little effect on pH response.

Table 4.	Photosynthetic	pН	changes	in	lagoon	cells,	autumn	1958
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Lagoon cells <sup>1</sup> and sampling conditions	Time (days)							
	1	2	3	5	7	10	12	15
Lagoon cell:	7. 7	7. 6	8. 4	8. 7	8. 5	9. 4	9. 5	10. 1.
2 <sup>3</sup>	8. 0	7. 7	8. 4	8. 8	8. 9	9. 2	9. 2	9. 6.
3 <sup>4</sup> 4 <sup>5</sup>	8. 1 8. 1	8. 0 7. 5	8. 5. 8. 2.	9. 1 10. 4	9. 3	9. 7	9. 7	10. 4.
Sampling time Weather	1:30 p.m_ Cloudy	11 a.m. Cloudy.	11 a.m Partly cloudy	11:30 a.m- Rain	10:30 a.m- Sunny	3 p.m Partly cloudy	11:30 a.m- Sunny	12 m. Sunny.
Temperature, degrees centigrade. Suspension solids, mg. per liter.		28	14 33	36	12 36	11 62	10 62	11. 70.

All lagoon cells filled with second-treatment plant effluent and seeded with equal volume of algal culture.
Intermittent aeration plus artificial illumination (400 foot-candles) in afternoon.
Intermittent aeration; no artificial illumination.

<sup>4</sup> No aeration; no illumination. <sup>5</sup> Intermittent aeration; air off after day 6.

case, it is the photosynthetic activity of the algae which governs the rate of removal.

Laboratory pilot-plant studies employing an illuminated contact unit followed by sedimentation have demonstrated that a process with a high rate of continuous flow is functionally feasible when light is not limiting. Orthophosphate concentrations can be reduced to less than 1 mg. per liter within 6 to 12 hours. This is equivalent to a 90 to 95 percent reduction in the phosphorus content of most sewage. Residual phosphate concentrations of about 3 to 5 mg. per liter were achieved with periods of contact as brief as 2 to 4 hours.

While a high treatment rate with the algal process appears to be functionally sound, economic considerations restrict the use of algae to the more leisurely conditions prevailing in oxidation ponds and sewage lagoons. Process costs are related to the cost of pH adjustment, which is a function of light requirements and the time required for detention. Where artificial illumination is used, power requirements and hence cost can be computed from a knowledge of lamp performance and the rate of light attenuation in the algal culture. In figure 5, cost of pH adjustment employing artificial and natural illumination is compared with the cost of pH adjustment by lime alone. Lime requirements were computed from titration curves of a number of treated sewages in the Seattle area. Economic considerations appear to preclude the use of artificial illumination.

Because of a high rate of light attenuation, it is exceedingly difficult to maintain adequate illumination of large-scale cultures. Theoretical considerations based on the Beer-Lambert law indicate that ordinarily it would not be possible to maintain light intensities above 100 foot-candles by natural or artificial means in depths much greater than 1 foot. Current practice is to construct oxidation ponds and lagoons with depths ranging from 3 to 5 feet.

As a practical matter, where thorough mixing occurs, the effect of employing depths greater than the depth involved in photosynthesis is roughly equivalent to illuminating the entire culture at a proportionately lesser intensity. The net photosynthetic response is thus reduced, and a longer contact time must be provided to achieve a given degree of pH adjustment. For example, it was found that 12 hours of contact time at 100 foot-candles was approximately equivalent to 10 to 12 days of lagoon retention time under field conditions prevailing in the Seattle area during the fall of the year.

Theoretically, an oxidation pond should be capable of very high efficiency in phosphorus removal. In order to realize this potential, active photosynthesis or high pH conditions, or both, must prevail for some time prior to discharge of sewage into the receiving water. Where biological fixation is the principal mode of phosphorus removal, the efficiency of the method will depend on detention time, growth rate, and cell-tissue concentration.

During field pilot-plant studies, algal concentrations in lagoon units with a depth of 3 to 4 feet varied from 25 to 50 mg. per liter. Average growth rate was equivalent to a rate of growth of 0.30 day<sup>-1</sup>. Thus, in order biologically to extract 5 mg. per liter of phosphorus (equivalent to 80 to 90 percent reduction for most sewage), a lagoon retention time of

Figure 5. Economic comparison of various methods of adjusting pH



Note: Costs of electrical energy were based on 1 cent per kilowatt-hour; power needs, on high-voltage fluorescent elements and a culture extinction coefficient of  $2 \times 10^{-8}$  cm.<sup>3</sup>/mg.; oxidation pond costs, on construction costs of 5 cents per cubic foot, a useful life of 20 years, and a liquid depth of 4 feet; and lime requirements, on titration curves for sewage in the Seattle area. Mechanical equipment costs were not considered.

14 to 28 days would be required. Theoretical retention time requirements for any other set of circumstances can be calculated from the data presented in figure 1. These considerations suggest that pond volume in excess of that employed in most oxidation ponds may be necessary where a high degree of phosphorus removal is an objective.

## **Summary and Conclusions**

The principle of employing algae as a means of removing phosphorus from sewage was studied in the laboratory and, on a pilot-plant scale, in the field. A process was developed in the laboratory whereby soluble phosphate reductions equivalent to 90 percent or more were achieved with contact times as brief as 6 to 12 hours. The process subsequently was studied in the field along with the behavior of conventional sewage lagoons. Among the findings were:

1. In the presence of adequate amounts of light, it is possible to realize rapid biological extraction of phosphates. Minimum light requirements appear to be in the vicinity of 100 to 200 foot-candles.

2. Under normal field conditions, adequate light intensities seldom prevail in algal cultures at depths much in excess of 1 foot. The use of deeper ponds, as is common practice today, serves in effect to decrease net illumination roughly in proportion to the ratio of light and dark volumes. Photosynthetic reaction times therefore must be markedly increased.

3. Adsorption and coagulation appear to play the major role in rapid removal of large amounts of phosphate. Metabolic conversion is the principal mechanism for removal of phosphate under the more leisurely conditions prevailing in oxidation ponds and sewage lagoons. In lagoons efficiency of phosphate removal is proportionate to detention time, other factors being equal.

4. Three algae, *Chlorella*, *Scenedesmus*, and *Stigeoclonium*, were grown in raw and treated sewage. Repeated re-use of these algae generally improved subsidence properties without noticeably impairing photosynthetic response. Settleability appeared to be influenced by the formation of insoluble phosphates at elevated pH. It appears that the photosynthetic pH shift may be employed as a means of enhancing the recovery of algae by sedimentation.

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