PHOSPHORUS REMOVAL FROM WASTEWATER BY PHOSPHORUS-STARVED ALGAE

by

Christine L. Thomas

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ABSTRACT

Two species of green algae, Selenastrum capricornutum and Scenedesmus sp. were evaluated for phosphorus removal from wastewater. Phosphorus-starved algae were used. The cells were exposed to varied levels of phosphorus in K_2HPO_4 solution, clarified effluent and mixed liquor. Affect of variables such as cell concentration, contact time, light and temperature on P removal were also evaluated.

Scenedesmus was capable of removing larger amounts of phosphorus per cell compared to Selenastrum. The rate of phosphorus removal was directly related to the concentration of orthophosphate in the substrate. Scenedesmus removed 99% of the phosphorus from a domestic wastewater (approximately 2.0 mg P/l) in less than 2 hours. Increased cell numbers and increased contact time resulted in greater phosphorus removal. There was no significant affect of light on the rate of phosphorus removal over a 24 hour period. Uptake efficiency decreased with a decrease in temperature. In a settling test, after phosphorus removal, 99% of Scenedesmus cells settled out by gravity compared to only 50% of Selenastrum cells.

APPROVED BY THE GRADUATE COMMITTEE OF:

Dr. Aga Razvi, Committee Chairman
Assistant Professor of Soil Science and Water Science

Dr. Theodore Roeder
Associate Professor of Water Science

Dr. Byron Shaw
Professor of Soil Science and Water Science

Dr. N. Earl Spangenberg
Associate Professor of Forestry and Water Science

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Anyone involved in a major undertaking has an easier task if there is an uninvolved party to share the triumphs and failures with. I would like to thank my husband, Stan, for sharing.

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INTRODUCTION

For many years, the main focus of wastewater treatment was on public health problems. Initially, the objective of most treatment processes was to remove solids and eliminate pathogenic organisms. By 1974, eighty-one percent of municipal plants employed secondary treatment. At that time, less than five percent of the plants practiced tertiary treatment for the removal of essential plant nutrients (Metcalf & Eddy, Inc., 1975).

The passage of Public Law 92-500 in 1972, has undoubtedly had an impact on the number of plants practicing tertiary treatment. This law calls for the elimination of pollutants discharged into navigable waters from point sources, by 1985. If this law is enforced, municipalities will be forced to adopt treatment methods which remove nutrients such as nitrogen and phosphorus.

Nitrogen and phosphorus are required by algae for growth. When discharged into lakes and streams, these nutrients allow algal populations to overcome natural nutrient limitations. This results in explosions in algal populations, often referred to as blooms. These are unsightly, may create taste and odor problems and generally make the receiving waters less desirable for industrial, recreational and domestic use. In addition, continued loading of excess nutrients speeds up the eutrophication or aging process in lakes (Wetzel, 1975).

Many studies indicate that phosphorus is more important than nitrogen in limiting algal growth (Fitzgerald et al, 1975; Sawyer, 1968). Since the Cyanophycophyta (blue green algae) possess the ability to fix nitrogen, limiting nitrogen discharge will not reduce blooms of these

organisms. Since phosphorus is the limiting nutrient in many aquatic situations, it would seem that phosphorus removal should be the focus of efforts to remove nutrients from wastewater. The objective of this study is to investigate a biological method of achieving phosphorus removal.

OBJECTIVES

The overall objective of this study is to determine the potential for phosphorus removal during tertiary treatment of wastewaters by use of phosphorus-starved algae.

Specific objectives are:

1. To compare two species of algae, which effectively remove phosphorus from wastewater, which are easily cultured and which are tolerant to sewage conditions.

2. To determine the best stage for incorporation of the phosphorus removal step into existing treatment facilities.

3. To determine the feasibility of separation of the cells from the sewage effluent.

LITERATURE REVIEW

Phosphorus in Wastewater

Phosphorus enters the municipal wastewater treatment system from various sources. Primarily, it originates in the by-products of human metabolism, food wastes and household detergents. Communities which receive the wastes of certain industries, such as slaughter houses or dairies, may have higher phosphorus loads.

Phosphorus in wastewater can be divided into two major categories: organic and inorganic. Each of these categories can be further subdivided into soluble and particulate phosphorus. The soluble inorganic forms are largely orthophosphates and polyphosphates. In a wastewater treatment plant, these forms are constantly interchanging. The polyphosphates are converted to orthophosphates which may then be changed into organic particulate phosphates by incorporation into microorganisms. A breakdown of dead microbial cells results in release of soluble organic and inorganic phosphates (Black & Veatch, 1976).

The average concentration of total phosphorus in domestic wastewater has been found to be 10 mg/l (Black & Veatch, 1976). In recent years, bans on phosphate detergents have resulted in a 50% reduction of this figure in some communities (Barth, 1978). A community which does not utilize phosphate detergents and with no specific P removal step in the wastewater treatment process, would allow approximately 2 mg/l of phosphorus to enter the receiving waters in the final effluent (Bouck, 1978). A community with a flow of 3 MGD and a discharge of 2 mg/l total P would discharge approximately 53 pounds per day into the receiving water. Under ideal conditions, this could

result in the production of over two tons of algal biomass for each day's discharge (Azad & Borchardt, 1970). These figures demonstrate the importance of phosphorus removal from wastewater.

As of June 1971, (prior to passage of PL92-500) sixteen states had effluent phosphorus standards. The range of limitations ran from 0.1 to 2.0 mg/l, with 1.0 mg/l being the limitation in many cases (Black & Veatch, 1976). As a result of these phosphorus reduction requirements, technology in this area has been expanding.

Current Phosphorus Removal Methods

Wastewater treatment facilities remove some phosphorus from wastewater whether or not phosphorus removal is the goal. As with most wastewater treatment processes, phosphorus removal techniques fall into three main categories: physical, chemical and biological. In most treatment plants, all three types play a role to some degree. In a conventional waste treatment plant, approximately 10 percent of the influent phosphorus (that portion corresponding to the insoluble particulate phosphorus) is removed by gravity in the primary settling tank (Metcalf & Eddy, 1979). Secondary treatment processes result in the removal of 20-40 percent of the influent phosphorus. This task is accomplished by bacteria which utilize some of the orthophosphate in the wastewater for growth and metabolism. At the same time, bacterial breakdown of organic constituents of the wastewater converts a large portion of the organic phosphorus to orthophosphate (Black & Veatch, 1976). The phosphorus which is incorporated into these cells is removed when the cells settle in the final clarifier.

The discussion in the previous paragraph dealt with the fate of phosphorus in the secondary treatment systems. In order to achieve phosphorus removal which is more in line with effluent phosphorus standards presently adopted by many states, it is necessary to employ additional treatment techniques. These techniques are referred to as advanced or tertiary treatment. They may be employed as part of the primary or secondary treatments or may follow the secondary treatment processes. The exact sequence depends upon factors such as the nature of the wastewater, the method of sludge disposal and the type of secondary treatment.

Physical Phosphorus Removal

Physical processes used for phosphorus removal are largely the same as those used for other types of wastes. They are based on the separation of insoluble particulate phosphorus from the wastewater. Often these processes are used following a chemical or biological step which converts soluble phosphorus into an insoluble or particulate form. Gravity or settling is the most common physical method. Other examples include centrifugation, vacuum filtration, sand filtration and on-land disposal (Metcalf & Eddy, 1979).

On-land disposal is a method which is becoming popular with small communities. In this method, effluent is irrigated onto soil, where phosphorus is adsorbed onto soil particles and later taken up by plants. This type of treatment usually follows either a primary or secondary treatment method and can reduce phosphorus levels in the effluent to less than 0.1 mg/l (Metcalf & Eddy, 1979). The major

drawback of this type of treatment is the large acreage required to treat the wastewater. For instance, Paw Paw, Michigan, a community of 3500 residents has a flow of 0.6 MGD. That community utilizes an aerated lagoon-crop irrigation process. The total facility covers 406 acres (Bush, 1979). Municipalities may have difficulty locating enough land of suitable soil type that is also affordable. In addition, care must be taken, with this type of system, to avoid nitrate contamination of the ground water.

Chemical Phosphorus Removal

Most chemical removal methods are based on the addition of a compound which reacts with the orthophosphates in the wastewater to form precipitates. Ionic forms of aluminum and iron as well as calcium in the form of lime have been found to be useful agents for this reaction. There are, however, several drawbacks to chemical removal methods. The greatest problem created by this method is the handling and disposal of the resulting sludge. A treatment plant with a flow of 1 MGD and an influent phosphorus level of 10 mg/l, which uses lime to remove phosphorus, could expect a sludge mass increase of 650 kg/d and a fivefold increase in sludge volume over the amount of sludge that would be produced by the activated sludge process alone (Metcalf & Eddy, 1979). Plants which treat phosphorus with alum can expect a sludge weight increase of 2.1 times the weight of the sludge from the biological processes in the plant (WPCF, 1977). These sludges are becoming increasingly hard to dispose of due to more stringent landfill limitations and a decrease in the number of landowners willing to

allow the spreading of sludge on their farm lands.

The second major drawback to chemical treatment is the cost of the chemicals. For instance, the current cost of alum is $100.00 per ton. The cost of lime is approximately $60.00 per ton, while liquid ferric chloride costs nearly $140.00 per ton (Posthuma, 1979). The amount of chemical required to treat the wastewater depends upon the characteristics of the wastes and climatic conditions. In addition, it is costly to handle the sludge, transport the chemicals, and install and maintain dosing equipment.

Pickle liquor is a liquid form of ionic iron which is sometimes available to municipalities at no charge. It is a waste product of the steel industry. While the price is right, there are still the factors of transportation and sludge handling to deal with. In addition, the material is corrosive and may cause the carryover of an iron floc into the effluent, which may be undesirable (Posthuma, 1979).

Biological Phosphorus Removal

Biological treatment requires the action of living organisms. Phosphorus is needed by organisms for metabolic activities. The organisms take up phosphorus, which becomes chemically bound within the cells. Then the cells must somehow be harvested to remove the phosphorus from the treatment system.

One method which utilizes a biological-chemical process is called PHOSTRIP (Matsch and Drnevich, 1979). This process uses bacteria in the conventional activated sludge process to concentrate phosphorus. The sludge from the mixed liquor is then subjected to anaerobic conditions for a short period of time. When exposed to anaerobic conditions,

most of the phosphorus is released into solution. The solution is then treated with lime to precipitate the phosphorus. The phosphorus-stripped bacteria are then recycled into the aeration basins. The stripped condition allows the organism to take up an excess amount of phosphorus, which accounts for the high degree of treatment obtainable. This method can produce an effluent with concentrations of less than 0.5 mg/l total P.

Another process known as "Activated Algae" employs algal cells for tertiary removal of phosphorus. A concentrated mixed liquor made up of photosynthesizing algal cells is used to remove phosphorus. It has been found that over 90 percent of the phosphorus removed by this method is actually precipitated in the form of apatite, when photosynthesis raises the pH of the mixed liquor. The major drawbacks to this system are the hydraulic retention time required; eight hours to remove 90 percent of a 10 mg/l concentration, and the cost of providing light to power the photosynthesis process (Doran, 1975). If a way could be found to remove phosphorus using less algal cells in a shorter retention time, perhaps this method would be more attractive. This requires a complete understanding of the parameters affecting algal utilization of phosphorus.

Algal Uptake of Phosphorus

Phosphorus is essential to the growth and metabolism of all cells. Cells store energy in the form of adenosine triphosphate (ATP) through a process known as phosphorylation. The splitting off of a phosphoric acid molecule from ATP releases a tremendous amount of energy to power

other metabolic processes (Giese, 1969). The only form of phosphorus which is directly usable by algae is the orthophosphate form, although some species produce the alkaline phosphatase enzyme, which hydrolyzes organic phosphates and allows indirect utilization of organic phosphorus by algal cells (Fogg, 1973).

There are many intracellular and extracellular factors which influence algal uptake of phosphorus. It is necessary to remember throughout the following discussion that there are two types of phosphorus uptake which need to be considered: growth related uptake which is that uptake required to support growth and metabolism and non-growth related uptake which is that uptake which occurs regardless of any increase in biomass. The second type, non-growth related uptake, is the one which will be emphasized in this study.

Since the process of photosynthesis requires the presence of intracellular phosphorus, it is not unreasonable to assume that light intensity has an effect upon growth related phosphorus uptake. The initial absorption of phosphorus has been found to be greater in the light (Wetzel, 1975). Conversely, it has been found that when P-starved Anacystis nidulans cells were exposed to P containing medium, in the darkness, that the phosphorus uptake was not significantly different in the dark than in the light (Batterton & Van Balen, 1967). However, this would only be true for non-growth related uptake, since light is required for photosynthesis. These results were also found to be true for Scenedesmus and Chlorella (Borchardt & Azad, 1968).

The effect of temperature on phosphorus uptake is a more complex situation. It has been found that more phosphorus is required to produce a given biomass at lower temperatures (Borchardt & Azad, 1968).

It is also reasonable to assume that growth related phosphorus uptake rates are depressed at lower temperatures due to a decrease in kinetic energy and a decrease in the rate of intracellular reactions (Giese, 1969). How temperature affects the uptake of phosphorus-starved algae is not clear. Little work has been done in this area.

Other factors also play a role in the growth related uptake of phosphorus. It has been found that an increase in soluble salts results in facilitation of the use of phosphorus by Microcystis aeruginosa (Shapiro, 1968). Studies indicate that the pH of the external cell medium may also affect the uptake of phosphorus (Kuhl, 1962).

Cell concentration also affects the uptake of phosphorus in growth related situations. Phosphorus uptake is increased as organism density increases, until density causes light to become a limiting factor (Borchardt & Azad, 1968). Phosphorus uptake is also affected by competition by other organisms. When phosphorus is assimilated into bacterial cells, it is not available for algal utilization. Bacteria have been found to outcompete algae for phosphorus in situations where phosphorus is limited (Rhee, 1973).

Phosphorus uptake velocity by algal cells is also a function of both internal and external phosphorus concentrations, while growth rate is controlled by the concentration of intracellular polyphosphates (Rhee, 1973). When algal cells reproduce by fission, the store of phosphorus within the cell is also divided. When the concentration of phosphorus in any given generation of daughter cells reaches a certain critical level, cell division ceases (Fogg, 1973). This critical level seems to be quite species specific and is widely discussed in the literature (Kuhl, 1974). When cells reach this critical level, they are

said to be phosphorus-starved and have been found to display certain characteristics including: increases in mean cell volume, increases in cell carbon, and a decrease in both cell nitrogen and cell protein (Fuhs et al, 1972).

In addition, algal cells possess the ability to absorb more phosphorus than they can use and store the excess in the form of polyphosphates. This phenomenon is referred to as "luxury uptake" (Fogg, 1973). When algal cells are starved for phosphorus and are suddenly exposed to an oversupply, a very rapid absorption of phosphorus occurs. The cells have the ability, under these conditions, to absorb up to 20% of their weight in phosphorus (Borchardt & Azad, 1968). It is this characteristic which may make algae an efficient vehicle for phosphorus removal from wastewater and is the principal used in this investigation.

Selection of an Algal Species

Since it is not within the scope of this project to investigate a large number of algal species as to their phosphorus absorbing capabilities, heavy reliance must be made upon the literature. Criteria for selection of the test organisms include the following: ease of culture, tolerance to northern climates and sewage conditions, ability to absorb the necessary levels of phosphorus and non-interference with treatment plant activities.

Chlorella and Scenedesmus are the most common members of the Chlorophycophyta found in association with sewage treatment facilities (Fair, Geyer, & Okun, 1968). Scenedesmus usually grows in colonies of four cells or less and therefore should present none of the problems

associated with filamentous growth in treatment facilities. Scenedesmus has been found to possess the ability to remove up to 20% of its weight in phosphorus from culture media (Borchardt & Azad, 1968). These characteristics make it an interesting prospect for further investigation.

It has been reported that Selenastrum capricornutum, another species of green alga, is able to deplete phosphorus in solution below 3 ug/l, when exposed in the P-starved condition (Brown, 1975). This species is easily cultured and readily available due to recommendation of its use in algal bioassays (APHA, 1976).

Certain of the Cyanophycophyta also possess the ability to take up large amounts of phosphorus. They have not been investigated in this undertaking for several reasons. Many of these organisms are multicellular, often forming large colonies or filaments. This characteristic would present problems in laboratory measurement of growth. In addition, filamentous or floating colonies would present problems in actual treatment plant facilities. Another drawback to the blue-greens is their ability to fix nitrogen. Green algae might reduce the nitrogen content of the sewage effluent, as a bonus to the phosphorus removal process. Lastly, the blue-greens are noted for production of toxic substances, which if released to the receiving water might be detrimental.

Summary of Literature Review

It has been demonstrated in the above discussion that phosphorus is present in the effluent of most municipal treatment systems in quantities that can adversely affect the quality of the receiving water.

It is probable that federal legislation will mandate upgrading of treatment facilities to include nutrient removal. Many of the treatment processes currently used are unsatisfactory due to economics or quality of the treatment. It has been shown that algae possess the potential for extremely high uptake rates. Selenastrum and Scenedesmus genera have been shown to meet selection criteria for investigation in the treatment process, mainly a high potential for phosphorus uptake. It is the purpose of this study to investigate this potential with regard to wastewater.

MATERIALS AND METHODS

Culturing Techniques

Unialgal cultures of Selenastrum capricornutum and Scenedesmus sp. were grown in 3000 ml Erlenmeyer flasks. The flasks contained 2 liters of PAAP medium (Bartsch, 1969)(Table 1). The cultures were incubated in an environmental chamber at 24 ± 2 degrees C and 400 foot-candles of "cool-white" flourescent light. Cultures were aerated with water-saturated air. When cultures reached late log/early stationary phase, they were transferred to P-free PAAP medium. This was done by centrifuging the cells and decanting the supernatent. The concentrated cells were then resuspended in 2 liters of P-free medium. Cultures were then reincubated until they reached late log/early stationary phase. Cell growth was monitored by direct cell count, using a Petroff-Hauser counting chamber. Three replicates were counted for each sample. When growth ceased in the P-free medium, cells were considered to be P-starved. No further growth occured when cultures were transferred to P-free medium for a second time, because of a lack of phosphorus in the medium (Sagher, 1976). P-starved cells were stored at 4 degrees C until needed for experimentation.

Phosphorus Analysis

All phosphorus analysis was done using a Technicon Autoanalyzer II. This apparatus analyzes for phosphorus using the ascorbic acid method (APHA, 1976). The instrument was calibrated to accept samples between .500 mg/l and .005 mg/l. Samples were diluted with acidified distilled

Table 1. Constituents of PAAP medium.

Nutrient	Reagent Source		Amount of Nutrient
		mg/l	mg/l
N	$NaNO_3$	85.00	14.00
P	K_2HPO_4	3.48	0.62
K	K_2HPO_4	----	1.56
Mg	$MgSO_4.7H_2O$		9.68
	$MgCl_2$	19.00	
S	$MgSO_4.7H_2O$	49.00	6.37
Ca	$CaCl_2.2H_2O$	14.70	4.01
C	Na_2CO_3	50.00	----
Fe	$FeCl_3$	0.32	0.11
B	H_3BO_3	0.618	0.11
Mn	$MnCl_2$	0.880	0.38
Zn	$ZnCl_2$	0.109	0.05
Co	$CoCl_2$	0.0026	0.0012
Cu	$CuCl_2$	0.00003	0.00001
Mo	$Na_2MoO_4.2H_2O$	0.0242	0.0096
--	$Na_2EDTA.2H_2O$	7.44	---------

water in order to meet these requirements (Hammermeister, 1978). Two replications of each sample were analyzed for phosphorus and errors were calculated to be less than 6% on the average.

Phosphorus Uptake Experimentation

Uptake From K_2HPO_4 Solution

Experiments were carried out in 500 ml Erlenmeyer flasks, at 21 degrees C. The cells required for a given experiment were concentrated into a 230 ml aliquot, to which was added 20 ml of a K_2HPO_4 solution to achieve the desired overall phosphorus concentration and a total volume of 250 ml. Samples were taken initially and at intervals, over a period of four hours, to determine uptake of the phosphorus from solution. This was done by swirling the flasks and by filtering a portion of the cell suspension through a 24 mm diameter glass fibre filter (Reeve Angel 934AH) with a pore size of .45 microns diameter. Two dilutions of the filtrate were made using acidified water. Acidified water is prepared using 955 ml distilled water and 5 ml sulfuric acid (Hammermeister, 1978). The diluted samples were stored in 5 ml polystyrene vials and refrigerated at 4 degrees C until analyzed. Various treatments were used within these experiments using Selenastrum capricornutum and Scenedesmus sp. as test organisms:

A. Variation of phosphorus concentration
B. Variation of cell numbers
C. Comparison of uptake in light and dark
D. Comparison of uptake at high and low temperatures

Phosphorus Uptake From Clarified Effluent

A 2 liter sample of unchlorinated, clarified effluent was obtained from the sewage treatment plant at Stevens Point, Wisconsin, an activated sludge facility. A 1230 ml sample of P-starved S. capricornutum culture, having a cell concentration of 1.3×10^7 cells/ml was centrifuged and concentrated to 50 ml. These cells were added to 200 ml of clarified effluent. The cell concentration at the start of this experiment was 6.4×10^{10} cells/l and the initial phosphorus concentration was 2.88 mg/l. Samples were taken and prepared for analysis using the above procedure. The same experiment was run using 1.5×10^9 cells/l of S. capricornutum. Scenedesmus sp. was also tested in the clarified effluent. Those cells were used in two concentrations: 5.9×10^9 cells/l and 4.0×10^8 cells/l.

Phosphorus Uptake From Mixed Liquor

Samples of mixed liquor from the aeration basin (final basin) of the Stevens Point facility were obtained. Experiments similar to those described above were run using 900 ml aliquots of mixed liquor and 100 ml of concentrated cells. In order to test the potential of the system for handling higher phosphorus loads, K_2HPO_4 was added to some of the experiments. The normal orthophosphate level in the mixed liquor at the Stevens Point facility was around 2 mg/l. Experiments were also run in the 13 mg/l and 24 mg/l ranges. The uptakes were conducted in 2 liter Erlenmeyer flasks at 21 degrees C, in the dark.

Constant aeration was necessary to duplicate conditions in the treatment facility.

It was felt that cells added to the mixed liquor would have a longer contact time, if added to the first aeration basin. In order to determine the potential of the algae for treatment, samples were obtained from the first tank and various cell levels were exposed to this medium. Sampling and analysis procedures were the same as for the above experiments. This experiment was conducted at 21 and 10 degrees C to test the effects of cold weather on uptake efficiency.

Cell Separation Experiments

The mixed liquor from two uptake experiments was used to determine how well settling would work as a means to remove algal cells from the sewage effluent. Three Imhoff cones were set up and filled with 1 liter samples. One contained mixed liquor. One contained Selenastrum capricornutum and mixed liquor and the third contained Scenedesmus sp. and mixed liquor from the uptake experiment. Determinations were made for total mixed liquor suspended solids, suspended solids, suspended solids in the supernatent after a thirty minute settling time, sludge volume index and number of algal cells in the supernatent. Procedures were used which are described in Standard Methods (APHA, 1976).

Another experiment was conducted to determine the potential of a semipermeable membrane as a means of separating the cells from the P-containing solution during the uptake process rather than after the P-uptake was completed. A cellulose acetate tubing was used. In

some experiments the cells were outside the tubing and in some the cells were inside the tubing. Experimental conditions were the same as in the direct contact experiments above.

Sludge Digestion

In order to determine the fate of the phosphorus in the sludge digester, a laboratory digestion was set up. Two flasks, each containing 100 ml of the sludge from each of the above Imhoff cones was innoculated with anaerobic organisms, sealed and vented with rubber tubing. The tubing was immersed in a beaker of water. The flasks were incubated at 37 degrees C and samples were removed for analysis at 14 and 21 days. The samples were filtered and the filtrate was analyzed for total and orthophosphate.

RESULTS AND DISCUSSION

This study, which was aimed at determining the potential of P-starved algae for removal of phosphorus from wastewater, was subdivided into the following smaller studies:

1. The effect of phosphorus concentration and other related conditions on algal phosphorus uptake in non-wastewater conditions.

2. The effect of wastewater environments on algal uptake of phosphorus.

3. Incorporation of the algal uptake process into existing secondary wastewater plants.

4. Physical separation of the algae from the wastewater during or after P uptake.

5. Release of absorbed phosphorus during anaerobic digestion of sludge containing the algae.

Phosphorus Uptake By Algae

Several factors affect algal phosphorus uptake from solution. Some of these factors include: the phosphorus content or physiological

state of cells that are actively involved in phosphorus uptake; the phosphorus concentration in the solution that the cells are exposed to; the number of cells actively involved in removing phosphorus from solution; whether or not light is available; temperature conditions and several other environmental factors such as nutrient availability, pH and presence of toxic substances.

In actual practice, the organisms in a wastewater treatment plant are exposed to a range of environmental conditions, such as varying concentrations of nutrients, variations in light availability, and fluctuating temperature conditions. It is necessary to study each one of these factors using the test organisms, in order to determine suitability of the organisms in a wastewater situation.

Phosphorus Content of Algal Cells - Effect on P Uptake

It is well documented in the literature that non-growth related P uptake is a function of cellular P levels (Borchardt and Azad, 1968). The rate of uptake is inversely related to the phosphorus level of the cells, all other parameters being equal. Since only cells with a very low P content (P-starved) were used throughout this study, experiments were not conducted to evaluate this factor.

Using PAAP media and culturing techniques described in the methods section, Selenastrum capricornutum cells were produced which had an internal cell P level of approximately 4×10^{-8} ug P/cell. For similar media conditions, Scenedesmus sp. cells were produced which had an internal cell P level of 4×10^{-7} ug P/cell. Algal cells with such low phosphorus levels are referred to as P-starved algae. The cell phosphorus levels of P-starved algae are near the minimum

amount necessary for the maintenance of normal cellular functions. These low cell phosphorus levels allow very rapid uptake of phosphorus from solution. This rapid uptake rate continues for at least a short period (4 hours) before an increase in cell phosphorus levels retards the phosphorus uptake rate (Sagher, 1976). The use of P-starved algal cells allows enough time for a continued rapid rate of removal of phosphorus from wastewater.

Phosphorus Concentration In Solution - Effect On P Uptake

An uptake experiment was conducted by exposing 6.4×10^{10} cells/l of Selenastrum capricornutum to two different levels of K_2HPO_4 in distilled water, 1.72 mg/l P and 8.62 mg/l P. The reaction vessels were incubated in the light at 22 degrees C.

Figure 1 shows that the cells were able to absorb a high percentage of the phosphorus in the high as well as the low concentrations within a short period of time. The relatively high concentrations of phosphorus initially resulted in a very rapid uptake. Later in the incubation period, the rate of removal levels off because of a lower concentration of phosphorus remaining in solution.

Uptake data for this experiment are shown in Table 2. The 6.4×10^{10} cells/l level of Selanastrum was able to reduce the level of phosphorus in solution from 1.72 mg P/l to below the detection limit (.005 mg/l) in ninety minutes. In the same period of time, the cells were able to remove 99.4% of the 8.62 mg/l P in the high concentration solution. After 15 minutes, the cells in the low concentration solution increased their cellular P level by 64%, as compared to 194% in

Figure 1. Phosphorus removal from K_2HPO_4 solution by 6.4×10^{10} cells/l of *Selenastrum capricornutum* exposed to varying phosphorus concentrations and incubated in the light at 22 degrees C.

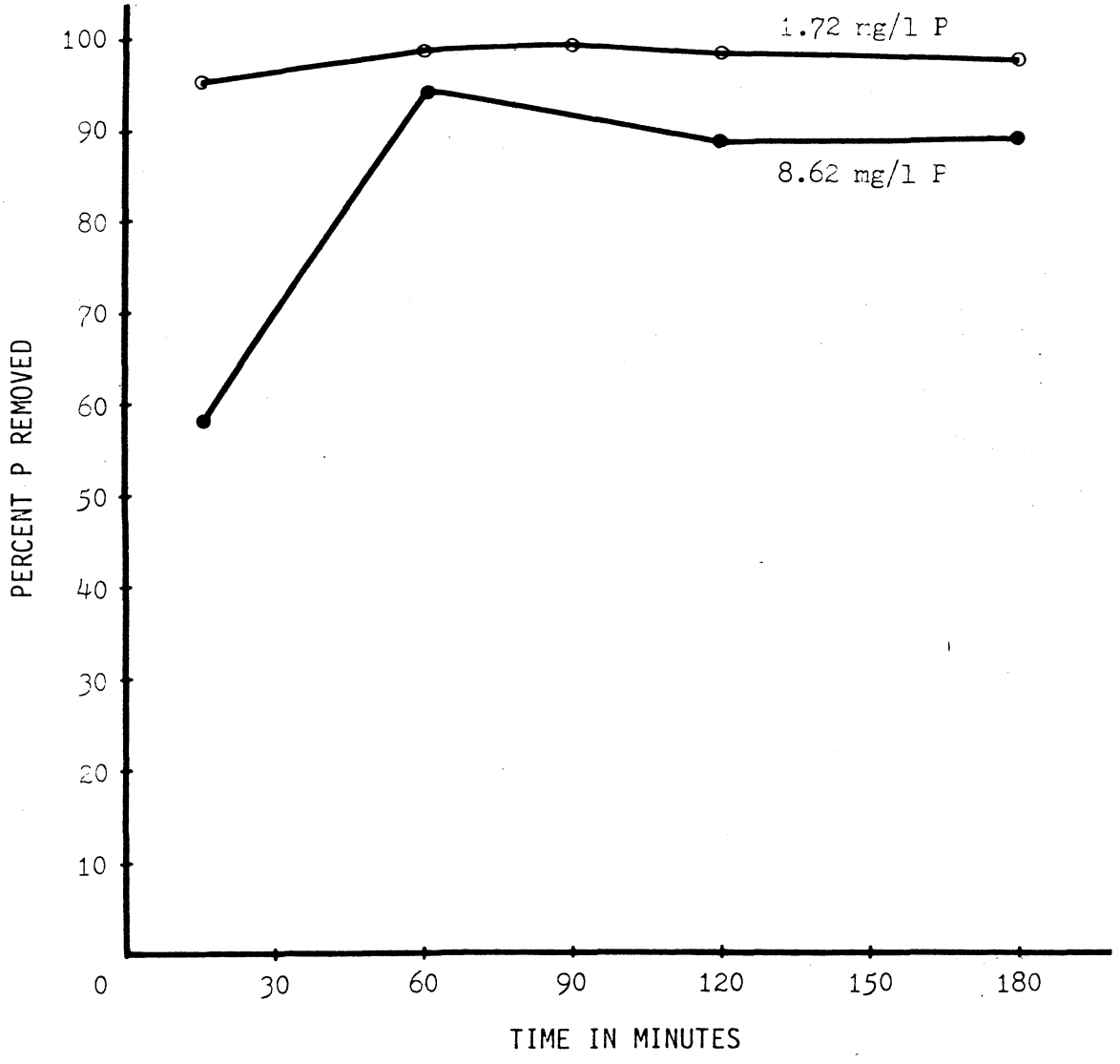

1.72 mg/l P
8.62 mg/l P
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
30
60
90
120
150
180
TIME IN MINUTES

Figure 1

Table 2. Phosphorus removal from K_2HPO_4 solution by 6.4 x 10^{10} cells/l of Selenastrum capricornutum exposed to varying phosphorus concentrations and incubated in the light at 22 degrees C.

	Low Phosphorus Concentration			High Phosphorus Concentration		
Time	P concentration in solution	P removed from solution	Increase in cell P	P concentration in solution	P removed	Increase in cell P
Minutes	mg/l	%	%	mg/l	%	%
0	1.72	0	0	8.62	0	0
15	0.08	95.4	64	3.65	57.6	194
60	0.02	98.9	66	0.52	94.0	316
90	BDL*	99	66	0.05	99.4	345
120	0.03	98.2	66	1.00	88.4	298
180	0.04	97.7	66	0.95	89.0	298
1200	0.03	98.2	66	1.08	88.0	298

*Below Detection Limit

the high concentration P solution. The increase in cellular P level was as high as 345% over the original level, at the end of 90 minutes, in the high P concentration solution.

At the higher concentration, after the ninety minute incubation period, the cell P levels began to decrease. Some of the phosphorus that was absorbed by the cells appears to seep back out of the cells into solution. After two hours, an equilibrium was maintained for 20 hours. This finding is consistent with other studies that found that algal cells tend to excrete/release phosphorus into solution to maintain an equilibrium level between external and internal cell P. This equilibrium level depends upon cell phosphorus levels (Fuhs, 1972) and the type of alga used. Such leaching could also occur at the low P concentration but may not be detectable by the analytical procedure used in this study.

There was no change in cell numbers over a 24 hour period of time, indicating that phosphorus uptake, in this case, is not related to growth. This was found to be true in other studies as well (Sagher, 1976).

An experiment similar to the one described above was conducted by using P-starved Scenedesmus sp. at a cell concentration of 2.9×10^9 cells/l. These cells were exposed to two different levels of P: 1.88 mg/l and 10.4 mg/l. Reaction vessels were incubated in the light at 22 degrees C.

Figure 2 demonstrates that the Scenedesmus cells were able to remove more than 95% of both levels of P in less than 4 hours. The uptake in the lower P concentration solution was much more gradual, as might be expected if Michaelis-Menten kinetics were considered. A lower concentration of substrate results in a lower rate of uptake (Giese, 1969).

Figure 2. Phosphorus removal from K_2HPO_4 solution by 2.9×10^9 cells/l of Scenedesmus sp. exposed to varying phosphorus concentrations and incubated in the light at 22 degrees C.

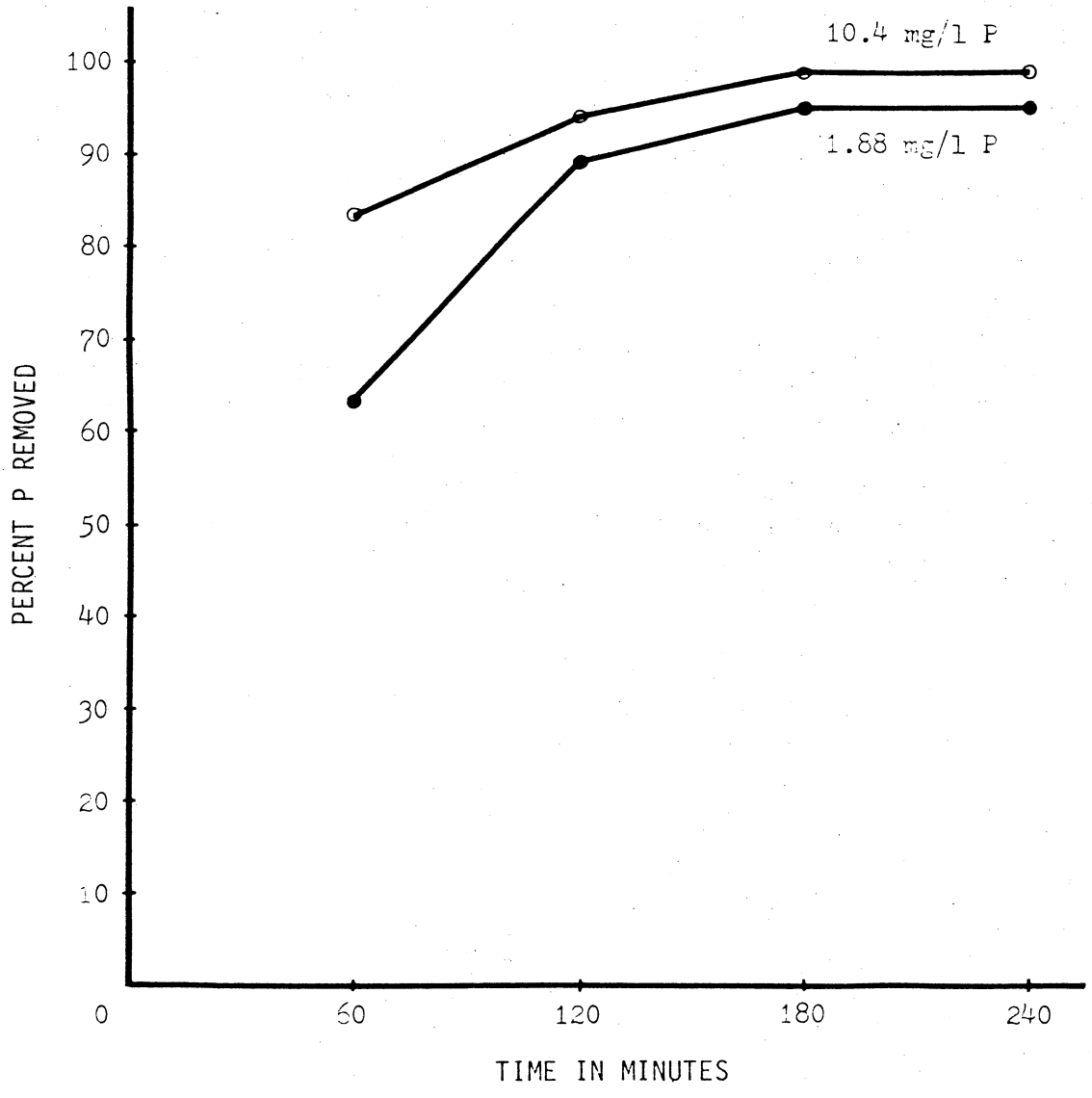

10.4 mg/l P
1.88 mg/l P
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
60
120
180
240
TIME IN MINUTES

Figure 2

Table 3 presents the uptake data for the Scenedesmus experiments. The "P Removal" columns show that the cells removed 95% of the P in the lower concentration solution and 99% of the P in the higher concentration solution. The cells in the higher concentration solution showed an increase in cell P of up to 888% over the initial level. Other studies have shown that Scenedesmus is capable of increasing its cell phosphorus by even greater amounts, if it is exposed to higher phosphorus levels, showing "luxury uptake" (Borchardt and Azad, 1970). The phosphorus concentration remaining in solution at both high and low initial P levels was 0.1 mg/l. This appears to be the equilibrium concentration for Scenedesmus sp. However, other studies indicate that Scenedesmus, at very low cell P levels, equilibrates at external concentrations of 0.01 mg/l (Brown, 1975).

Both Selenastrum and Scenedesmus studies show that the rate of P uptake is related to the concentration of phosphorus in solution. As the concentration of P in solution declines, the rate of uptake declines. For the P concentrations used in this study, Selenastrum was able to deplete the phosphorus in the media to a much lower level than Scenedesmus.

The data from these experiments indicates that either species is capable of taking up levels of phosphorus commonly found in municipal wastewater, with a greater than 90% removal in 4 hours, a time well within the contact/detention of the waste in the aeration stages of a sewage treatment plant. Other factors which affect phosphorus uptake must be examined in order to determine which species is more suitable.

Table 3. Phosphorus removal from K_2HPO_4 solution by 2.9×10^9 cells/l of Scenedesmus sp. exposed to varying phosphorus concentrations and incubated in the light at 22 degrees C.

	Low Phosphorus Concentration			High Phosphorus Concentration		
Time	P concentration in solution	P removed from solution	Increase in cell P	P concentration in solution	P removed from solution	Increase in cell P
Minutes	mg/l	%	%	mg/l	%	%
0	1.88	0	0	10.4	0	0
60	.69	63	102	1.74	83	746
120	.21	89	144	.64	94	841
180	.10	95	153	.10	99	888
240	.09	95	154	.12	99	886

Effect Of Cell Numbers On P-Uptake

To determine the effect of cell numbers on P removal rate, P-starved Selenastrum capricornutum at three different cell levels (1.3×10^{10}, 3.9×10^{10}, 6.4×10^{10} cells/l) was exposed to approximately 1.74 mg P/l, in K_2HPO_4 solution. The cells were incubated at 22 degrees C, in the light, during the course of the experiment.

Figure 3 and Table 4 demonstrate a very rapid utilization of most of the available phosphorus at the higher cell concentration. The slope of P uptake is more gradual for the lower cell numbers.

The above experiment was repeated using 4×10^8 cells/l, 2.9×10^9 cells/l and 6.4×10^9 cells/l of P-starved Scenedesmus sp. Figure 4 and Table 5 show the same type of trends that were displayed by Selenastrum. The higher the cell numbers, the more rapid the depletion of phosphorus. This results in a decline in uptake rates and a leveling of the graph.

Scenedesmus cells in the lowest concentration were able to increase their phosphorus from 4×10^{-7} ug P/cell to 3.9×10^{-6} ug P/cell in four hours. That is almost a six hundred percent increase over the next higher cell level.

The amount of P that was absorbed per cell at each of the three cell levels was nearly the same at the end of fifteen minutes. This indicates that for P-starved cells (identical P levels) P uptake is controlled by the concentration of P in the media and not the number of cells. The absolute amount of phosphorus removed per unit time, however; is expected to be greater for a larger population of cells because of an increased surface area available for absorption. This

Figure 3. Phosphorus removal from 1.74 mg P/l K_2HPO_4 solution by varied cell levels of Selenastrum capricornutum, incubated in the light at 22 degrees C.

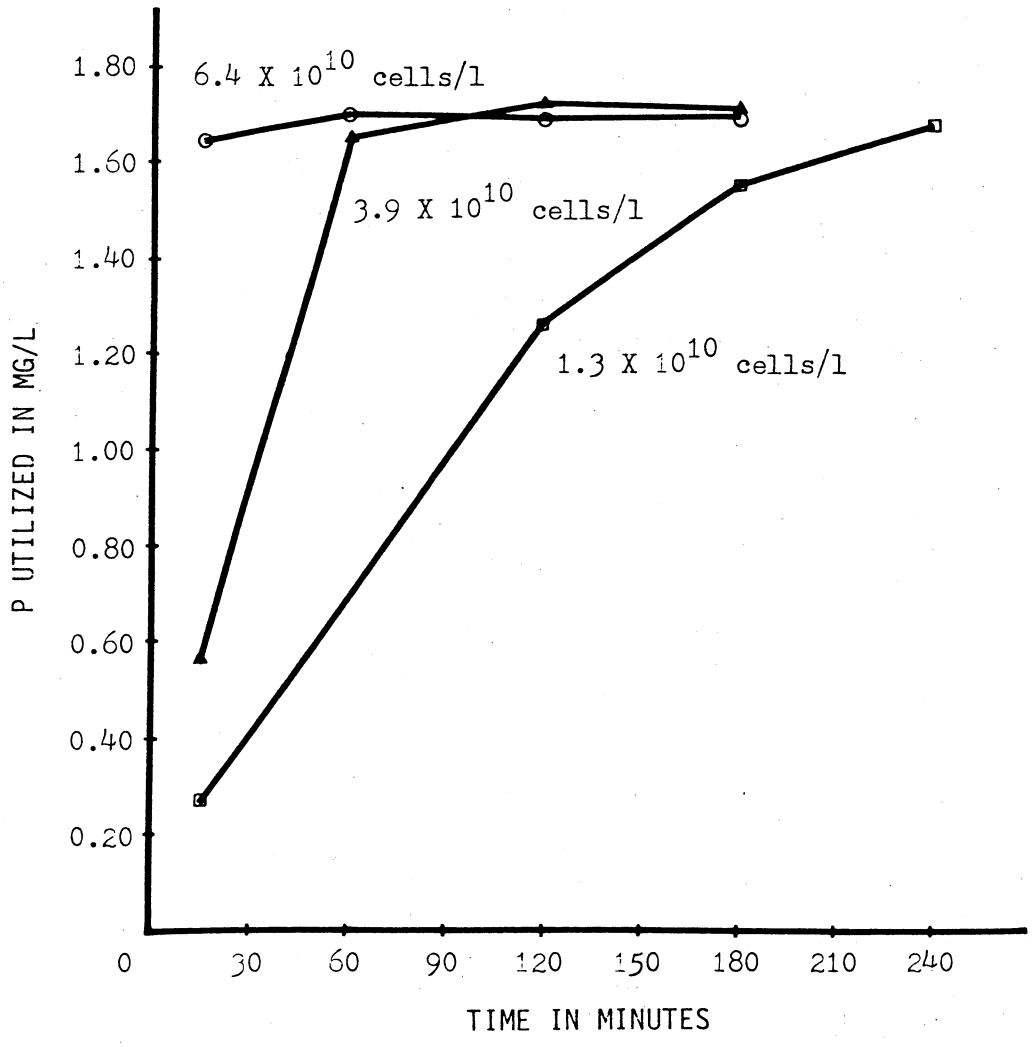

1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
P UTILIZED IN MG/L
6.4 X 10^10 cells/l
3.9 X 10^10 cells/l
1.3 X 10^10 cells/l
0
30
60
90
120
150
180
210
240
TIME IN MINUTES

Figure 3

Table 4. Phosphorus removal from 1.74 mg P/l K_2HPO_4 solution by varied cell levels of Selenastrum capricornutum, incubated in the light at 22 degrees C.

	1.3×10^{10} cells/l		3.9×10^{10} cells/l		6.4×10^{10} cells/l	
Time	P in solution	P removed/cell	P in solution	P removed/cell	P in solution	P removed/cell
Minutes	mg/l	$\times 10^{-8}$ ug	mg/l	$\times 10^{-8}$ ug	mg/l	$\times 10^{-8}$ ug
0	1.74	0	1.75	0	1.72	0
15	1.47	2.1	1.2	1.9	.07	2.5
60	1.18	4.3	.10	4.2	.02	2.6
120	0.58	8.9	.03	4.4	.03	2.6
180	0.18	12.0	.04	4.4	.02	2.6
240	0.06	13.0	BDL*	---	BDL	---

*Below Detection Limit

Figure 4. Phosphorus removal from 1.75 mg P/l K_2HPO_4 solution by varied levels of Scenedesmus sp., incubated in the light at 22 degrees C.

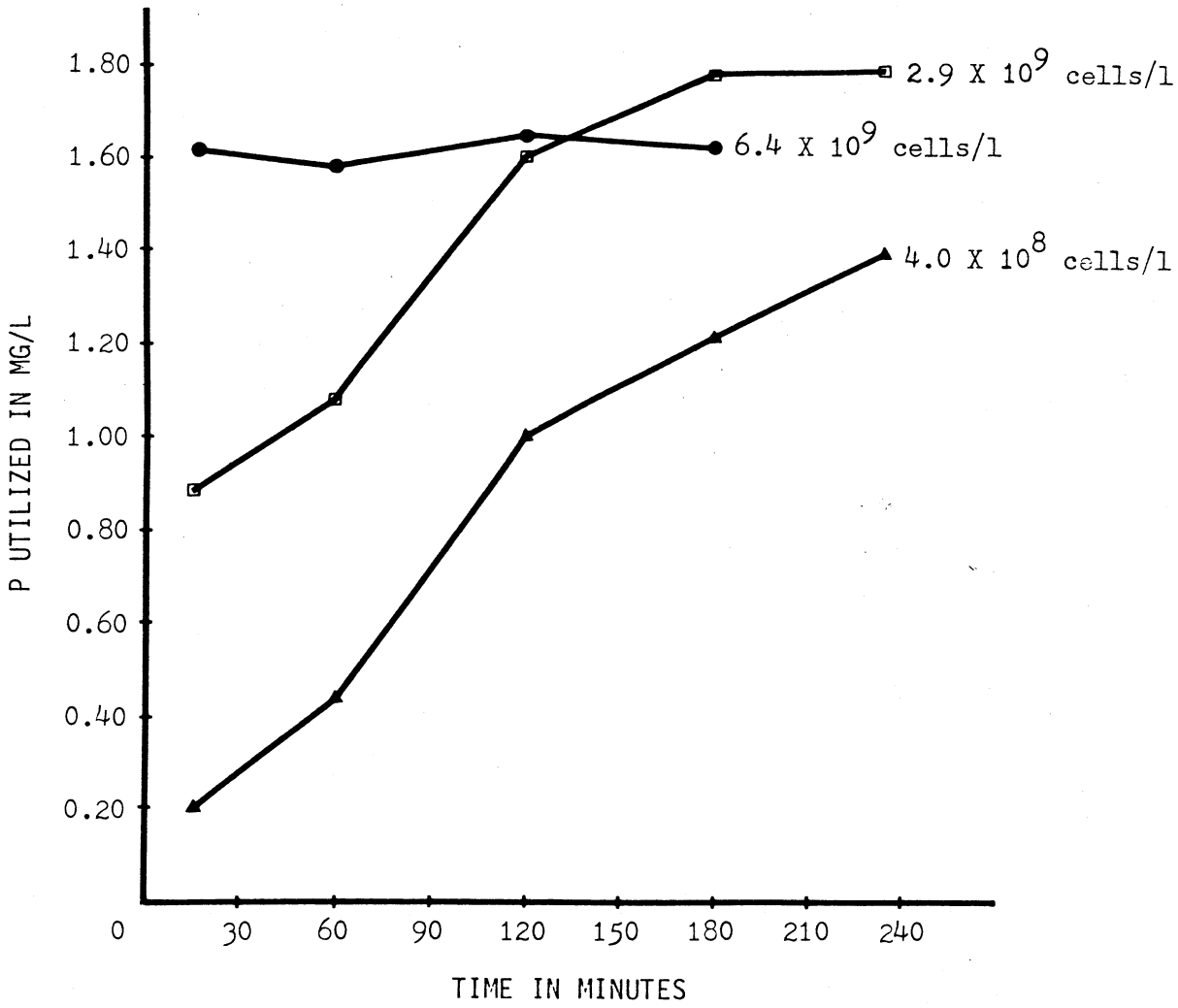

1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0
30
60
90
120
150
180
210
240
P UTILIZED IN MG/L
TIME IN MINUTES
2.9 X 10^9 cells/l
6.4 X 10^9 cells/l
4.0 X 10^8 cells/l

Figure 4

Table 5. Phosphorus removal from 1.75 mg P/l K_2HPO_4 solution by varied cell levels of Scenedesmus sp. incubated in the light at 22 degrees C.

	4×10^8 cells/l		2.9×10^9 cells/l		6.4×10^9 cells/l	
Time	P in solution	Premoved/cell	P in solution	Premoved/cell	P in solution	P removed/cell
Minutes	mg/l	$\times 10^{-8}$ ug	mg/l	$\times 10^{-8}$ ug	mg/l	$\times 10^{-8}$ ug
0	1.70	0	1.88	0	1.73	0
15	1.50	50	1.00	30	.11	25
60	1.26	110	.69	40	.15	24
120	.68	260	.21	57	.08	26
180	.44	310	.10	61	.10	25
240	.30	350	.09	61	---	--

would be true only if the level of P in solution was not limiting. The increased P removal at higher cell levels results in a rapidly diminishing P concentration in solution, which results in a retarded rate of P uptake by these cells. In a wastewater treatment plant, phosphorus is less likely to become limiting because of a continuous input of phosphorus in the influent. A higher algal cell level in a wastewater treatment plant is likely to remove greater amounts of P per unit time and yet maintain a low equilibrium P concentration in the effluent.

At the lower cell levels, the amount of P removed/unit time by all the cells in a flask is much lower than that of the higher cell numbers. This leaves a relatively high P concentration in solution, which the algae respond to. This exposure to higher concentrations of P, for a longer period of time, results in higher rates of P uptake/cell, compared to cells in high cell count flasks. This increased uptake rate, coupled with more available P/cell results in a higher P/cell in the low cell flasks than in the high cell flasks. This increased cell P level could offset the increased rate of P uptake, due to exposure to higher P concentrations, if the cells were to be actively growing. This increased cell P level may not be a factor for at least 24 hours. Use of low cell concentrations in a wastewater treatment plant could adequately remove P, if contact time were long enough, but slightly higher equilibrium P concentrations would be expected because of the higher cell P levels.

Phosphorus Uptake In The Dark

Research by Brown (1975) has shown that P-starved Selenastrum is capable of removing P from solution, in the dark, at a rate comparable to cells exposed to P in the light. However, this study was carried out over a short period of time. In order to use algae in the dark to remove P from wastewater, it was necessary to compare the ability of these cells to remove P over extended periods of continuous darkness.

Two experimental flasks were set up. Each contained 3.58×10^{10} cells/l of P-starved Selenastrum capricornutum and 9 mg/l P. One flask was incubated at 22 degrees C in the darkness and the other was incubated at 22 degrees C in the light.

Figure 5 shows the removal results from the two flasks. The graph shows comparable uptake between the light and dark flasks, over a period of 20 hours. However, there is a slightly lower rate of uptake in the dark in the later stages of incubation. This would be expected if the P uptake is not related to growth. Cells which are incubated in the light will continue to remove P, because of growth initiated by photosynthesis. In the dark, where photosynthesis and therefore growth is nonexistent, P uptake does not continue.

In a wastewater treatment plant, light is not always available. During hours of darkness and when the density of solids in the mixed liquor is high, photosynthesis would be limited by light availability. Based on these results, use of P-starved algae for phosphorus removal would be applicable if the organisms used display uptake characteristics similar to Selenastrum.

Figure 5. Effect of light vs. dark on the uptake of phosphorus from K_2HPO_4 solution by 3.58×10^{10} cells/l of Selenastrum capricornutum.

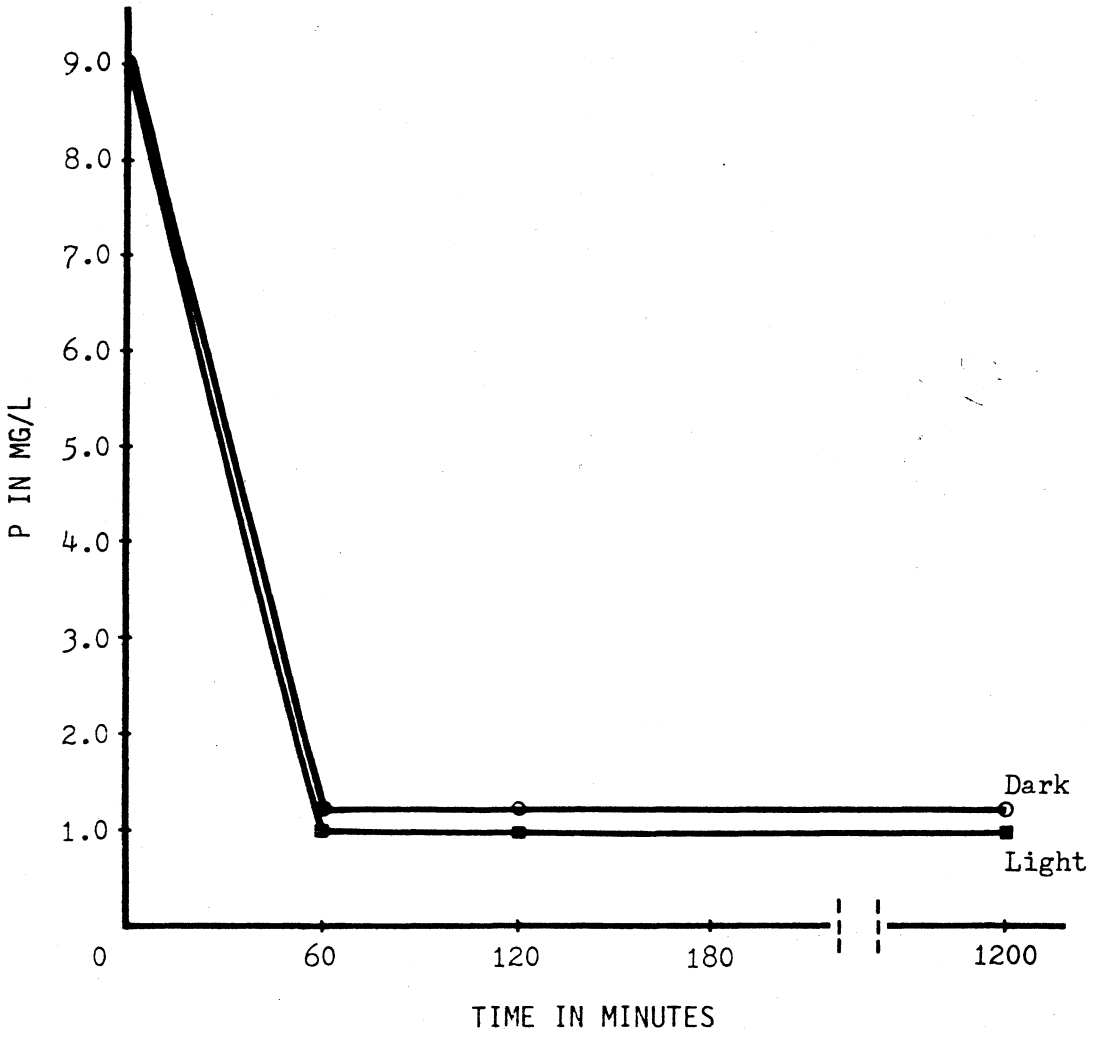

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
P IN MG/L
Dark
Light
0
60
120
180
1200
TIME IN MINUTES

Figure 5

Table 6. Effect of temperature on phosphorus removal from K_2HPO_4 solution by Selenastrum capricornutum and Scenedesmus sp. at 1 x 10^{10} cells/l and 9 mg P/l, in the dark.

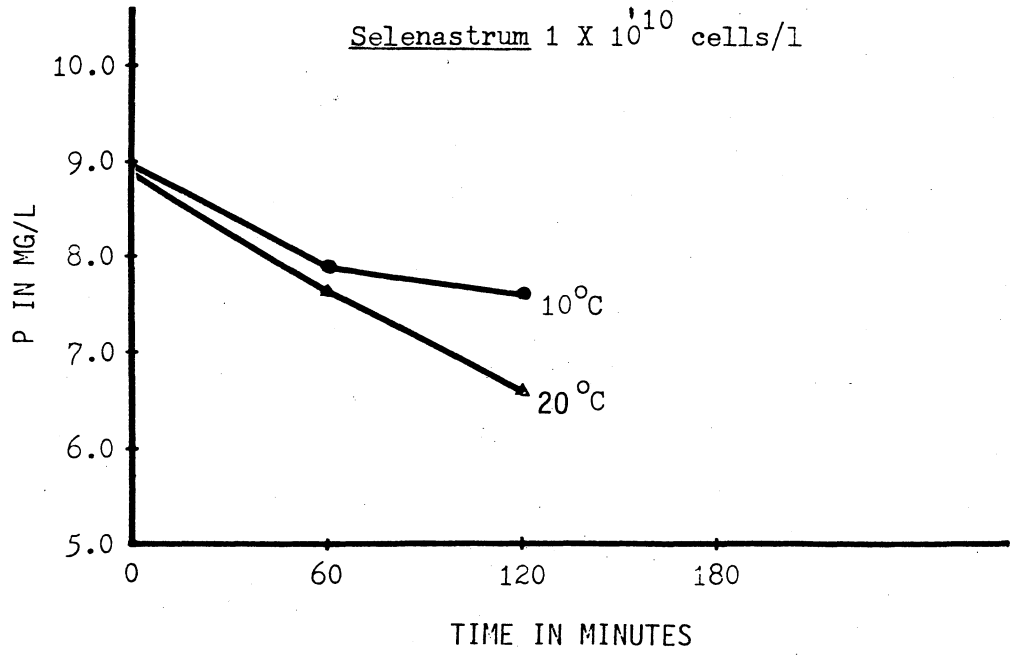
	Selenastrum				Scenedesmus			
	10 degrees C.		22 degrees C.		10 degrees C.		22 degrees C.	
Time	P in solution	P/cell removed	P in solution	P/cell removed	P in solution	P/cell removed	P in solution	P/cell removed
Minutes	mg/l	x 10^{-8}ug	mg/l	x 10^{-8}ug	mg/l	x 10^{-8}ug	mg/l	x 10^{-8}ug
0	8.9	0	8.8	0	9.1	0	9.2	0
60	7.9	10	7.6	12	7.6	15	6.9	23
120	7.6	13	6.6	22	7.3	18	5.8	34

Effect Of Temperature On P Uptake

In order to determine whether or not this method of treatment would be adequate in winter months of northern climates, a set of experiments was conducted. 1.0×10^{10} cells/l of P-starved Selenastrum capricornutum was exposed to 9 mg/l of P in K_2HPO_4 solution. One flask was incubated at 10 degrees C and an identical flask was incubated at 20 degrees C. An identical experiment was set up using Scenedesmus sp. The same number of cells of Scenedesmus (1.0×10^{10} cells/l) was used.

Table 6 and Figure 6 show the P removal data for the two species at the high and low temperatures. The data indicates a depressed treatment at 10 degrees C, for both Selenastrum and Scenedesmus. This is consistent with Van't Hoff's Rule (Giese, 1969). The Q_{10} factor for both experiments appears to approach 2. The Q_{10} factor is much lower in the first 60-90 minutes of treatment, probably due to the fact that the cells start out at a temperature warmer than 10 degrees C, due to the mixing of 100 ml of cells at room temperature with 900 ml of substrate at 10 degrees C. These figures indicate that adequate winter treatment could be obtained by increasing the number of cells exposed.

Figure 6. Effect of temperature on phosphorus removal from K_2HPO_4 solution by *Selenastrum capricornutum* and *Scenedesmus sp.* at 1×10^{10} cells/l and 9 mg/l, in the dark.


Selenastrum 1 X 10^10 cells/l
10.0
9.0
8.0
7.0
6.0
5.0
P IN MG/L
10°C
20°C
0
60
120
180
TIME IN MINUTES

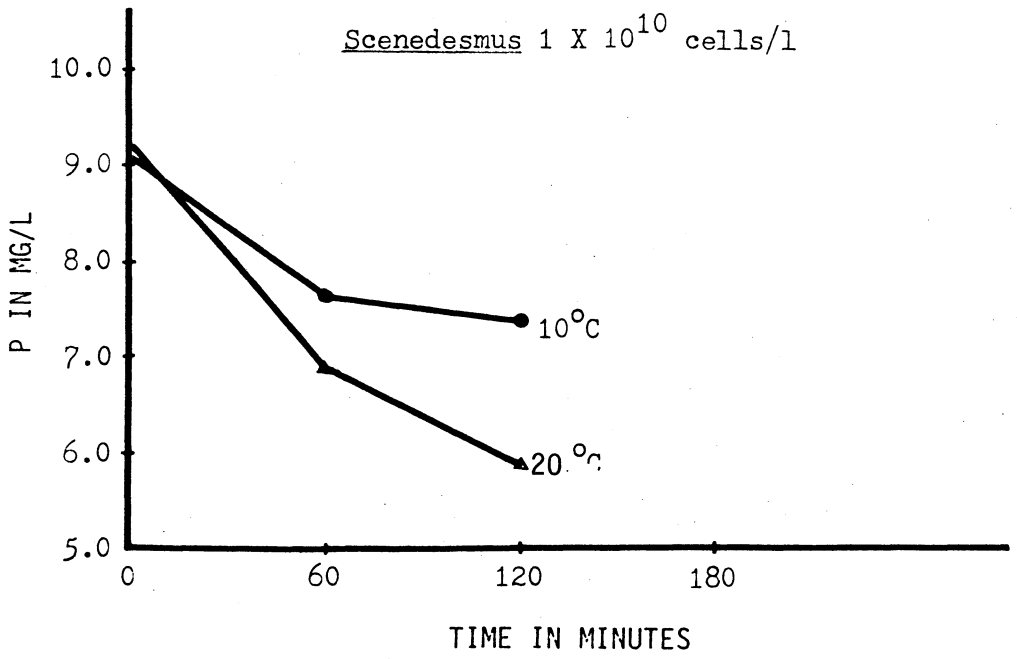

Scenedesmus 1 X 10^10 cells/l
10.0
9.0
8.0
7.0
6.0
5.0
P IN MG/L
10°C
20°C
0
60
120
180
TIME IN MINUTES

Figure 6

Phosphorus Removal From Wastewater

Removal From Clarified Effluent

In the preceeding chapter, it was shown that both Selenastrum and Scenedesmus are capable of rapid removal of P from solution. These solutions did not contain any particulate material other than the algae, nor did they have any waste products, competitive organisms or other substances that are likely to be present in domestic wastewater. The effect of these variables needs to be investigated, in order to determine the feasability of incorporation of this method of P removal into existing treatment systems.

If cells were introduced after the final clarifier, there would be a great advantage, in that a minimum of interference could be expected, due to a low suspended solids content. Also, algal cells would be much easier to recover and reuse at this stage. The major disadvantages to adding cells at this stage would be an increased hydraulic retention time requiring construction of additional tanks.

Two cell levels of Selenastrum capricornutum (1.5×10^{10} cells/l and 6.4×10^{10} cells/l were added to the clarified effluent and incubated in the dark at 22 degrees C for two hours. In a separate experiment, two cell levels of Scenedesmus sp. (4×10^{8} cells/l and 5.9×10^{9} cells/l) were exposed to clarified effluent, under the same conditions. The samples each contained 2.88 mg P/l. Samples were taken and analyzed for orthophosphates, over the two hour incubation period.

The results for both experiments are presented in Table 7 and Figure 7. Both cell levels of Selenastrum show a very rapid P removal, with greater than 96% removal in less than 60 minutes, for the low cell concentration and 99% removal in the same time period for the high concentration. As might be expected, the removal rate was more rapid in the flask containing the higher cell concentration.

Table 7 and Figure 7 show the results of exposing two cell levels of Scenedesmus to clarified effluent. As with Selenastrum, an increase in the cell numbers resulted in an increase in the rate of P removal. Both cell levels removed phosphorus below the .5mg/l mark in less than 60 minutes. This demonstrates that both organisms could adequately treat the effluent at these cell levels, by increasing the retention time of the wastewater by 1 and 1/2 to 2 hours. Additional time would be necessary for separation of the cells.

Removal From Mixed Liquor

The next set of experiments was done to determine whether or not algal cells could be added to the mixed liquor and still achieve the desired removal. Addition of cells to the aeration basins would eliminate the advantage of recycling the algal cells, but would also eliminate the disadvantage of construction of additional treatment tanks. Also, treatment time might be increased without any increase in hydraulic retention time.

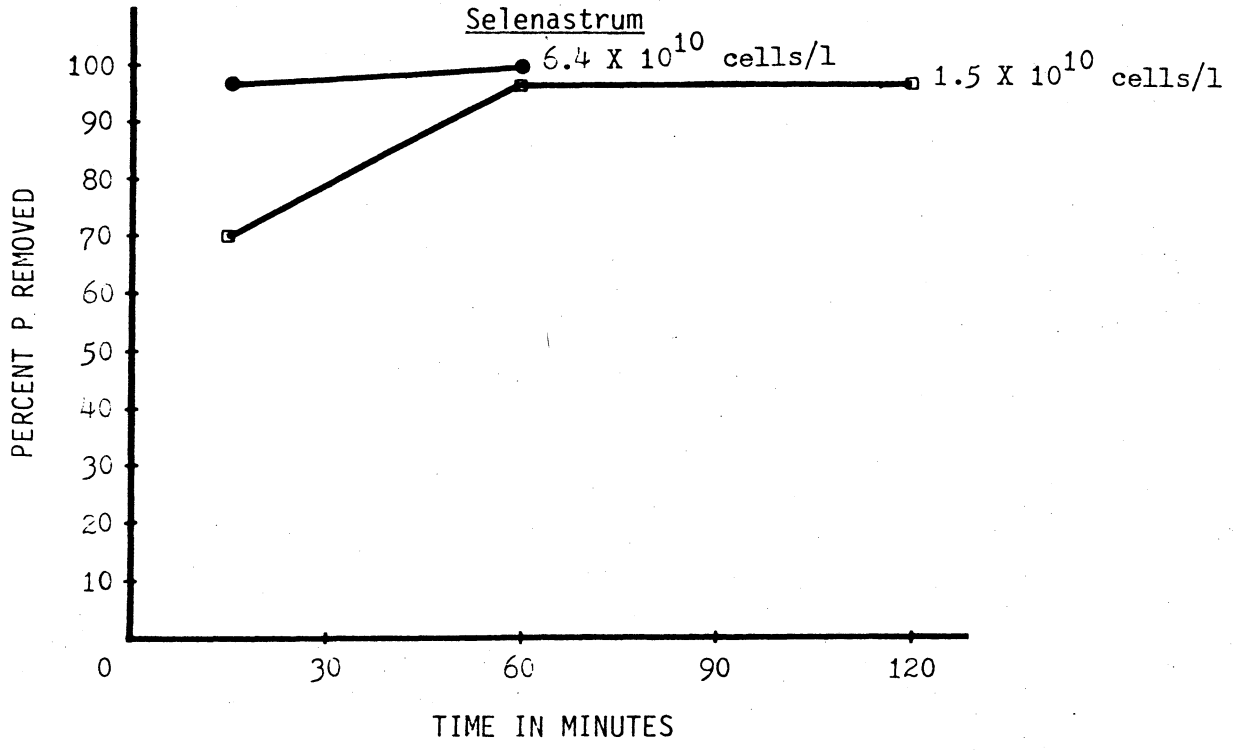
Mixed liquor was spiked with various levels of orthophosphate (zero, 10.3, and 21.2 mg/l) and exposed to 5×10^{10} cells/l of Selenastrum capricornutum. The cells and substrate were incubated in the dark at 22 degrees C.

Table 7. Phosphorus removal from clarified effluent containing 2.88 mg P/l, using various cell levels of Selenastrum capricornutum and Scenedesmus sp, incubated in the dark at 22 degrees C.

	Selenastrum						Scenedesmus					
	1.5×10^{10} cells/l			6.4×10^{10} cells/l			4×10^{8} cells/l			5.9×10^{9} cells/l		
Time	P in solution	P Removal	Increase In cell P	P in solution	P Removal	Increase in cell P	P in solution	P Removal	Increase cell P	P in solution	P Removal	Increase cell P
Minutes	mg/l	%	%	mg/l	%	%	mg/l	%	%	mg/l	%	%
0	2.88	0	0	2.88	0	0	2.88	0	0	2.88	0	0
15	.86	70	336	.10	96	108	2.10	27	487	.42	85	104
60	.10	96	462	.03	99	111	.84	71	1275	.22	92	112
120	.10	96	462	BDL*	--	---	.23	92	1655	.12	96	117

*Below Detection Limit

Figure 7. Phosphorus removal from clarified effluent containing 2.88 mg P/l, using various cell levels of Selenastrum capricornutum and Scenedesmus sp., incubated in the dark at 22 degrees C.


Selenastrum
6.4 X 10^10 cells/l
1.5 X 10^10 cells/l
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
30
60
90
120
TIME IN MINUTES

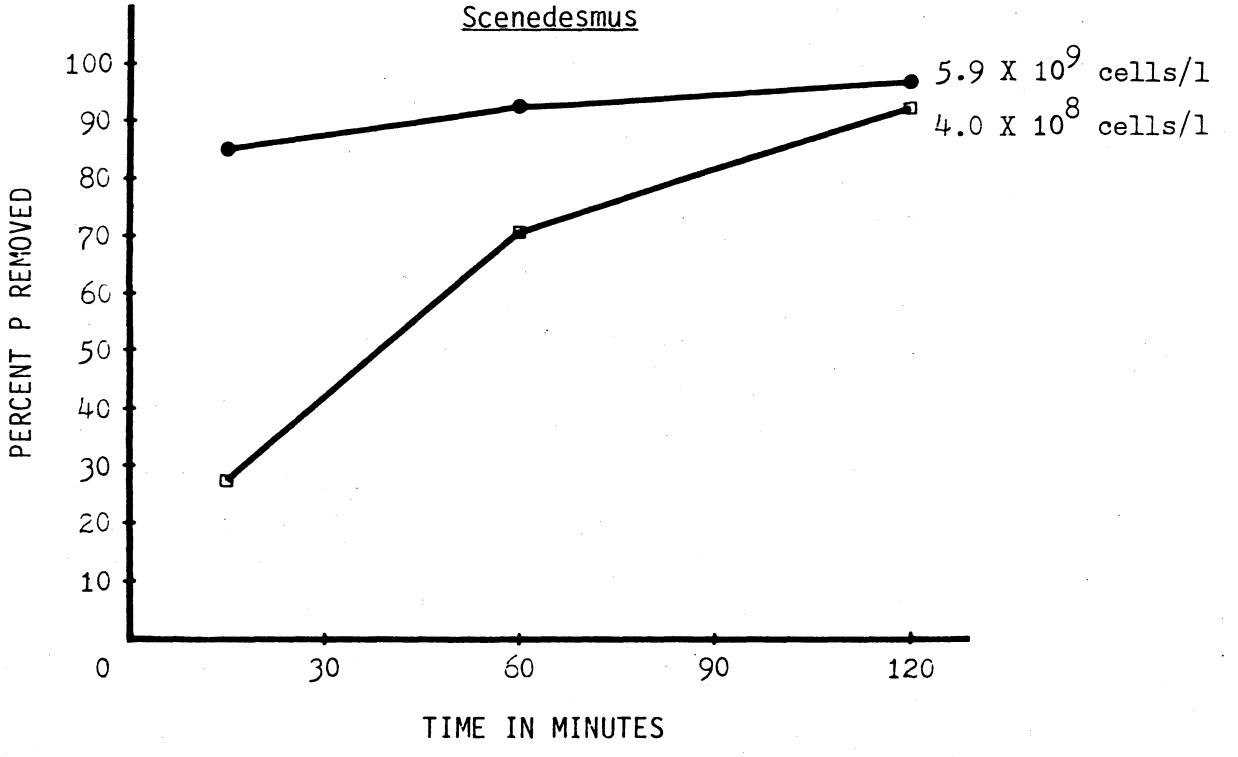

Scenedesmus
5.9 X 10^9 cells/l
4.0 X 10^8 cells/l
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
30
60
90
120
TIME IN MINUTES

Figure 7

Figure 8 and Table 8 depict the results of this series of uptakes. The cells absorbed 95% or more of the phosphorus from all three flasks in less than 2 hours. In three hours, Selenastrum was able to remove 99.6% of 22.99 mg P/l, resulting in an 1100% increase in internal cell P. After 21 hours, a solution equilibrium was established at .168 mg/l P.

The same experiment was run using 1.3×10^9 cells/l of Scenedesmus sp. Figure 9 and Table 9 show the results of this uptake. Scenedesmus was able to remove P down to the detection limit from the 1.71 mg P/l sample (unspiked activated sludge), in two hours. While the cells in the highest concentration, 22.99 mg P/l were able to remove only 64% of the P, this resulted in an increase in cellular P of 2844%. Nine mg/l of P were absorbed from that solution in 2 hours. This was more than twice the amount of orthophosphate found in average domestic wastewater. The cells removed P down to the detection limit, from the 22.99 mg P/l solution in 21 hours, with a 4400% increase in cell P over the initial level.

The above data give some indication of the potential which these cells have for phosphorus removal. Selenastrum was able to remove 4.6×10^{-7} ug P/cell before stabilizing, while Scenedesmus removed 1.8×10^{-5} ug P/cell (40 times the amount absorbed by Selenastrum) and may be capable of more. Figure 10 demonstrates the actual P absorption per cell for the 22.99 mg P/l mixed liquor study, for both species.

In previous experiments, phosphorus removal from K_2HPO_4 solution, clarified effluent and mixed liquor has been examined. Figure 11 is a summary of 2 hour uptake data for Selenastrum at 6.4×10^{10} cells/l and Scenedesmus at 4×10^8 cells/l, in the three substrates. In both

Figure 8. Phosphorus removal from mixed liquor by 5×10^{10} cells/l of Selenastrum capricornutum, exposed to varying phosphorus concentrations and incubated in the dark at 22 degrees C.

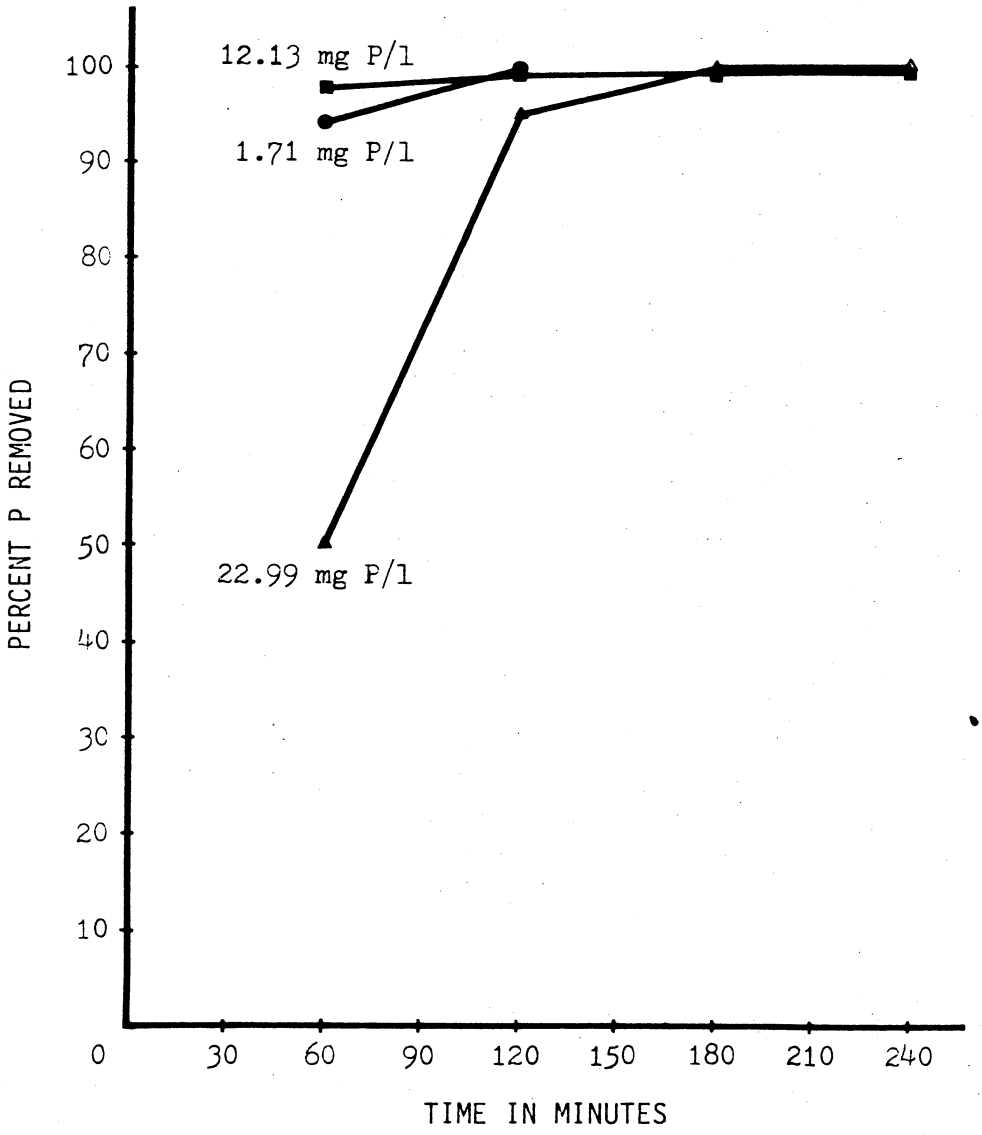

12.13 mg P/l
1.71 mg P/l
22.99 mg P/l
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
30
60
90
120
150
180
210
240
TIME IN MINUTES

Figure 8

Table 8. Phosphorus removal from mixed liquor by 5 x 10^{10} cells/l of Selenastrum capricornutum, exposed to varying phosphorus concentrations and incubated in the dark at 22 degrees C.

	1.71 mg P/l			12.13 mg P/l			22.99 mg P/l		
Time	P in solution	P Removed	Cell P Increase	P in solution	P Removed	Cell P Increase	P in solution	P Removed	Cell P Increase
Minutes	mg/l	%	%	mg/l	%	%	mg/l	%	%
0	1.71	0	0	12.13	0	0	22.99	0	0
60	.10	94	80	.28	97.7	592	11.49	50	575
120	BDL*	--	--	.12	99.0	600	1.13	95	1093
180	---	--	--	.08	99.3	602	.10	99.6	1144
240	---	--	--	.08	99.3	602	.09	99.6	1145
1260	---	--	--	--	--	--	.168	99.2	>1100

* Below Detection Limit

Figure 9. Phosphorus removal from mixed liquor by 1.3×10^9 cells/l of of Scenedesmus sp. exposed to varying phosphorus concentrations and incubated in the dark at 22 degrees C.

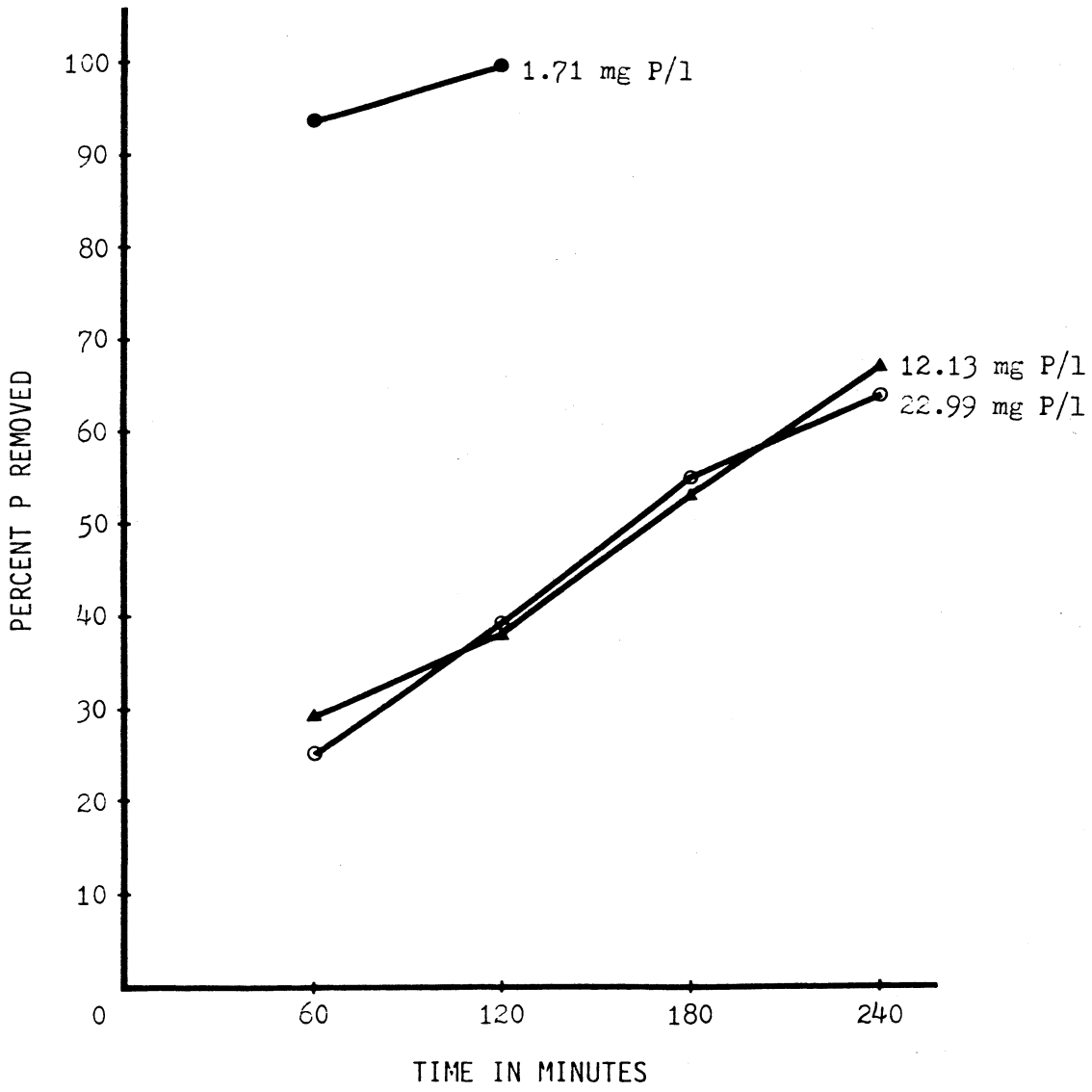

100
90
80
70
60
50
40
30
20
10
0
PERCENT P REMOVED
1.71 mg P/l
12.13 mg P/l
22.99 mg P/l
60
120
180
240
TIME IN MINUTES

Figure 9

Table 9. Phosphorus removal from mixed liquor by 1.3×10^9 cells/l of Scenedesmus sp. exposed to varying phosphorus concentrations and incubated in the dark at 22 degrees C.

	1.71 mg P/l			12.13 mg P/l			22.99 mg P/l		
Time	P in solution	P Removed	Cell P Increase	P in Solution	P Removed	Cell P Increase	P in Solution	P Removed	Cell P Increase
Minutes	mg/l	%	%	mg/l	%	%	mg/l	%	%
0	1.71	0	0	12.13	0	0	22.99	0	0
60	.105	93.8	308	8.55	29	688	17.30	25	1094
120	BDL*	--	--	7.46	38	898	14.0	39	1729
180	--	--	--	5.68	53	1240	10.3	55	2440
240	--	--	--	4.0	67	1563	8.2	64	2844
1260	---	--	--	--	--	--	BDL*	>99	4400

*Below Detection Limit

Figure 10. Phosphorus absorbed per cell of Selenastrum and Scenedesmus from activated sludge with a phosphorus concentration of 22.99 mg P/l, incubated in the dark at 22 degrees C.

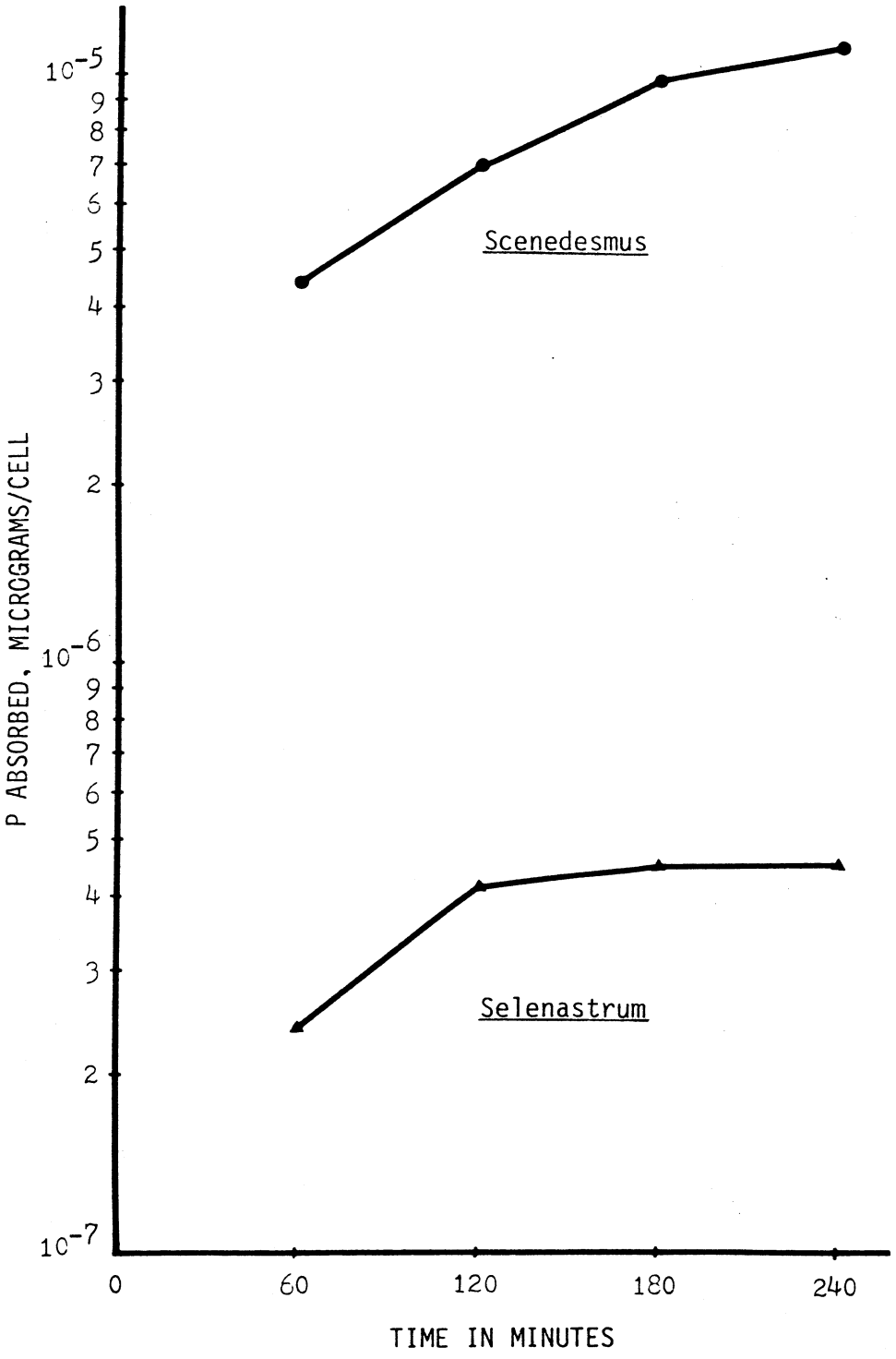

P ABSORBED, MICROGRAMS/CELL
10-5
9
8
7
6
5
4
3
2
10-6
9
8
7
6
5
4
3
2
10-7
Scenedesmus
Selenastrum
0
60
120
180
240
TIME IN MINUTES

Figure 10

Figure 11. Comparison of phosphorus removal from K_2HPO_4 solution, clarified effluent, and mixed liquor, by Selenastrum and Scenedesmus, at equal initial phosphorus levels.

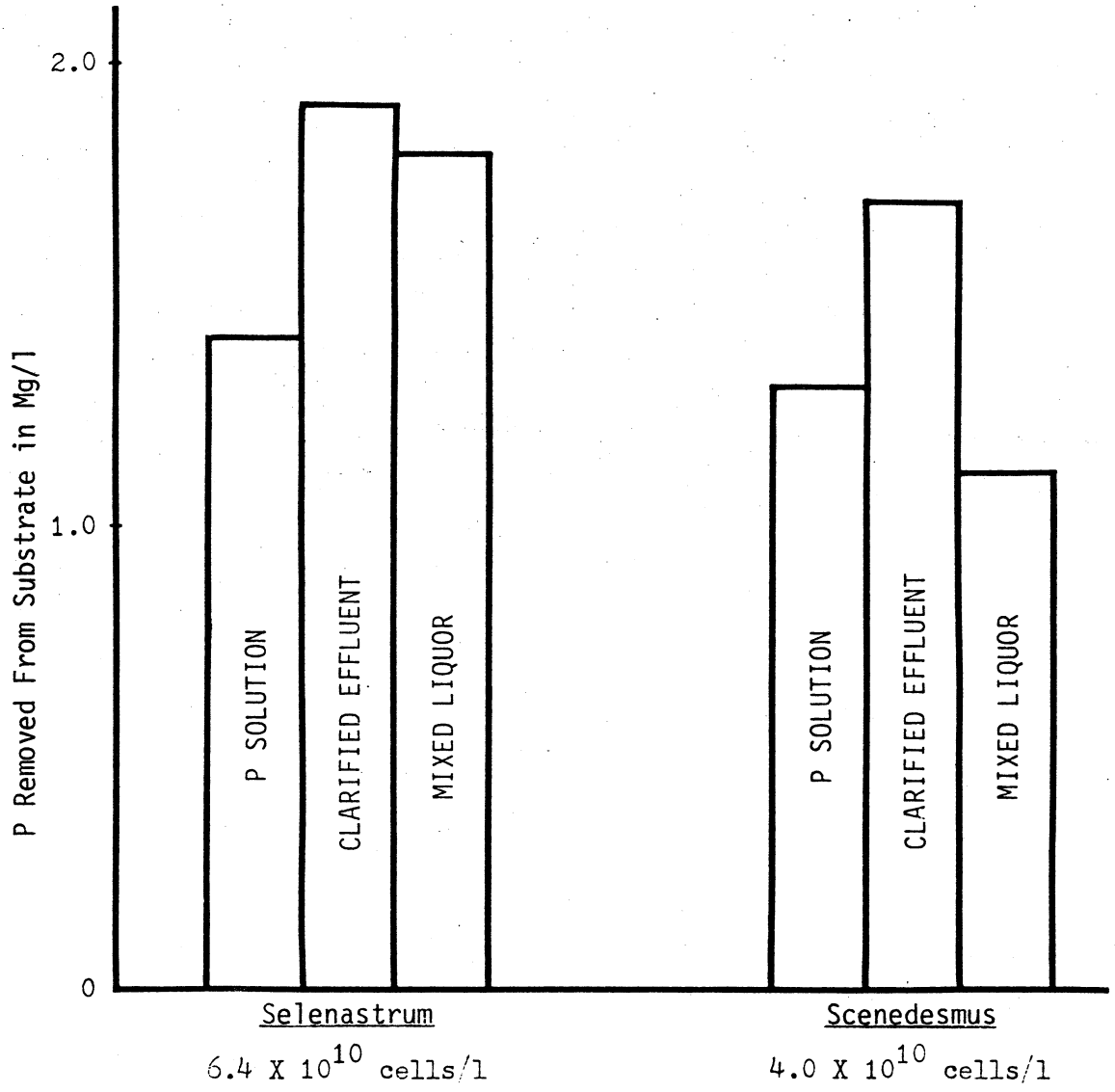

2.0
1.0
0
P Removed From Substrate in Mg/l
P SOLUTION
CLARIFIED EFFLUENT
MIXED LIQUOR
P SOLUTION
CLARIFIED EFFLUENT
MIXED LIQUOR
Selenastrum
6.4 X 10^10 cells/l
Scenedesmus
4.0 X 10^10 cells/l

Figure 11

cases, the clarified effluent substrate shows the most complete removal, while the removal in the mixed liquor appears to be slightly reduced. The increased removal in the clarified effluent compared to the P solution is probably due to the presence of ions which enhance phosphorus uptake (Fogg, 1973). The reduced removal in the mixed liquor may be due to interference by the mixed liquor suspended solids which may block the P absorbing surfaces in the cells. It should be pointed out that analysis in these studies were primarily restricted to inorganic orthophosphate in solution. In reality, the polyphosphate and also the organic phosphates would be hydrolyzed to release P. This study is therefore, probably an underestimate of the P uptake potential of the cells. Scenedesmus has a relatively high phosphatase activity (Fitzgerald, 1975) and these enzymes could hydrolyze a fair amount of organic phosphorus, resulting in an increase in orthophosphate.

The above experiments demonstrate that treatment is a function of cell type, cell numbers and exposure time. If a way could be found to increase the exposure time, fewer cells would be needed. Since cell production is the most expensive part of the process, due to energy costs, a reduction in cell numbers would be desirable. Cell numbers could be decreased if exposure time was increased. In an activated sludge plant, the average retention time in the aeration basins is 4-6 hours. If the algal cells were introduced in the first aeration basin, the cells would be exposed to the phosphorus for 4-6 hours, in addition to the time in the final clarifier. The following sets of uptakes were conducted by using lower cell concentrations than have been used in previous experiments and by increasing the contact time.

P-starved Selenastrum capricornutum, at two different cell concentrations (1×10^{10} cells/l and 5.9×10^{9} cells/l) was exposed to

mixed liquor from the first aeration basin of the sewage treatment plant at Stevens Point. The reaction vessels were incubated in the dark at 22 degrees C.

Table 10 and Figure 12 show the results of this uptake. The 1 x 10^{10} cells/l level of Selenastrum was able to achieve 96% removal in four hours, while the lower cell level was able to achieve only 89% removal in the same time period. This indicates that the lowest number of cells for acceptable treatment probably lies somewhere in between, unless contact time was extended to six hours.

The same experiment was conducted using two cell levels of P-starved Scenedesmus sp. (2.5 x 10^8 cells/l and 8 x 10^8 cells/l). Table 10 and Figure 12 depict the results of this experiment, as well. The lower concentration of cells (2.5 x 10^8 cells/l) removed only 60% of the orthophosphate, after four hours. This is not an adequate level of treatment, indicating that either the cell level must be increased or the contact time must be extended. The higher concentration of cells (8 x 10^8 cells/l) adequately removed 99% of the orthophosphate from the mixed liquor in four hours.

The above studies confirm that it is possible to achieve adequate removal by a reduced number of cells of both species of algae at a longer contact time.

The P uptake which has been discussed thus far has been in terms of orthophosphate. Since phosphorus effluent standards are in terms of total P, an experiment was done to determine how much organic P and polyphosphate would remain in the effluent after treatment by the P-starved algae method.

7.5 x 10^9 cells/l of Selenastrum and 1 x 10^8 cells/l of Scenedesmus were exposed to activated sludge for four hours, at 22 degrees C.

Table 10. Uptake of phosphorus from mixed liquor from the first aeration basin by Selenastrum capricornutum and Scenendesmus sp. in the dark, at 22 degrees C.

	Selenastrum						Scenedesmus					
	1×10^{10} cells/l			5×10^{9} cells/l			2.5×10^{8} cells/l			8×10^{8} cells/l		
Time	P in Solution	P Removal	Cell P Increase	P in Solution	P Removal	Cell P Increase	P in Solution	P Removal	Cell P Increase	P in Solution	P Removal	Cell P Increase
Minutes	mg/l	%	%	mg/l	%	%	mg/l	%	%	mg/l	%	%
0	2.03	0	0	2.03	0	0	2.03	0	0	2.03	0	0
120	.15	93	470	.61	70	710	1.40	31	630	.16	92	584
240	.08	96	487	.23	89	900	.83	59	1200	.01	99	631

Figure 12. Uptake of phosphorus from mixed liquor from the first aeration basin by Selenastrum capricornutum and Scenedesmus sp. in the dark, at 22 degrees C.

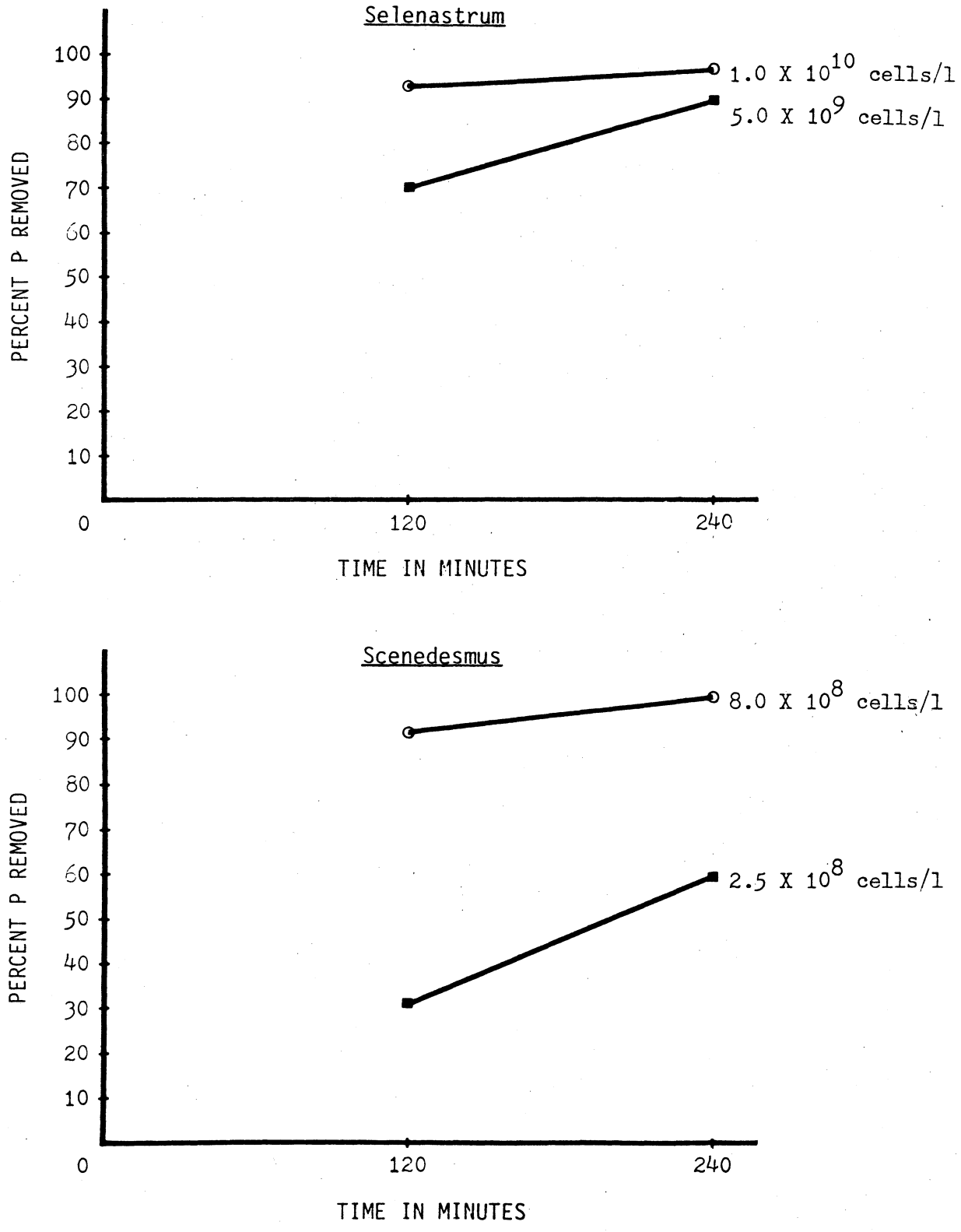

Selenastrum
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
120
240
TIME IN MINUTES
1.0 X 10^10 cells/l
5.0 X 10^9 cells/l
Scenedesmus
PERCENT P REMOVED
100
90
80
70
60
50
40
30
20
10
0
120
240
TIME IN MINUTES
8.0 X 10^8 cells/l
2.5 X 10^8 cells/l

Figure 12

Orthophosphate and total phosphate analysis was performed on samples taken initially, at two hours and at four hours. Figure 13 represents the data from these analyses. The organic fraction of the total P in the solution shows a doubling over the four hour time period, from .06 mg/l to .12 mg/l, in the Selenastrum sample. This is probably due to breakdown of bacterial or algal cells.

The organic fraction of the total P in the Scenedesmus sample shows an increase at two hours and a decrease to the original level at four hours. The probable reason for this is that Scenedesmus displays phosphatase activity. When the level of orthophosphate in solution reaches a certain level, the cellular enzymes attack the organic P in solution. This feature of Scenedesmus may make it a preferable candidate over Selenastrum.

It should be noted, however, that the organic fraction of the total P was quite small, in both cases. The lowest degree of treatment still produced an end product with a total P of less than 0.3 mg/l. This would probably be an acceptable degree of treatment in most communities.

Figure 13. Comparison between orthophosphates and organic P and polyphosphates remaining in solution after P uptake by Scenedesmus and Selenastrum from activated sludge.

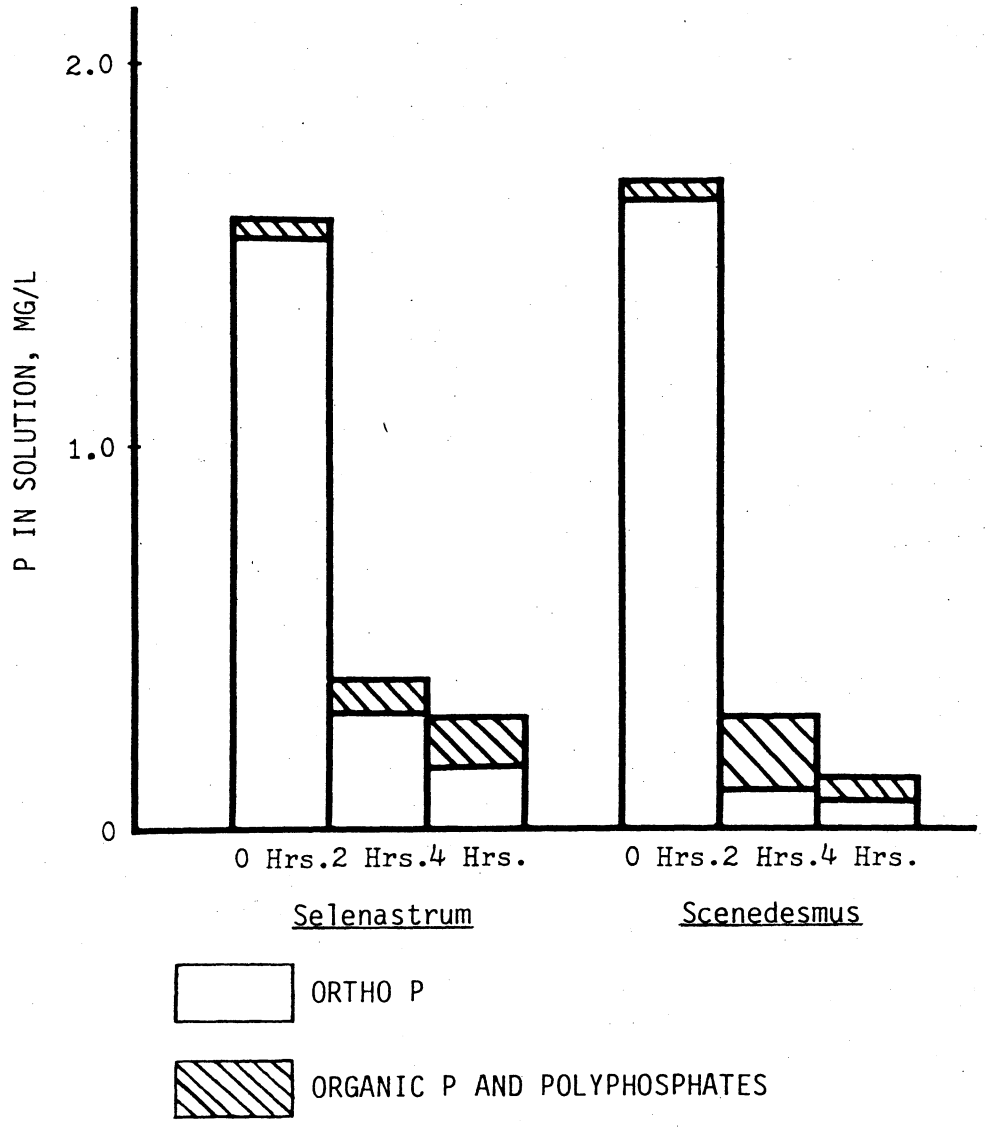

2.0
1.0
0
P IN SOLUTION, MG/L
0 Hrs.2 Hrs.4 Hrs.
0 Hrs.2 Hrs.4 Hrs.
Selenastrum
Scenedesmus
ORTHO P
ORGANIC P AND POLYPHOSPHATES

Figure 13

Nitrogen Removal

In order to determine whether or not algae would remove inorganic nitrogen from wastewater, ammonia and nitrate analysis was performed on samples from two of the phosphorus uptake experiments. 5×10^7 cells/l of P-starved Selenastrum was exposed to 22.22 mg/l of ammonium nitrogen and .8 mg/l nitrate nitrogen. These were the actual amounts of nitrogen (inorganic) present in the activated sludge. After four hours of exposure, there was 3.96 mg/l of ammonium N and .9 mg/l of nitrate N remaining in solution. After 21 hours of exposure, there was no detectable ammonium N and 0.10 mg/l of nitrate N remaining in solution.

1.3×10^9 cells/l of Scenedesmus were exposed to the same levels of inorganic N in activated sludge as described above. After four hours of exposure, 8.03 mg/l of ammonium N and 1.50 mg/l nitrate N remained in solution.

In the above uptakes the nitrate N increases after four hours. This is probably due to oxidation of the ammonium ions during aeration of the mixed liquor. It was found in the Selenastrum experiment, that all detectable ammonium nitrogen was used up after 21 hours. The nitrate N was reduced to 0.1 mg/l. This finding is consistent with the literature which reports that most algae preferentially utilize ammonium N and reduce nitrate N to ammonium N, which is then utilized only after all ammonium N ions are absorbed (Stewart, 1974). The results of this nitrogen uptake suggest that a bonus of the phosphorus removal process might be a reduction in nitrogen in the effluent.

Cell Separation Experimentation

Having discovered that it is possible to remove phosphorus from wastewater using Scenedesmus and Selenastrum, the next step was to separate the algal cells from the wastewater. A series of measurements was made to determine whether or not sedimentation was an effective means of cell separation. Another experiment was set up to determine the feasability of physically separating the cells.

Sedimentation of Cells-Activated Sludge

Two phosphorus uptake experiments were set up. One exposed 1 x 10^8 cells/l of Scenedesmus to activated sludge and the other exposed 7.5 x 10^9 cells/l of Selenastrum to activated sludge. The reaction vessels were incubated at 22 degrees C for four hours, in the dark. The orthophosphate in the Scenedesmus flask was reduced from 1.62 mg P/l to 0.06 mg P/l. The Selenastrum cells reduced the orthophosphate from 1.62 mg/l to .17 mg/l in four hours. After the uptakes were completed, the algae and mixed liquor were mixed and samples analyzed to determine the mixed liquor suspended solids (MLSS). This analysis was also performed on a control sample of mixed liquor which contained no algae. Results of this analysis are displayed in Table 11 and Figure 14. The mixed liquor suspended solids in the Scenedesmus sample was much higher than the control, due to the addition of a large number of relatively large cells. The MLSS of the Selenastrum sample was slightly less than the control. The samples and control were transferred to 1 liter Imhoff cones. Sludge Volume Index, Suspended Solids in the 30 minute supernatant and percent of cells settled were determined.

Suspended Solids In The Supernatant

After 30 minutes of settling, the suspended solids remaining in the supernatant of the control cone was 60 mg/l. The Scenedesmus sample had a residual suspended solids of 85 mg/l while the Selenastrum sample displayed a suspended solids level of 220 mg/l. Since the MLSS of the samples were not equal at the start of the experiment, it is better to compare percent suspended solids remaining in the supernatant, than to compare absolute numbers. This information is located on Table 11. The Scenedesmus sample settled 95% of the solids, while Selenastrum settled only 85% of the solids. The control cone showed 96% settling. This indicates much poorer settling in the Selenastrum cone, than in the other two.

The "% cells removed" column gives a good indication of what is not settling. More than 99% of the Scenedesmus cells settled, while only 53% of the Selenastrum cells settled. It is necessary to note that the object of all this is to remove phosphorus from wastewater. If only half of the Selenastrum cells settle, that means that half the absorbed phosphorus might enter the receiving water. This means that while Selenastrum is capable of adequate treatment, when incorporated into existing activated sludge systems, an additional step would be necessary for the complete removal of algal cells from the final effluent, in order to provide adequate treatment. Scenedesmus, however, is capable of adequate removal of P and settles out with the activated sludge. Based on this, Scenedesmus is probably a better choice for P removal methodology when incorporated into existing activated sludge plants.

Sludge Volume Index

The Sludge-Volume Index (SVI) is "the volume in milliliters occupied by one gram dry weight of activated sludge-mixed liquor solids; after settling in a 1000 ml graduated cylinder, for 30 minutes" (Metcalf & Eddy, 1979). This volume is used to determine how much sludge to return and also as an indication of the settling characteristics of the sludge. A good quality sludge will have an SVI of 40-150 (WPCF, 1977).

Table 11 and Figure 14 demonstrate the SVI data for this settling experiment. The SVI's for all three samples are quite low. This may be due to the aeration of the samples which may have allowed better floc formation than would occur in an actual treatment plant.

It would appear that the samples which contain Scenedesmus have a slightly reduced SVI. It is probably premature to make a statement to this effect based on one experiment, but at least the data indicates that the addition of the algal cells does not have a detrimental effect on the SVI.

Sedimentation of Cells-Clarified Effluent

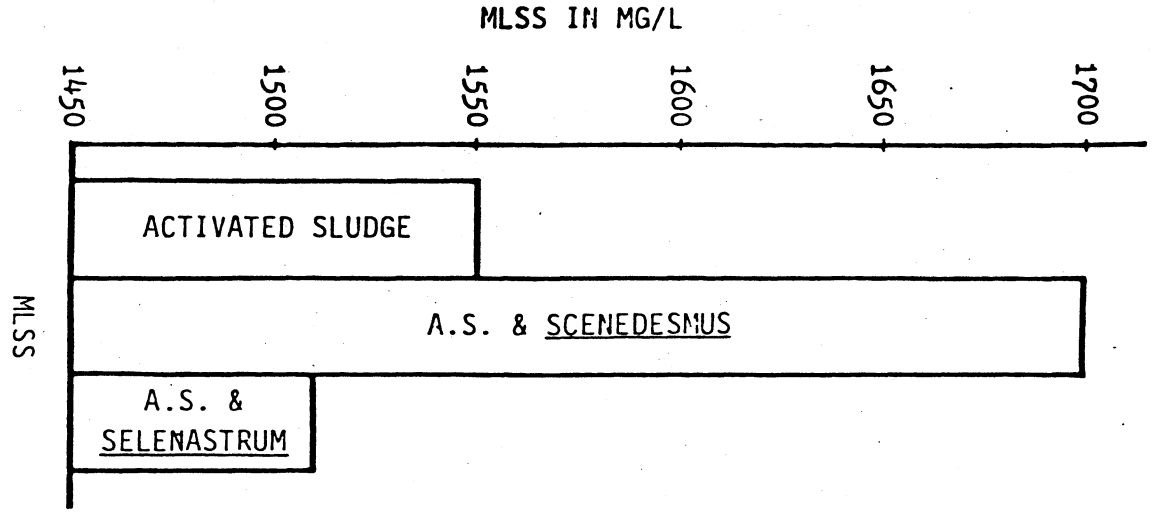
Two Imhoff cones were set up, one containing 1 liter of clarified effluent and 8×10^8 cells of Scenedesmus. The other cone contained 1 liter of clarified effluent and 7.2×10^9 cells of Selenastrum. After 24 hours, only 6% of the Scenedesmus cells had settled and less than 2% of the Selenastrum cells had settled. A time schedule of this type is just not practical, therefore, exposure of the cells to the clarified

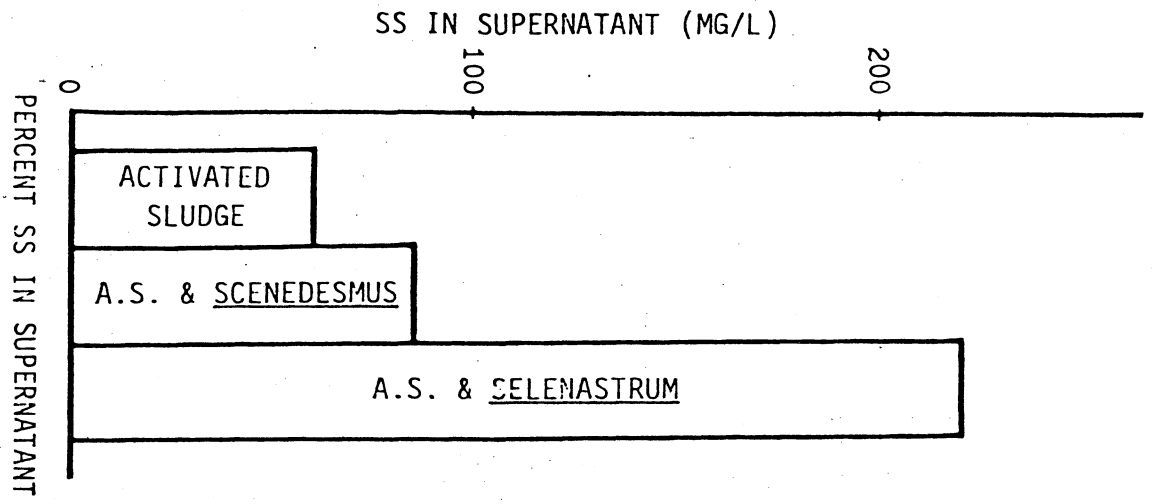
Table 11. Data collected from cell settling experimentation.

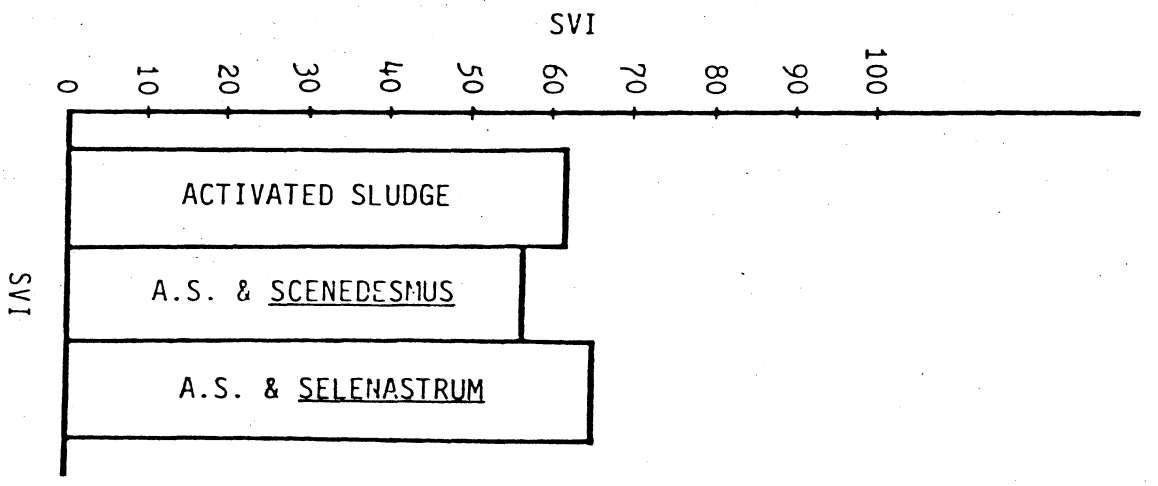
Type of Alga Added	MLSS mg/l	SUPERNATANT			
		SS mg/l	% SS Remaining	% Cells Removed	SVI
Control (none)	1550	60	4	N.A.*	61.3
Scenedesmus 1×10^8 cells/l	1700	85	5	99	55.8
Selenastrum 7.5×10^9 cells/l	1510	220	15	53	66

*Not Applicable

Figure 14. Characteristics of sludge and supernatant resulting from cell settling experimentation.


MLSS IN MG/L
1450
1500
1550
1600
1650
1700
MLSS
ACTIVATED SLUDGE
A.S. & SCENEDESMUS
A.S. & SELENASTRUM


SS IN SUPERNATANT (MG/L)
0
100
200
PERCENT SS IN SUPERNATANT
ACTIVATED SLUDGE
A.S. & SCENEDESMUS
A.S. & SELENASTRUM


SVI
0
10
20
30
40
50
60
70
80
90
100
SVI
ACTIVATED SLUDGE
A.S. & SCENEDESMUS
A.S. & SELENASTRUM

effluent might necessitate centrifugation or another means of separating the cells from the effluent. The probable reason for this slower rate of settling, as compared to the activated sludge samples is that the algal cells in the activated sludge attach to bacterial flocs and gravity has a greater effect on the larger flocs.

Membrane Separation

Another means of cell separation using a semipermeable membrane was also used. The cells were enclosed in cellulose acetate tubing, which was immersed in a P-containing solution. Theoretically, the P ions would flow across the membrane and be absorbed by the cells, thus preserving the concentration gradient. The advantage would be that 100% of the cells would be separated from the effluent without the necessity of an energy-requiring mechanical separation step.

Several variations were tried, including pre-soaking the membranes, reversing the membranes, and allowing a continuous flow of fresh cells to flow through the tubing. In all cases, a 10.4 mg P/l solution was used. The maximum amount of P which crossed the membrane in any experiment was 6% or 0.62 mg P/l. This represents an extremely poor removal. Even if a better removal rate had been obtained with the use of the membranes, it is probable that this method of treatment is not practical for wide application. There would be many problems associated with the use of membranes, including: clogging by suspended solids and bacterial growth, tearing of membranes, replacement of membranes and reduced treatment efficiency due to a low surface area exposed to the phosphorus.

Sludge Digestion

In order to determine what would happen to the phosphorus when the algal cells undergo anaerobic digestion, the settled sludge from the Imhoff cones in the cell sedimentation experiment was digested anaerobically, for 24 days, in the dark, at 37 degrees C. Samples were taken initially, at 7 days and at 24 days. The samples were filtered and the filtrate was analyzed for orthophosphates.

The data from the phosphorus analysis is located in Table 12. The "initial solids" column represents the amount of solids which made up the sludge. In the case of the activated sludge sample, it is largely made up of bacterial cells. In the cases of the other two samples, it represents the bacteria and that amount of algal cells which settled. The "P absorbed By Algae" column represents the amount of P which was absorbed by the algal cells and which then settled with the bacterial floc. If all the algal cells and bacterial cells digested, the Scenedesmus sample should contain 15 mg P/l more after digestion, than the control sample and the digested Selenastrum sample should contain 7 mg/l more orthophosphate than the control sample. This was not the case. After 24 days of digestion, the Scenedesmus sample showed the lowest increase in orthophosphate, while the Selenastrum and control samples showed higher increases in orthophosphate.

The "mg Ortho P Increase/mg Initial Solids" column probably best represents what actually happened. The control sample showed an increase of 3.4×10^{-3} mg P/mg solids. This is probably due to the decay of aerobic bacteria. The Selenastrum sample shows an increase of

Table 12. Seepage of orthophosphates from digesting sludge into sludge supernatant when sludge is digested anaerobically, at 37 degrees C, in the dark.

	Initial			7 Days	24 Days		
	Ortho P	Solids	Absorbed P	Ortho P	Ortho P	Net Increase	Increase
	mg/l	mg/l	mg	mg/l	mg/l	mg/l	mg P/ mg solids
A.S* (control)	2.94	1496	N.A.	35	54	51.06	3.4×10^{-3}
Scenedesmus	1.15	16150	1.54	24	41	39.85	2.5×10^{-3}
Selenastrum	1.62	14880	.72	40	56	54.38	3.6×10^{-3}

*activated sludge

only 6% over the control sample, while the Scenedesmus sample shows a decrease of 36% from the control sample. The Scenedesmus samples and the Selenastrum samples maintained a greenish color throughout the digestion, even though they were incubated in darkness. This indicates that the cells did not completely digest. It may be that the Scenedesmus cells did not digest and actually continued to absorb or adsorb phosphorus as it was released by the bacterial cells.

What this means to an actual treatment plant situation, is that supernatant from the sludge digester could be returned to the aeration basins, without causing a build up of phosphorus in the system. It also means that the digested sludge would contain a higher percentage of phosphorus and would therefore be a more desirable commodity for agricultural use.

CONCLUSIONS

1. Selenastrum capricornutum and Scenedesmus sp. were both capable of removal of adequate amounts of phosphorus from wastewater. The following was found to be true for both species:

   a. The initial rate of P removal was directly related to the concentration of orthophosphate in the wastewater.

   b. The rate of P removal by cells incubated in the dark compares favorably to cells incubated in the light. This assures that adequate P removal would occur at night, with no additional lighting.

   c. No obvious problems exist in culturing the algal species.

   d. Increased cell numbers and increased contact time resulted in a greater P removal.

   e. Rate of uptake was found to be reduced at lower temperatures. This could affect treatment efficiency in winter months. However, this could be overcome by increasing cell numbers in the winter.

2. Scenedesmus is capable of removing larger amounts of P/cell/unit time than Selenastrum.

3. When the algae are introduced in the aeration basins, the suspended solids and total P, after settling, (in the resulting effluent) was much lower when Scenedesmus was used than when Selenastrum was used.

4. Greater amounts of orthophosphate appear to have been removed from clarified effluent as compared to mixed liquor. The increased contact time without need for increased plant hydraulic retention time made possible by introduction of the cells into the aeration basin compensates for the more rapid treatment possible in the clarifier effluent.

5. Digester supernatant, which would be recycled, does not release absorbed P. This results in increased P in the sludge.

6. Considering the above findings together, it can be concluded that Scenedesmus is the preferred species over Selenastrum. Best treatment is achieved by introducing Scenedesmus into the first aeration basin of activated sludge facilities. This allows increased contact time and also complete removal of cells, resulting in a better quality effluent.

FUTURE RESEARCH

This project is an indication of the potential of the P-starved algae for phosphorus removal from wastewater. There are several areas which need more intensive research before this method could actually be put "on-line." The following is a list of some suggested future projects:

1. Investigation of P removal efficiency when cells are exposed to wastes from different types of treatment plants.

2. Construction of a pilot plant, possibly in conjunction with an existing treatment plant to:

   a. Investigate methods of culturing Scenedesmus to increase cell production.

   b. Determine the rate and extent of P removal in a continuous flow system.

   c. Determine costs involved in production of P-starved algae and P removal.

   d. Study the effect of the treatment on settling properties and quality of the settled sludge.

   e. Study the affects on sludge digestion and the resulting supernatant.

3. A laboratory and pilot plant study to determine rates and extent of nitrogen removal.

4. Examine the effects of P-starved algae on the resultant BOD in the activated sludge process, in the lab as well as in the pilot plant.

LITERATURE CITED

American Public Health Association. 1976. Standard Methods for the Examination of Water and Wastewater. 14th edition. American Public Health Association, New York.

Azad, Hardam S. and J. A. Borchardt, 1970. Variations in phosphorus uptake by algae. Environmental Science and Technology.

Barth, E. F. 1978. Implementation of nitrogen control processes in Advances in Water and Wastewater Treatment: Biological Nutrient Removal. Wanielista, Martin P. and Eckenfelder, W. Wesley Jr. 1978. Ann Arbor Science Publishers, Inc.

Bartsch, A. F. 1969. Provisional Algal Assay Procedures. Joint Ind./ Govt. Task Force on Eutrophication P.O. Box 3011. Grand Central Station. New York. 10017.

Batterton, J. C. and C. Van Balen. 1967. Phosphorus deficiency and phosphorus uptake in the blue green alga Anacystis nidulans. Canadian Journal of Microbiology. 14:341-348.

Black and Veatch. 1976. Process Design Manual for Phosphorus Removal. U. S. Environmental Protection Agency.

Borchardt, J. A. and H. S. Azad. 1968. Biological extraction of nutrients. Journal of Water Pollution Federation. Volume 40, No. 10.

Bouck, David W. 1978. Nutrient removal in three stage processing. in Advances in Water and Wastewater Treatment: Biological Nutrient Removal. Wanielista, Martin P. and W. Wesley Eckenfelder, Jr. Ann Arbor Science Publishers, Inc.

Brown, E. J. 1975. Kinetics of P uptake by aquatic microorganisms. PH.D. Thesis, University of Wisconsin. Madison, Wis.

Bush, Harry L. 1979. Superintendent of Public Works, Paw Paw, Michigan. Personal Communication.

Doran, M. D. 1975. Phosphorus removal by activated algae. M.S. Thesis, University of Wisconsin. Madison, Wis.

Fair, G. M., Geyer, J. C. and D. A. Okun. 1968. Water and Wastewater Engineering. Volume 2. p34-10.

Fitzgerald, G. P., M. S. Torrey and G. G. Gerloff. 1975. Green Bay self-purifies algal nutrients. Water and Sewage Works. Volume 122. No. 6.

Fogg, G. E. 1973. Phosphorus in primary aquatic plants. Water Research. Volume 7. p91.

Fuhs, G. W., S. D. Demmerk, and E. Cunelli and M. Chen. 1972. Characterization of phosphorus limited plankton algae. Limnology and Oceanography. Special Proceedings. 113-133.

Giese, Arthur C. 1969. Cell Physiology. W. B. Saunders Co.

Hammermeister, Dean. 1978. University of Wisconsin, Stevens Point, Wisconsin. Environmental Task Force. Personal Communication.

Kuhl, Adolf. 1962. Inorganic phosphorus uptake and metabolism. in Physiology and Biochemistry of Algae. Edited by Ralph Lewin. Academic Press.

Kuhl, Adolf. 1974. Phosphorus. in Algal Physiology and Biochemistry. edited by W.D.P. Stewart.

Matsch, L. C. and Drnevich, R. F. 1979. Phostrip: a biological chemical system for removing phosphorus. in Advances in Water and Wastewater Treatment; Biological Nutrient Removal. Wanielista, Martin P. and W. Wesley Eckenfelder Jr. Ann Arbor Science Publishers, Inc.

Metcalf and Eddy, Inc. 1975. Report to the national commission on water quality. An assessment of technologies and costs for publicly owned treatment works. Vols. 1 and 2, prepared under P. L. 92-500, Boston.

Metcalf and Eddy, Inc. 1979. Wastewater Engineering. McGraw Hill Book. C.

Posthuma, A. 1979. Williams and Works Engineers and Surveyors, Grand Rapids, Michigan. Personal Communication.

Public Law 92-500. 1972. Federal Water Pollution Control Act of 1972. U. S. Federal Water Pollution Control Administration. U. S. Government Printing Office.

Rhee, G. Y. 1972. Competition between an alga and an aquatic bacterium for phosphate. Limnology and Oceanography. Vol 17. p505-514.

Rhee, G. Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in Scenedesmus sp. Journal of Phycology. Vol 9. p495-506.

Sawyer, C. N. 1968. The need for nutrient control. Journal of the Water Pollution Control Federation. Volume 40, No. 3. p363-370.

Sagher, Aga. 1976. Availability of soil runoff phosphorus to algae. PH.D. Thesis. University of Wisconsin, Madison, Wisconsin.

Shapiro, Joseph. 1968. Studies on natural factors affecting phosphate absorption and utilization by algae. Water Research. Volume 2 No. 1. p21-23.

Stewart, W.D.P. 1974. Algal Physiology and Biochemistry. Chapter 21 by I. Morris. p584-585.

Water Pollution Control Federation. 1977. Wastewater Treatment Plant Design. Lancaster Press, Inc.

Wetzel, Robert G. 1975. Limnology. W. B. Saunders Co. p243-245.