# THE USE OF CHEMOSTATS
# FOR THE STUDY OF ALGAL DYNAMICS

by

Charles M. Weiss

Professor of Environmental Biology
Department of Environmental Sciences and Engineering
School of Public Health
University of North Carolina
Chapel Hill, North Carolina

## THE USE OF CHEMOSTATS FOR THE STUDY OF ALGAL DYNAMICS

The use of chemostats for algal bioassay of nutrient elements is justifiable with reference to obtaining information relating to growth dynamics. However its routine use may not be justified due to its inherent difficulty of operation. In theory its advantages are based upon the concept of a steady state operation in comparison to the continuously varying environmental conditions of the batch assay, e.g. changes in nutrient concentration. The chemostat is claimed to be useful for assaying transient conditions because algal growth in the chemostat is operating under dynamic conditions at constant nutrient and cell concentrations. Because of the sensitivity of response to subtle changes in the environment, it theoretically provides a basis for assessment of abrupt or subtle changes in the environment.

The disadvantages of the chemostat relate primarily to the problems involved in operating it as a routine assay technique. Questionable results are generally obtained at very low nutrient concentrations equivalent to oligotrophic situations. These results in part may be attributable to fluctuations in flow rate as well as growth that may occur on influent lines and containers. Chemostats operating at low nutrient concentrations are functioning under difficult experimental conditions, since these concentrations are difficult to measure accurately. The maintenance of constant operating conditions in chemostats require almost constant attention as well appreciation of the theoretical basis of chemostat operation which may be generally beyond comprehension of the average technical staff. The equipment required to maintain constant operating conditions is generally complicated, subject to frequent failure and malfunction and generally leads to a high degree of frustration by the operating personnel since long runs may be wiped out due to failure at critical times. The operation of a chemostat is theoretically based on the uniform dispersion and suspension of the organisms throughout the media. If attachment organisms are present in the culture which grow on the wall of the chemostats, interpretation of data becomes most difficult and unreliable.

A chemostat in theory is basically a model of a highly simplified ecosystem. The Michaelis-Menton and Monad modifications of the growth of single cell organisms in suitable substrate concentrations, provides a theoretical basis for describing changes in cell numbers and mass. When these concepts are applied to the growth of algae in a chemostat, the growth response appears to follow particularly at high nutrient concentrations, such as would be found in the effluent of sewage treatment plants or in polluted streams or lakes. These high nutrient concentrations are those which permit short residence times in the chemostat probably no longer than a maximum five to seven days. The theoretical concepts do not, however, appear applicable to the growth of organisms at nutrient concentrations which require long residence times. Under these conditions the rate of multiplication of cells is so slow that washout and multiplication rate are essentially the same and no change in numbers or mass can be readily measured. The chemostat then becomes basically a batch test and the hydraulic complexity of the chemostat is not justified.

In developing a model of algal growth or response to environmental factors, it needs to be considered that the organisms in question are extracting from the environment both elemental materials, as well as molecular configurations which are then reassembled by biochemical processes into new cellular constituents which finally results in the situation where the cell divides forming two new cells. Thus, the simplest model to describe algal response to nutrient levels in the aquatic environment consists of A, representing the particular algal species; B, the elemental and molecular substances utilized in cell multiplication and the reaction of A + B giving rise to C which is cell number or mass. The reaction can be described as A + B = n + 1. The excess of 1 is that number or mass which is considered undesirable.

In this simplest of algal growth models unknown factors include both the nature of the species under consideration, the rate at which it uses specific elemental or molecular substances and that number or mass of the particular species which is considered undesirable. At the elemental level of nutrient requirement, the quantities and state of oxidation of nitrogen and phosphorus is a variable which is still not clearly understood for the many species of algae that have been described as responding with high multiplication rates when certain critical nutrient levels have been reached or exceeded. The requirements for specific molecules, such as vitamins as a requirement for growth, is another unknown in the algal growth mechanism and particularly with reference to rates of growth which may lead to undesirable conditions. Thus, the ultimate choice of assay procedure, chemostat or batch, will depend on the nature of the response to be measured and which most accurately relates to the characteristics of the growth material.

DISCUSSION

MODELS OF ALGAL POPULATION DYNAMICS

BASED ON THE CHEMOSTAT APPROACH

SANDERS: We are looking at one of the problems, most of these chemostats are not sterile. At least they start off sterile and they don't remain sterile over a period of weeks, so you have a fixed system, and one part of the system is not responsive.

WEISS: That is quite correct. The system we are trying to establish does require that the test water be preceded by fresh water. Most of the water we use has been treated by filtration.

WATTS: You have used the word, verify, and I think this is a good word to scrub from your vocabulary. I would suggest that the word you want to put in is adequacy, because if you ever want to verify a model, you really want to test its adequacy. Only by looking for lack of this (adequacy) do you start to learn. The other thing, I think biologists start to raise their eyebrows when anybody says, I had to change the parameter when I took this model over to another lake. This seems to suggest that they are saying this casts a lot of doubt upon the problem. I would say this is not the case, the thing is that when you have to start changing the structure of a model, then you are certainly casting doubt upon the model. When you have to change the parameter, these parameters are functions of other things and this is giving you information. We should look for why these parameters are having to be changed. These so-called constants in your equations, What are they actually constants of?

BELLA: Let's assume, for instance, that the algae did have a vertical distribution, against reality. That would mean they could grow vertically.

They could produce, simply because the only algae that contribute to the growth are the ones that are lightened.

O'CONNER: That is what I tried to get over. I knew it wasn't very adequate. The average over the depth. This is the growth.

BELLA: But if your algae are all located down at the bottom, your argument that your time scale allows you to better make the assumption of complete mixing. I don't think that argument is quite as valid here. If you didn't have that complete mixing the growth would be low regardless of your time scale.

MOREAU: Would you comment on how you arrived at this resolution of time in terms of weekly averages specifically in the case which we are looking at here?

O'CONNER: Specifically, in the case we are looking at here, we zeroed in immediately on how good it was. The question is, now that we have gone this far, why don't we go a little further and sample next year's? You could probably sample three times a week, but I think you would probably get a feedback from the modeling effort, saying that it doesn't agree with the data.

MOREAU: Could you comment on resolution of time in terms of other kinds of water quality considerations?

O'CONNER: In that respect, something like the surface elements, like detergents accumulating in the environment. You can deal with the lakes in a series of five tanks, and go through the same procedures.

PATTEN: I would like to get some clarification on mixing and growth relation.

O'CONNER: We took that equation and took it to the system two ways:

laterally and vertically, so now the model looks like top layer and bottom layer.

FRUH: Going back to the assay as a model in adequacy, is it part of your plan now that somebody should go back into the lakes and check whether your growth rate and production mass are similar to the lake itself?

WEISS: The original concept, the procedure, the PAAP test, was to use the batch test as an assay procedure for the establishing of nutrient levels in certain water. The contract was distributed around the country in order to evaluate local conditions. There were many kinds of problems involved in putting the chemostat together. In the original design, the chemostat and batch test were to be checked against the surface waters which were to be prepared by certain standard procedures we have been able to perform simple experiments funneling through membranes.

FRUH: Do these tests give you the nutrients?

WEISS: Your question was, and this is what is answered by the test itself, as far as this contract work, will the two tests give us essentially the same information and will one be easier to use than the other?

BARTSCH: Let me respond to your question, also. Obviously one of the key questions with respect to assaying procedures is whether or not and to what extent in judgment you apply this information to natural conditions. With respect to this particular effort, we would not have reached that stage of application yet, but we can point to some brilliant applications where the procedure has been applied in the field. Dr. Shapiro has done some work on an estuary in the Port of Huron. In connection with this program for the improved assay procedures, we contemplate that we will have reached an appropriate point when the major effort will shift over to determining how to apply this knowledge to the field. We can already foresee many difficult problems. One, for example, Case Three, we call it: How in the world do you take a sample from nature and say to it, Now what would you be like if we reduced the phosphorus content by fifty percent? How in the world do you do that without changing some of the other qualities of this sample? We have some ideas, but please tell me, write to me, call me collect - These procedures, which are provisional, are the same as the PAAP Test, except that we hope eventually to remove the first key, which is where it is official. These techniques are now delineated in a document which I would be willing to make available to any of you who intend to use it, and if you have that kind of desire, show some hands and I will put a pad up here and get your name and address.