CHAPTER - I

INTRODUCTION

Low cost waste stabilization ponds may be broadly classified under three heads : aerobic, anaerobic and facultative depending upon loading, mixing, depth and the nature of biological activity. Of the three types, aerobic stabilization ponds may be designed on two main concepts: (a) minimum depth with maximum algal production and (b) induced mechanical mixing or aeration without necessarily involving photosynthetic oxygenation(Gloyna, 1965, 1971).

(a) Minimum depth with maximum algal production:

This type of pond is called the high-rate oxidation pond which has passed the experimental stage of development. The basis for development is the conversion of as much carbon dioxide into algal cell material as possible. In this manner both maximum protein and oxygen production can be obtained. According to Gloyna (1971), it would appear that this type of pond requires unusual amount of of attention and therefore uneconomical. When there is a market for plant protein in the near future he thinks that it will become very popular.

But the future of the world is closely linked up with the urgent need for mass production of cheap protein and pure water which are in short supply on account of increasing population at the rate of 1.9% and increasing industrial development. So, new techniques and new sources of supply must be sought. This can be met by treating waste-waters to such an extent that they can be used straightway for water treatment for drinking purposes, irrigation or industrial purposes. At the same time the fertilizing elements - nitrogen and phosphorus - from the wastes may be profitably used in the large scale manufacture of edible protein for animals and men; and thus, also indirectly prevent eutrophication of receiving waters. All these triple benefits can be had economically and at one stroke by employing the high-rate aerobic lagoon process, in which the principle of photosynthetic oxygenation is solely employed. This process, Oswald (1962) hails as " The Coming Industry of Controlled Photosynthesis".

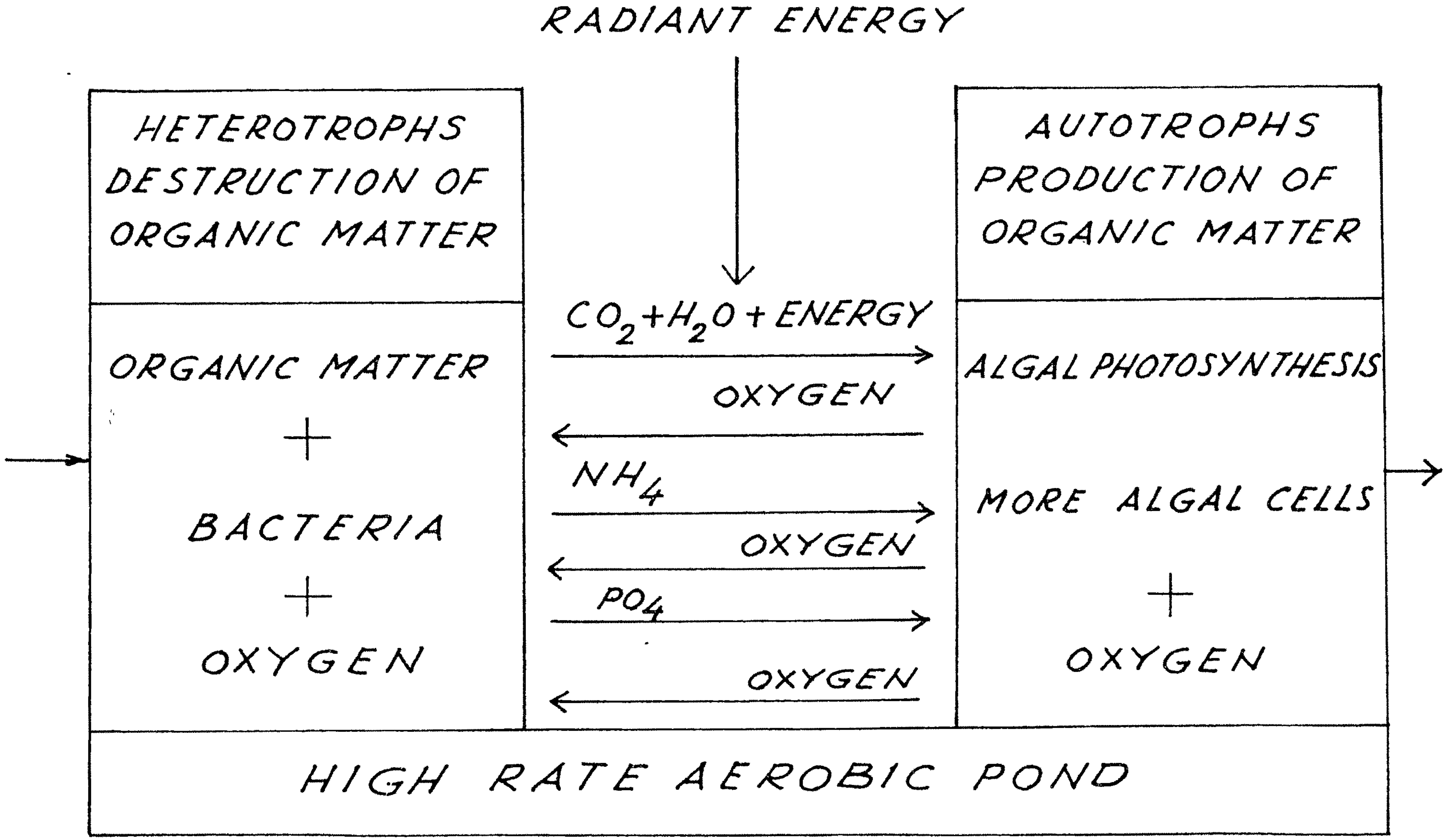
(b) Induced mechanical mixing and aeration:

This can be achieved in four ways :(i) recirculation of oxygen rich waters from the effluent of a waste stabilization pond system to the influent area; (ii) induced surface aeration; (iii) compressed air, and (iv) combination of the above three processes. Of these four methods, ponds fitted with surface aerators and compressed air systems known as "aerated lagoons" are becoming very popular. In other words, another concept which is equally important and gathering momentum, is the adoption of a really low-cost waste treatment system for all kinds of colourless and coloured wastes by which satisfactory BOD removals with sharply increased organic loadings can be had with the most economical utilization of land. These factors seem to have contributed to the development of aerated lagoon process since 1960, which is really a precursor of the oxidation ditch process and which leads further into the extended aeration activated sludge process.

High-rate aerobic ponds (Fig. 1)

The concept of high-rate oxidation pond system had its origin and development in the University of California (Gotaas and Oswald 1955 and Oswald and Gotaas 1957). It has been studied in New Zealand (Hicks 1958), Taiwan (Soong 1961), in the Pirana Sewage Farm at Ahmedabad, India (Ganapati et al 1965) and in Australia (McGarry 1967). A high-rate pond research programme has recently been initiated in Bangkok, Thailand at the Asian Institute of Technology (McGarry and Tangkasame 1971) and " Studies on Sewage Purification " in the Biochemistry Department of the M.S. University, Baroda under an AmPL-480 Research Programme

Fig. 1



RADIANT ENERGY
HETEROTROPHS DESTRUCTION OF ORGANIC MATTER
ORGANIC MATTER + BACTERIA + OXYGEN
CO2+H2O+ENERGY
OXYGEN
NH4
OXYGEN
PO4
OXYGEN
AUTOTROPHS PRODUCTION OF ORGANIC MATTER
ALGAL PHOTOSYNTHESIS
MORE ALGAL CELLS + OXYGEN
HIGH RATE AEROBIC POND

(Ramakrishnan and Ganapati, 1974) to study conditions for a maximum yield of algae and reduction of sewage ~~economics.~~ treatment costs.

Method of working:

Gotaas and Oswald (1955); Gotaas and Golueke, (1957); Oswald and Gotaas, (1957); Oswald, et al (1957; 1958); Golueke et al. (1958); and Oswald et al. (1959 a and b) have demonstrated successfully the working of high-rate aerobic lagoons. It must be shallow enough (9" - 18") to permit the entry of light almost to the bottom of the pond; and it must be mixed at least once a day to maintain the deposited sludge in an aerobic condition by creating a flow velocity of about 1.5 feet per second. This velocity is considered sufficient to generate the required tractive force to resuspend both deposited algae and sludge with the oxygen-rich supernatant layers. A healthy sludge comparable to activated sludge is maintained in the pond, provided mixing is carried out for about three hours a day. Following an initial accumulation, the volume of aerobic sludge does not increase, but rather remains constant indicating essentially that "total oxidation" is taking place. During mixing, the sludge is suspended throughout the pond volume, but within 15 minutes after mixing ceases, more than 80% of the sludge settles and is again distributed over the pond bottom so that sunlight entering the surface is not obscured. The algae do not adhere to the sludge nor do they become incorporated in it. Rather they remain suspended to continue the synthesis in sun-light unless an extremely high pH brings about autoflocculation.

Quality of reclaimed water:

Oswald and his colleagues showed that as a result of culturing algae in sewage using the high-rate aerobic process:-

(a) an effluent fit for industrial use may be produced at the location where such water is required;

(b) a water fit for domestic or irrigation purposes may be produced provided the influent waste water is low in sodium and sulphate; and

(c) fertility compounds such as phosphate, ammonia and carbon dioxide are removed from sewage and no nitrate is formed. So algal free-effluent may be discharged into streams or lakes.

It is possible to grow continuous crops of algae on sewage in a form acceptable to livestock feed. Yields of vegetable protein per acre may be 20 - 40 times those obtained in most of the conventional forms of cropping. Thus, an acre devoted to algal culture might eliminate the necessity for irrigating 30 acres of crop land, and hence an equivalent amount of water might be released for other purposes. This is a very important point in these days of water scarcity especially in India.

A highly purified algal product is essential for human consumption, but one with impurities such as sand might be quite adequate for livestock feed. The cost of algae depends upon the production techniques employed and may vary from \$ 0.25 to 0.50 per lb to \$ 0.03 to 0.06 per lb in the States.

Processing of algae from high-rate aerobic ponds:

This involves three stages; (a) initial concentration or removal; (b) dewatering and (c) final drying. Golueke et al. (1964) have studied in detail all these aspects. They found that in the initial concentration phase, chemical precipitation with alum or hydrated lime or a polyvalent cationic synthetic organic polymer proved to be quite suitable. Centrifugation, passing the algal suspension through an ion exchange column, floatation, vacuum filtration, passage through a charged field and sonic vibration were the other methods tried for initial concentration of algae. Of these, excellent removal was obtained with the use of ion exchange columns, but this process was economically unsatisfactory either because of practical difficulties or because of economics.

After initial concentration, dewatering is necessary to remove the high moisture content of the algal concentrates obtained either by centrifugation or chemical precipitation; and also because of the perishable nature of the algal product and heat lability of its vitamins, algae must be dried rapidly with a minimum amount of exposure to high temperatures with vacuum filtration, continuous solid bowl centrifuge, or industrial gravity filter, algal slurry can be dewatered. Also, in specially prepared sand beds of 6400 sq. feet of sand bed per acre of pond surface, algal slurry can be dewatered and air dried in a single operation in summer months. This method is a simple and low cost operation but the algae are contaminated with 2 - 3 % of sand particles.

In the case of chemical precipitation with alum or lime, the algal paste is dried as a thin film on a heated drum. Such drying apparently improves the digestibility of the product and at the same time produces an essentially sterile product. So, the cost of algal production will vary widely with the quality of the final product, capacity of the production plant, and the algal concentration of the pond effluent.

Fish culture:

Before harvesting the algae from high-rate oxidation ponds, the final effluent may be passed through a series of specially prepared fish-ponds in which special varieties of Indian carps and exotic species which thrive solely on algae may be stocked. Indian carps like Rohu, Mrigal, Barbus etc., and exotic species like Telapia mosambica, Chinese Silver carp, Chanos-chanos can be easily grown before the algae are harvested. In some of the ponds, the growth of Zoo-plankton may also be encouraged so that such species of carps which feed on Zoo-plankton may be stocked in them. In this way, the Fisheries Department of each State can obtain a good yield of fish protein also, from high-rate sewage oxidation ponds in their Sewage Farms now in existence in most of the towns.

Microbiological aspects

It has also been observed that waste treatment plants which base their entire operation on micro-organisms within them, have been designed for the past fifty years with almost no consideration for the biochemical reactions brought about by the various micro-organisms (Mckinney, 1962). Parker (1962) has also stated that no detailed study of the dominant bacterial species occuring in different types of oxidation ponds has been made so far. Studies of bacteria involved in the normal functioning of waste treatment processes are relatively few in number. Although reports which list the specific organisms involved in aerobic oxidation in stabilization ponds are not available, it is extremely likely that the aerobic bacteria of ponds, which are mainly contained in a yellow-brown flocculent sludge (the substance created during bio-flocculation) differ but little from those found in activated sludge or in trickling filter slimes (Oswald, 1960). In the same paper(Oswald 1960, p.384) he has stated that in the case of the high rate aerobic ponds: "A healthy sludge comparable to activated sludge is maintained in the pond, provided mixing is carried out for about three hours a day. Following an initial accumulation, the volume of aerobic sludge does not increase, but rather remains constant indicating essentially that total oxidation is taking place".

It is not known whether a specific microflora is responsible for "total oxidation" of organic matter in this case or whether the phenomenon is associated with any other biochemical process. The advocates propounding the theory of "total oxidation" of organic matter by bacteria have not advanced any biochemical proofs of their contention.

It will require therefore, renewed investigation of a more exhaustive nature to establish definitely that in some cases alone like the activated sludge and trickling filter processes bacteria "partly oxidise" organic matter and in other cases (high-rate oxidation ponds) completely oxidize the organic matter.

The algae are available as a direct source of food for Zoo-plankton and fish and also as an agent helping to maintain the fertility of aquatic soil. Thus they are very important as a bio-geo-chemical agent and as a transformer of energy and therefore must be harvested for useful purposes. They also require careful studies in high-rate aerobic ponds.

Again the specialized treatment structures imposed by the mechanical aspects of the three different systems of treatment have been arrived at by purely empirical procedures and are, perhaps, responsible for causing differences in the patterns of microbial degradation of organic matter. This is indicated by the strikingly different periods of time taken for purification in each case. In the case of the activated sludge process where huge quantities of the floc are in constant turbulent motion sweeping through the liquid to be purified, the contact period between the organic wastes and the activated sludge when stabilization is brought about is 4 - 6 hours. The contact period between wastes passing through a trickling filter and microbial surfaces on filter stones is approximately thirty seconds - according to the studies made at the Robert A. Taft Engineering centre and at the Purdue University (Mckinney and Pfeffer 1965). In the case of the oxidation pond neither of the above phenomenon occurs. The time required for the organic wastes to be purified by algal-bacterial symbiosis is ordinarily 20 - 30 days in this comparatively sluggish process which is lessened to 2 - 6 days in the high-rate oxidation pond (McGauhey 1960). The structural features of the three treatment systems would therefore seem to determine largely what species accomplish treatment (Lackey and Smith 1956). The types of bacteria concerned in algal-bacterial symbiosis and the pattern or pathways of degradation of the soluble organic substrates are still unknown (Golueke et al 1959) although enormous literature has been published on certain other aspects of the low cost waste treatment systems.

These facts may be considered sufficiently convincing to justify a detailed biochemical study of algal-bacterial symbiosis in high-rate aerobic pond systems with varying detention periods and algae. So, attempts were made in this thesis to study the following aspects such as (a) the degree of purification attained; (b) algal bio-mass production; (c) photosynthetic oxygen production; (d) CO_2 requirement for algal biomass production; (e) input-output energy balance; (f) O_2 requirement for bio-oxidation of sewage organic matter; (g) CO_2 production from organic matter and (h) the types organisms concerned using Baroda settled raw sewage and the green alga Scenedesmus obliqus Microcystis aeruginosa and mixed algae with varying detention periods; and the results of the same are discussed in this thesis. We are concerned with the normally photosynthetic algae requiring only inorganic nutrients, CO_2 and H_2O which are technically classed as autotrophic-photo-lithotrophs.

Basis of Claim for Ph.D.

According to O.Ph.D. 9 of the University Ph.D. Ordinances, - a candidate submitting his thesis for Ph.D. has to state whether " the work is based upon the discovery of new facts by the candidate or of new relations of facts observed by Others - and how the work tends to the general advancement of knowledge".

I submit that my thesis is not based on the discovery of new facts or original data but is based upon the discovery

<u>of new relations of facts observed by others</u>; and the thesis does tend to the general advancement of knowledge pertaining to strictly aerobic oxidation ponds as indicated below:

My thesis is entitled "Studies on algal-bacterial symbiosis in high-rate oxidation ponds using <u>Scenedesmus obliqus</u>. In order to evaluate "algal-bacterial symbiosis" (or how one helps the other) in my experiments with the several algae using Baroda sewage, it is necessary to know the quantity of CO_2 liberated during total bioxidation of sewage organic matter for the production of each of the algal biomasses experimented; and also in turn how much of the photosynthetic oxygen is liberated by each of the algal biomass during photosynthesis for total bacterial oxidation of sewage organic matter.

But it is not possible to estimate directly either of the two gases in the eco-system during "algal-bacterial symbiosis" for two reasons (i) they are not phased metabolic processes (i.e. one taking place after the other) but they are considered to be not only almost simultaneous or concurrent but are also stated to be used up as soon as they are liberated in the closed aquatic ecosystem (Oswald, 1960). Even granting that it is possible to estimate them directly by means of isotopes, there are no no facilities for such work in our laboratory. Further according to Fogg (1970,p.162) "the radio carbon method suffers from more uncertainties". Therefore the method suggested in this thesis appears to be more appropriate than isotope method for the conditions existing in the eco-system. So, resort to short-cut methods which could be verified, if need be, when facilities for work with labelled compounds are available in other laboratories was made.

A. New relations of facts observed between COD and algal biomass;

So, attempts were made to estimate the quantities of the two gases indirectly by discovering new relations of facts observed by other Scientists. This is the first time that such an attempt has been made in the history of oxidation pond literature for establishing new relations of facts observed by others from the two most indespensable parameters - COD and algal biomass- which have been estimated actually in my laboratory experiments. The main metabolic reaction taking place in oxidation ponds is "algal-bacterial symbiosis", and therefore an attempt has been made to dissect the mechanism of "algal-bacterial symbiosis" into its two component parts. (a) bacterial oxidation and (b) algal photosynthesis, assuming that the two reactions are taking place independently in order to evaluate quantitatively how one has helped the other. So, the results of our experiments have been discussed under 3 heads :

1. Bacterial oxidation of sewage organic matter.
2. Algal photosynthesis and
3. Algal-bacterial symbiosis.

In each of these cases new relations of facts observed by others have been established and they are detailed below:-

1. <u>Bacterial oxidation of Organic matter.</u>

a) <u>Facts observed by others:</u>

i) Porges (1960) has furnished a factor for estimating approximately the quantity of organic matter from COD values. COD values divided by 1.2 gives the corresponding values of the quantity of bio-degradable organic matter in waste water.

ii) Oswald, Hee and Gotaas (1958) have experimentally found that the bacterial oxidation of sewage organic matter in high-rate oxidation ponds to follow the equation.

$C_{11}H_{29}O_7N + 14O_2 + H^+ \dashrightarrow 11CO_2 + 13H_2O + NH_4$

(Organic matter)

or 287 gm Organic matter + 448gm $O_2 \dashrightarrow$ 484gm CO_2 + $13H_2O$ + NH_4

$\therefore$ 1 gm of organic matter will produce 484/287 = 1.69 gm of CO_2 and will require 448/287 = 1.56 gm of O_2

Combining the facts observed by Porges (1960) and by Oswald et al (1958) and using COD values, new relations of facts have been established for estimating CO_2 liberated and O_2 received during bacterial oxidation of organic matter in pages 89 and 90.

2. Algal photosynthesis:

(a) Facts observed by others:

i) Myers (1962) has found that 1.8g. of CO_2 are used up during the formation of 1.0g of algal dry matter.

ii) Oswald and Gotaas (1957) have found that 1.6gm of O_2 will be released during the formation of 1.0gm of algal biomass.

Combining the facts observed by Myers (1962) and Oswald and Gotaas (1957) and using the algal biomass values, new relations of facts have been established for estimating the quantities of CO_2 used up for algal-biomass production and photosynthetic O_2 released during algal-biomass formation on pages 101.

3. Algal-Bacterial Symbiosis:

For the first time, bacterial oxidation is quantitatively related to algal-photosynthesis in oxidation ponds, and how much extra CO_2 from other sources is obtained for algal biomass is indicated on pages 110 and 111.

B. New relations observed between the values for bacterial biomass calculated according to two formulae established by Scientists of repute in the world of Sewage purification.

a) Facts observed by Others:

i) Sawyer (1956) has suggested a formula for estimating total bacterial growth in Activated sludge process of sewage treatment. It is :

Total Bacterial growth formed = 0.5 x BOD_5 used up(mg/l).

ii) Mckinney (1962) has furnished different formulae for calculating total bacterial mass, active bacterial mass and decreasing bacterial mass in an activated sludge process. According to him :

Total bacterial mass = COD used up (mg/l)/2.13

$$\text{Active bacterial mass} = \frac{S}{(1 + K_3 . t)}, \text{ where}$$

S = total bacterial mass;

K_3 = 0.006;

t = time in hour

$\therefore$ Decreasing bacterial mass = total bacterial mass - active bacterial mass

The above authors have applied these formulae for the activated sludge process of sewage treatment and not for oxidation pond method of sewage treatment. I have applied the above formulae to oxidation pond method for the first time. It may be argued that the quantity of bacterial sludge produced in high-rate aerobic ponds cannot be compared to that produced in activated sludge process as the two systems of treatment are different and would not be expected to yield the same quantity of sludge. But it has to be pointed out that it is agreed by one and all that the end results of all biological treatment processes are the same. Such being the case the bacterial solids formed in 4 - 6 hours in the activated sludge process required 6 days in high-rate aerobic ponds so that the total percentage of BOD removal is the same, in both the treatments. If the efficiency of purification is the same then it cannot be stated that the quantity of bacterial sludge formed will not be the same in both cases. So, I have tried to apply the formulae used in activated sludge process to the oxidation pond method in order to find out how they work. I get new interesting relations of facts observed by them.

b) New relations observed:

From the data shown on pages 85 to 87, it is shown for the first time that the values calculated according to Sawyer work out to about 2/3 of the corresponding values of total bacterial mass calculated according to Mckinney.

C. New Correlation between bacterial biomass and algal biomass.

The quantity of algal biomass produced in a high-rate aerobic pond seems to be nearly twice the quantity of total bacterial biomass calculated according to Mckinney (Vide p.88). The application of the facts mentioned by others in a, b and c has thus led to new knowledge or understanding of the process of algal-bacterial symbiosis in high-rate oxidation ponds, which was not known before. Although various chemical and biological changes in waste water to which algae had been added have been measured and reported by many others in the past, knowledge of this process thus gained was not sufficient to predict the resulting changes. For the first time in this thesis, the new relations of facts observed by others have been used for predicting the resulting changes.

D. <u>How the thesis tends to the general advancement of knowledge.</u>

1. Most of the literature on oxidation ponds deal with either facultative or anaerobic type of oxidation pond and practically very few deal with purely or strictly aerobic type of oxidation ponds. Even these few references do not deal with the biochemical aspects. This thesis deals with the biochemistry of algal-bacterial symbiosis for the first time.

2. Very little is known about the basic principles underlying the absence of huge bacterial sludge in this treatment system. This thesis furnishes that information.

3. There are no references to show that any alga in domestic waste water will support enough algal cells to provide its own requirement of oxygen by photosynthesis for bacterial oxidation of organic

matter and thus purify the waste water to the desired degree. This information is furnished in this thesis.

4. Significant difference in composition of the bacterial flora during assimilatory and endogenous phases has been found (p. 63).

5. The mechanism of purification in high-rate aerobic ponds is not a case of "de jure" total oxidation, but is based on the "extended aeration principle".

6. This thesis shows a method of culturing any alga on a large scale from an organic liquid waste for removing nutrients of biological significance. The method can be used for harvesting any alga-nitrogen fixing and non-nitrogen fixing on a large scale for agricultural purposes.

7. Large-scale manufacture of sea weeds useful for industrial purposes from sea water fertilized with the sewage of a coastal town is feasible in shallow ponds similar to salt pans, following the methods described in this thesis.