CHAPTER - II

LITERATURE REVIEW

Definition of Symbiosis:

"A number of terms have been employed to designate relationship between organisms. One of them is 'symbiosis' which has been used both in a broad and restricted sense. According to Burkholder (1952) the relationship where two or more species live together with mutual benefit is termed 'symbiosis' which is used in a restricted sense. But Caullery (1952) has used the word 'symbiosis' to designate the intimate and constant association of two organisms with mutual relationship assuring them of reciprocal benefit. In contrast Leach (1940) has interpreted 'symbiosis' in a broader sense to include all associations in which dissimilar organisms live together in closed spatial relationship without any benefit or detriment to either party of the association. Again 'symbiosis' in a restricted sense is used to designate relationship between taxonomically different organisms, in which there is a permanent or a semi-permanent physical union between the individuals. Relationships which do not involve a union of the participating organisms will be generally termed non-symbiotic". (de Vay 1956).

In Encyclopaedia Britannica (1957) 'symbiosis' is defined as "the general term for the partnership of dissimilar organisms that have become established by evolution. In this discussion three major types of symbiosis are recognized: (i) commensalism in which food or space is shared without evident benefit or harm to either organism or in which the benefit relation is one-sided without harm to the host, (ii) mutualism in which both host and guest (or the two partners) are obviously or apparently benefited and (iii) parasitism in which the host is subject to varying degrees of injury. The term 'symbiosis" has been widely used in the restricted sense of mutualism. The broader definition corresponds both to the original definition of symbiosis by Heinrich de Bary (1879) and to modern usage".

In the same book commensalism is defined as "representing a level of relationship, a degree removed from that in aggregations of animals about a common food supply".

Pierre Van Beneden (1876) refers in the same book to commensal "as an organism that requires from his neighbour a simple place on board his vessel and does not part-take of his provisions. The messmate does not live at the expense of his host, all he desires is a home or his friend's superfluities".

"The support and protection supplied by the fungus combined with the carbohydrates synthesis of algae, makes possible the invasion of inhospitable rock and other adverse environment by Lichen".

In Peare's Cyclopaeda (1970-71), 'symbiosis' is defined as follows: When two organisms living together and both derive mutual benefit from the association, live together, the partnership is known as 'symbiosis'.

De Bary (1879) has defined 'symbiosis' and it has been quoted by Hungate (1963) as an association in which two different species live together in a closed spatial and physiological relationship. If they are reciprocally dependent, the relationship is mutualism. If the two species occur independently of each other in nature, "their relationship has not evolved to the point of survival advantage, and it is questionable that it is mutualism".

"Many mutually beneficial relationships exist between species without the close physical association that is the characteristic feature of symbiosis. If close spatial connection (at least during some essential phase of the cycle) is not retained as a criterian of ~~of~~ symbiosis, loose relationships such as production of oxygen by a plant and its utilization by an animal are included and the term looses both meaning and usefulness".

Oswald and Gotaas have used the word 'symbiosis' in the following contexts:

i) Ludwig, H.F., Oswald, W.J., Gotaas, H.B., and Lynch, V. (1951):

"Algae symbiosis in oxidation ponds. I. Growth characteristics of <u>Euglena gracilis</u> cultured in sewage." Sewage and Industrial Wastes, Vol.23, p. 1337.

ii) Gotaas, H.B., and Oswald, W.J. (1951):

"Algae symbiosis in oxidation ponds. Second progress report." Institution of Engineering Research Bulletin, Series, 44(3), p. 1-58, Univ. of California.

iii) Oswald, W.J., Gotaas, H.B., Ludwig, H.F. and Lynch, V. (1953):

"Algae symbiosis in oxidation ponds. II. Growth characteristics of <u>Chlorella pyrenoidosa</u> cultured in sewage." Sewage and Industrial Wastes, Vol.25, p. 26.

iv) Oswald, W.J., Gotaas, H.B., Ludwig, H.F. and Lynch, V. (1953):

"Algae symbiosis in oxidation ponds. III. Photosynthetic oxygenation." Sewage and Industrial Wastes, Vol.25, p. 692.

v) Gotaas, H.B. and Oswald, W.J. (1952):

"Algal-bacterial symbiosis in sewage oxidation ponds. Third progress report." Institute of Engineering Research. Series 44(4), p. 1-45. Univ. of California.

vi) Gotaas, H.B. and Oswald, W.J. (1954):

"Algal-bacterial symbiosis in sewage oxidation ponds. Fifth progress report." Institute of Engineering Research. Series 44(5), p. 1-88. Univ. of California.

In all these cases Oswald and Gotaas seem to have used the term in a very very broad sense as a beneficial relationship between algae and bacteria in sewage oxidation ponds, although the mutually beneficial relationship exists without the close physical association that is the chracteristic feature of 'symbiosis' according to Hungate (1963). We have followed the examples of the two eminent workers in the usage of the term 'symbiosis' in this thesis.

<u>"Algal-bacterial symbiosis" in high-rate oxidation ponds:</u>

How algae and bacteria, when cultured together in high-rate aerobic ponds, derive mutually beneficial help during the period of their partnership is explained below.

It is well known that organic matter in sewage is most rapidly oxidized in activated sludge process and in trickling filters. There is also much evidence to show that fresh algal organic matter is also most rapidly synthesised on a sustained basis by green algae (Burlew, 1953; Krauss, 1956; Tamiya, 1957). At about this time a new approach to waste water treatment was furnished by Gotaas and Oswald (1955), and Gotaas, Oswald and Ludwig (1954). The possibility that green algae might be grown directly in sewage in order to provide oxygen in the place and at the time it was needed for biochemical stabilization of waste organic matter involved is a relatively new approach to waste treatment. Laboratory and Pilot plant investigations of sewage carried out during 1951-1955 by Ludwig et al (1951), Oswald et al (1953-a,b) and Gotaas and Oswald (1955) in the Public Health Engineering Research Laboratories of the University of California, have furnished ample experimental evidence for the beneficial relationship between algae and bacteria in new or existing sewage aerobic oxidation ponds, for it is generally recognized that the principal products of aerobic bacterial oxidation of organic matter are carbon-dioxide, ammonia and water which, except for the additional requirement of light energy, are exactly identical to the principal requirements for algal photosynthesis.

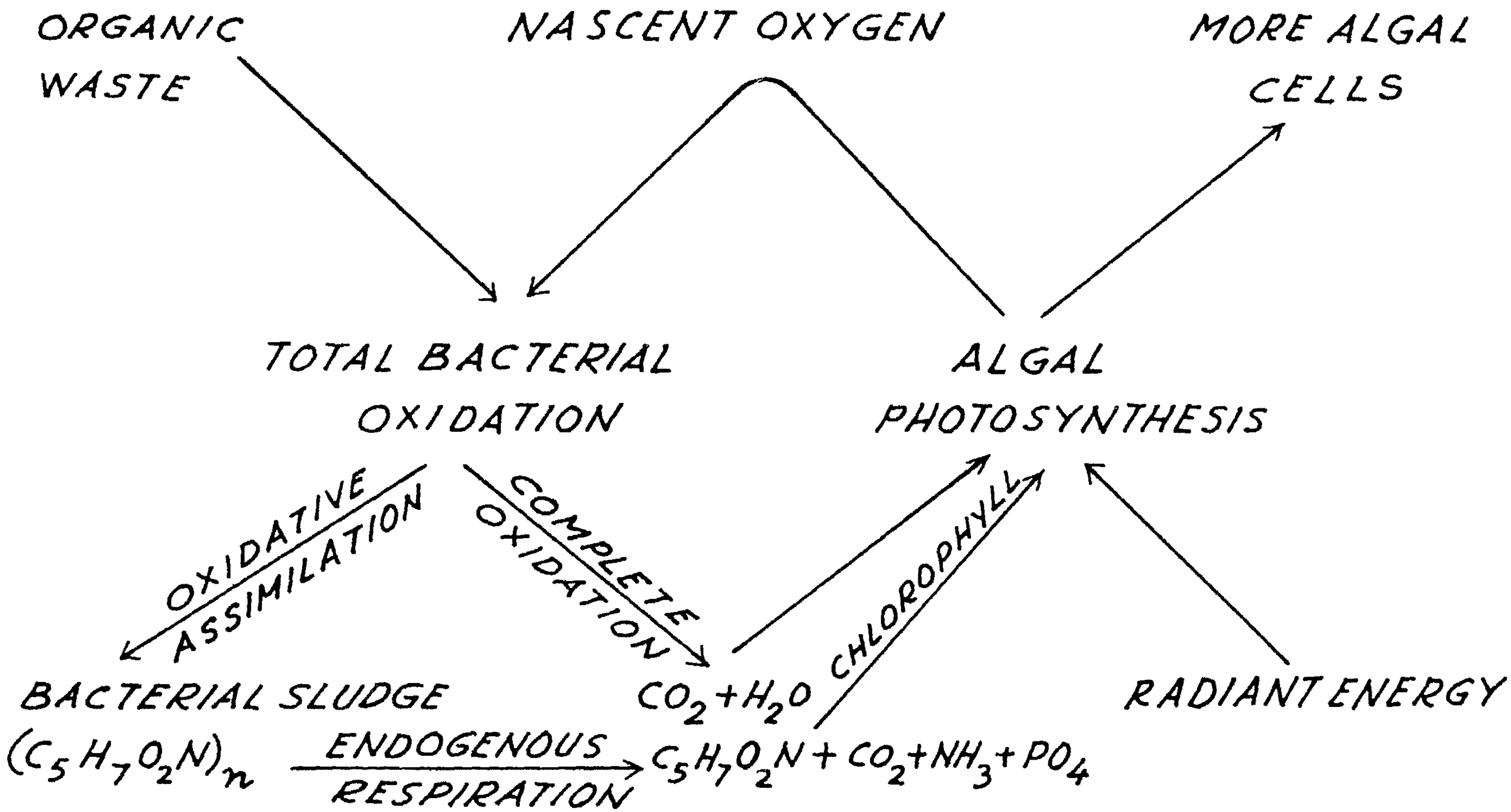
Thus, in theory the decomposition of organic matter by bacteria may occur at the same time that new organic matter is being synthesised by algae, provided that light is available as the energy source. Under such circumstances the efficiency of oxygen utilization is greater because oxygen is used as soon as it is formed. Oswald, Gotaas, Ludwig and Lynch (1953-a, p. 692) have collected considerable evidence from their experiments on high-rate ponds indicating that the species of algae which are effective in photosynthetic oxygen production utilize ammonia as the principal source of nitrogen with which to build their proteinaceous material from sewage. At moderately long detention periods of 3 to 6 days when light and temperature are optimum, almost all the ammonia nitrogen appears in the form of algal cell materials. Thus nitrogen is conserved and at the same time BOD is considerably reduced. Recirculation is very important to photosynthetic oxygen production as it permits the seeding of influent with algal cells, and produces good overlapping of bacterial oxidation and photosynthetic reduction thereby preventing loss of CO_2 and NH_3 from the bacterial phase and also providing an efficient outlet for the oxygen liberated by algal growth (Oswald, Gotaas, Ludwig and Lynch, 1953-b, p.26). This overlapping produces more abundant growth of bacteria and would produce more abundant growths of algae, were it not for the problem of sludge deposition. Algae tend to remain dispersed in the solution while bacteria tend to form a floc which together with coagulated sewage colloids containing a large part of carbon and nitrogen settles quite rapidly to the bottom of the pond. Principal benefits of recirculation are : (i) seeding and (ii) aeration of the influent.

If continuous vertical mixing is used some aerobic sludge may be carried with the pond effluents. This material may be readily removed from algal suspension by sedimentation and returned to the pond. The retention of sludge is advantageous as it allows more complete oxidation of organic matter, resulting in increased algal growths and improved removal of suspended solids other than algae (Oswald and Gotaas, 1957).

Further studies since then by a host of scientists in U.S.A. -- chiefly Oswald and Gotaas (1957), Oswald (1960, 1962, 1963, 1964), Oswald and Golueke (1960, 1968), Oswald et al (1958, 1959-a,b; 1961, 1964), Golueke et al (1958, 1959), Golueke (1960), McGarry (1971) - have established beyond a shadow of doubt that mutual benefits have accrued between algae and bacteria, when they are cultured together in aerobic sewage oxidation ponds resulting in huge algal production, nutrients removal and purification of sewage. These studies have also provided some basic principles which can be used for engineering

Fig. 1

BIOCHEMISTRY OF "ALGAL-BACTERIAL SYMBIOSIS" IN HIGH RATE AEROBIC POND SYSTEM (MODIFIED AFTER OSWALD & GOTAAS 1958)



design of the process and for the production of operational performance of new or existing ponds. Based on these principles the design creteria have been formulated by these authors.

The cycle of photosynthetic O_2 production and bacterial oxidation of organic matter is shown in Fig.1. It will be seen from a study of the figure that organic matter entering the system as sewage is oxidized by sewage bacteria using oxygen liberated by algae. The algae in their turn utilizing solar energy are simultaneously synthesising fresh organic matter in the form of algal cells using carbondioxide and ammonia produced by bacteria. Although this entire reaction may take place in a closed system, some carbon-dioxide is normally drawn into the cycle from the atmosphere and excess oxygen may be lost.

In order to develop practical equations for the design of such a unit, it is desirable to assume that it will be so operated that all the oxygen required by bacteria will result from the development of the algal biomass and all the carbon-dioxide required for algal synthesis will result from the bacterial oxidation of sewage organic matter. To evaluate these factors and to express them in simple euqations, certain assumptions based on well known facts have been made.

<u>High-rate aerobic ponds in use:</u>

The high-rate oxidation pond has been studied in New Zealand (Hicks, 1958); Taiwan (Soong 1961); at Ahmedabad, India (Ganapati *et al*. 1965) and in Australia (McGarry, 1967). A high-rate pond research programme has also been recently initiated at Bangkok in Thailand at the Asian Institute of Technology (McGarry and Tangkasame 1971). In the last place research has been done at laboratory and pilot plant scales. Twenty-four experimental ponds were constructed for the treatment of diluted settled night soil. Twenty-seven pond conditions were studied with combinations of the levels of variables such as loading, depth and detention period. The ponds were mixed daily by brooms at 9.00 a.m. and 7.00 p.m.

Efficient waste-water treatment and high yields of algae are achieved through the operation of a high-rate pond at 2000 lbs BOD/acre/day loading, 17.7" depth and one day detention time. Under these conditions effluent BOD/algae removed is lower than 10 mg/l and an acre of pond can produce about 100,000 lb or 45,400 Kg of algal dry weight per year. At solar energy levels of 480 gram-calories/sq.cm/day, 2800 lb/day (1270 Kg/day) of dried algae (with less than 10% moisture) can be produced on one acre.

An urban model has been suggested incorporating re-cycling of reclaimed clarified pond effluent (after treating with alum or alum plus poly-electrolytes) for household cleaning purposes. Potable drinking water would be supplied through a separate distribution system. Use of such a dual distribution system would effect a two-third reduction of conventional water supply requirements.

Ganapati et al. (1965) have described the type of high-rate aerobic lagoons which were in use in the Pirana Sewage Farm at Ahmedabad, India. In the extensive farm of 2850 acres on the eastern banks of the river Sabarmati, broad irrigation was carried out on 2500 acres and the remaining 350 acres at the farthest end of the farm were converted into 280 plots of "solar drying beds" where, raw settled sewage, not required for irrigation was stored to a depth of 2 to 27 inches and allowed to percolate and to evaporate in the sandy soil on the bank of the river. The algal solids were removed finally from each bed and sold as manure.

Impounding raw settled sewage (which had travelled over a distance of six miles in narrow open channels with a self cleaning velocity) was practised as a distinct treatment method since 1932. Two distinct processes were taking place in the ponds; (a) synthesis of profuse green and blue-green algal organisms making use of the fertilising elements of sewage, and (b) concomitant release of large quantities of dissolved oxygen The 280 ponds were examined for their physico-chemical and microbiological conditions during the different seasons of 1961-62; and the results have been published already.

The so-called "solar drying beds" of Ahmedabad resembled in important respects the high-rate aerobic ponds in their smaller area and depth with detention periods of less than one week, where stabilization of sewage was brought about solely by the photo-physiological action of green and blue-green algal organisms (Ganapati et al. 1965).

The Activated Algae :

Mckinney and Waheb (1968) have introduced the concept of "activated algae" and suggested that a combination of bacteria and algae in a controlled treatment process would remove the algal nutrients from waste waters, which would otherwise stimulate algal blooms in receiving waters. Goodman and Weis (1968) have reported on the preliminary development of the full-scale "activated algae" process, discussed the difficulties involved and the lack of data to date to evaluate properly the feasibility of such a large scale treatment system. Humenik and Hanna (1969) demonstrated that a symbiotic algal-bacterial culture could be developed and maintained so that the culture could remove nutrients efficiently in a properly designed system using Chlorella pyrenoidosa as the algal organism. Maximum and most consistent removal of influent COD and organic N was obtained during unaerated operation with daily harvesting of algae. But no appreciable phosphorus uptake was observed. Supplemental aeration did not improve nutrient removal, and it was considered as a wasted energy input for an optimally functioning symbiotic algal-bacterial culture. The culture of the bio-mass growth was a natural mixture of algae and activated sludge in which Chlorella became integrally enmeshed within the bacterial matrix. The algal-bacterial floc settled very rapidly under optimum conditions yielding a clear supernatant. This process is still in the experimental stage.

The need for further research on "algal-bacterial symbiosis:"

Golueke (1960) states: "An extensive knowledge of organisms involved in the process for the treatment of waste in a high-rate oxidation ponds is required. This is true because effective biological control requires an optimum relationship between the environment and the biotic community concerned; and this can be accomplished only by providing proper environmental conditions. To establish a relationship, it is necessary to know the nature and the extent of the influence of principal environmental factors to which an algal-bacterial community is subjected in an oxidation pond .....".

"There is paucity of information in the literature on the effect of these environmental factors either individually or collectively on such organisms when living as members of a biotic community". His statement holds good even today, and also justifies this thesis; which deals with the nature and extent of influence of the principal ecological factors chiefly CO_2 and O_2 on algal-bacterial symbiosis in high-rate oxidation ponds studied under laboratory conditions.