a) Definition of Symbiosis:

The relationship between organisms has been discussed in several ways. One of them is designated as "symbiosis" to describe the intimate and constant association of two types of organisms with reciprocal benefits. There are three types of symbiotic relationships such as "Neutrlistic", "Mutualistic" and "Antagonistic" (De Ley 1960). "Neutrlistic symbiosis is the relationship existing between two organisms when they have little or no effect on one another. If one or both participants benefit from the relationship without any injurious effects on the other, this relationship is called "mutualistic. In "antagonistic" symbiosis one of the two participants may or may not derive any benefit from the relationship or one of them may be deleterious to the other. So, it will be seen from the above that algal-bacterial symbiosis in high-rate aerobic ponds may be any one of the three types depending upon the dominant algae and bacteria present in the waste, and it would appear to be mutualistic.
b) Algal-bacterial Symbiosis:

There are still some scientists who regard algal-bacterial symbiosis in aerobic oxidation ponds as a fiction. Experimental and cited evidences are furnished below to convince them that it is a fact and not a fiction.

It is well known that organic matter in sewage is most rapidly oxidized in the two conventional aerobic methods of biological waste-water treatment (Eckenfelder and Weston, 1956; Eckenfelder and Porges, 1957). 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; Tamiya, 1957). Laboratory and Pilot plant investigations of sewage carried out during 1951-1955 by Ludwig et al. (1951), Oswald et al (1953,a,b), Gotaas and Oswald (1955) in the Public Health Engineering Research Laboratories of the University of California, have furnished ample experimental evidence that algal-bacterial symbiosis is actually taking place 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 CO₂, NH₃, H₂O 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 oxidation ponds indicating that the species of algae which are effective in photosynthetic oxygen production utilize NH$_3$ as the principal source of N with which to build their proteinaceous material from sewage. At moderately long detention periods of 3 to 4 days when light and temperature are optimum, almost all the NH$_3$-N appears in the form of algal cell materials. Thus N 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/algae growth (Oswald, Gotaas, Ludwig and Lynch, 1953-a; p.26). This
overlapping produces more abundant growth of bacteria and would produce more abundant growth 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 floe which together with coagulated sewage colloids containing a large part of C and N 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).

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

The cycle of photosynthetic $O_2$ production and bacterial oxidation of organic matter is shown below:

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Organic waste  Dissolved $O_2$  Excess algae
   |                       |       |
  Bacterial oxidation   Algal photosynthesis
    |                               |
Excess bacteria + $CO_2 + H_2O + NH_3$  Solar energy
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It will be seen from a study of the figure that organic matter entering the system as sewage is oxidized by sewage bacteria using $O_2$ liberated by algae. The algae in their turn utilizing solar energy are simultaneously synthesising fresh organic matter in the form of algal cells using $CO_2$ and $NH_3$ produced by bacteria. Although this entire reaction may take place in a closed system, some $CO_2$ is normally drawn into the cycle from the atmosphere and excess $O_2$ may be lost.
The basic principles on which algal-bacterial symbiosis takes place in a high-rate aerobic pond are shown in the above diagram. In order to develop practical equations for the design of such a unit, it is desirable to assume that it will be operated such that all the $O_2$ required by bacteria will result from the development of the algal bio-mass and all the $CO_2$ required for algal synthesis will result from the bacterial oxidation of sewage organic matter. To evaluate these factors and to express them in simple equations, certain assumptions based on well known facts have been made.

c) **Nutritional aspects of Algae:**

Fink and Herold (1957, a,b) have carried out animal feeding experiments with sewage-grown algae and found how beneficial they were in preventing action on liver necrosis. Cook (1962) has also estimated nutritive value of sewage grown algae.

d) **Activated algae:**

McKinney and Waheb (1968) have introduced the concept of "activated algae" and suggested that combination of bacteria and algae in a controlled treatment process would remove the algal nutrients from waste water
which would otherwise stimulate algal blooms in receiving waters. Goodman and Weiss (1968) reported on the preliminary development of a 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; 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 which 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 biomass 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.

e) **High-rate oxidation pond at various places:**

The high-rate oxidation pond has been studied as
stated previously 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. to 7.00 p.m.

Efficient waste water treatment and high yields of algae are achieved through the operation of a high-rate pond at 200 lbs BOD/acre/day loading, 17.7" depth and 1 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 45.00 kg of algae/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 that incorporates recycling of reclaimed, clarified effluent (after treating
with alum or alum plus poly-electrolytes) for house-hold 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.

"Research on the system under tropical conditions is now required in the fields of animal nutrition, product processing, market analysis, and process economics, both at the pilot plant and phototype scales" (McGarry and Tangkasame 1971).

Ganapati et al (1965) have described the types 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 bank 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 pond; (a) synthesis of profuse green and blue-green algal organisms making use of the fertilizing elements of sewage; and (b) concomitant release of large quantities of dissolved oxygen. The 282 ponds were examined for their physico-chemical and micro-biological 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 of 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).

f) The need for research into the biochemical aspects of high-rate aerobic pond:

Golueke (1960) states "An extensive knowledge of the ecology of the organisms involved in the process for the treatment of waste in a high-rate oxidation pond is required. This is true because effective biological control requires an optimum relationship between environ-
ment 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 extent of the influence of the principal environmental factors to which an algal-bacterial community is subject 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 member of a biotic community". His statement holds good even today; and therefore justifies this thesis, which deals with the nature and extent of influence of the principal ecological factors chiefly CO₂ and O₂ on algal-bacterial symbiosis in high-rate oxidation ponds studied under laboratory conditions.

At the time of registration, I was working on the alga *Scenedesmus obliquus*. But later on I could find time to work with few other algae for confirming my results. So the title of the thesis includes the name of only one alga though this thesis includes the results of several algae.