----: SECTION - A :----

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 the biological activity. The distinguishing characteristics, concepts and performances of each of the three main classifications have been described in detail by Gloyna (1971). Of the three types of ponds, aerobic stabilization ponds have been further subdivided into two main types: aerobic pond and aerated lagoons. The working principle of the former is that they are operated mainly on photosynthetic oxygenation while the latter is operated on induced mechanical mixing. This thesis will be dealing only with high-rate aerobic ponds with minimum depth and maximum algal production.

This type of pond has passed the experimental stage of development. Both with pilot scale ponds (Oswald and Gotaas 1957; Oswald *et al.* 1958) and in field investigations (Gotaas and Golueke 1957; Oswald *et al.* 1959-a), it has been clearly demonstrated on a practical scale that it is possible to develop large quantities of algal cell material with minimum bacterial sludge production and to oxygenate domestic sewage in very shallow two-acre ponds. Still algal blooms proved sometimes difficult to sustain under field conditions. So, Oswald et al. (1959-b) carried out further investigations for the failures as stated earlier, developed an economically feasible method of preventing such failures and of algal recovery through careful attention to details of design and operation; and thereby reclaimed the fertility elements in the sewage as protein concentrates for livestock feed. They obtained yields of vegetable protein per acre from 20 to 40 times those obtained in most of the conventional forms of cropping on pasture or agricultural land. For these reasons perhaps Gloyna (1971, p. 84) thought that this type of pond required greater attention for maximum algal production than self-regulated conventional ponds and therefore were 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 consisting of about 4000 million population 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 2% and increasing industrial development. So new techniques and new sources of supply must be sought. This can be mėt partly by treating the vast volume of waste waters produced by the world population to such an extent that they can be used straightway for water treatment for drinking, 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 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": "In the next generation, the lines of development of controlled photosynthesis are expected to include waste disposal, water reclaimation, food production and perhaps power production. Use of controlled photosynthesis for maintaining the environment of space ships suitable for human habitation is an interesting application which is now under intensive study at the University of California and elsewhere".

So, for these reasons this process of high-rate aerobic treatment deserves careful considerations and efforts must be taken to make this process as popular as possible. But, it has been observed generally that waste treatment plants which base their entire operation on micro-organisms within them, have been designed for the past fifty years or so 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 bioflocculation) differ but little from those found in activated sludge or in trickling filter slimes (22)" (Oswald 1960). In the same paper Oswald (1960, p. 384) has added 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 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 the trickling filter processes bacteria "partly oxidise" organic matter and in other cases (high-rate oxidation pond) "totally oxidise" the organic matter.

Algae are valuable 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 algae are very important as a bio-geo-chemical agent and as a transformer of energy and therefore must be harvested for useful purposes. Their cultivation also require careful studies in high-rate aerobic ponds.

Again, the specialised treatment structures imposed by the mechanical aspects of the three well-known 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 bacterial 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 the 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 oxidation pond neither of the above phenomenon occurs. The time required for organic wastes to be purified by "algal-bacterial symbiosis" is ordinarily 20-30 days in the comparatively sluggish conventional process which is lessened to 2 to 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 (Lacky and Smith 1956). The types of bacteria concerned in "algal-bacterial symbiosis" and the pattern of pathways of degradation of the soluble organic substrates are still unknown (Golueke 1960) although enormous literature has been published on certain engineering and design aspects of the low cost waste treatment systems (Arceivala et al. 1969; Gloyna 1971).

These facts may be considered sufficiently convincing to justify a thorough biochemical and microbiological study of "algal-bacterial symbiosis" in high-rate aerobic ponds systems with varying detention periods.

So, attempts were made in this laboratory to study some of the above aspects particularly the microbial chemistry of "algal-bacterial symbiosis" under laboratory conditions using Baroda settled sewage and three different types of green and blue-green algae* technically defined as autotrophic photo-lithotrophs with constant light intensity for 24 hours of the day with varying detention periods. The term microbial chemistry is used

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* Although the title of the thesis carries one type, I have carried out experiments with three different types of algae.

in the sense of understanding the changes brought about by microbes or low forms of life i.e. algae and bacteria in the physico-chemical and biochemical conditions of the environment in the high-rate aerobic pond ecosystem studied. Three series of experiments were carried out with three different algal forms to study : (a) the degree of purification in terms of organic matter removal and inorganic nutrients removal, (b) amount of algal bio-mass production. The main metabolic reactions taking place in high-rate aerobic ponds is the "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, (b) algal photosynthesis and to evaluate quantitatively how one has helped the other. These constitute " Section - A" of the thesis. In "Section - B" the results of our studies made on catalase activity and its relation to certain important waste water parameters are discussed.

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