C H A P T E R

II

LITERATURE REVIEW

MICROBIAL METABOLISM IN ACTIVATED SLUDGE PROCESS

Microorganisms of various types are seen in all aerobic waste treatment systems such as the activated sludge, the trickling filter and the oxidation pond. They are bacteria, fungi, algae, protozoa and other higher animals. They may be broadly grouped under three heads : reducers, consumers and producers.

REDUCERS

Voluminous data are available regarding the role of bacteria (or reducers) in the most popular and well-known method of sewage purification viz., the activated sludge process. It may be worth while at this stage to analyse the available data of this process under several heads so that it may be useful later on for a proper understanding of the mechanisms involved in the oxidation pond method of sewage treatment about which this thesis deals.

In the aerobic biological treatment of sewage and other organic wastes by the activated sludge process,microbial metabolism is the basic mechanism which seems to take place in two stages : nutrient utilization, phase I and flocculation, phase II (Heukelekian and Schulhof 1938; Tenney and Stumm 1965).

Nutrient Utilization Phase I

In the first phase, organic matter is partially oxidised for free energy for assimilation of the remainder into cellular constituents. During the second phase flocculation of the newly synthetised cells takes place. A review of the important literature dealing with phase I under the heads : (a) relative distribution of the quality and quantity of the bacterial cells; (b) microbial adaptation; and (c) changes in microbial population, is given. Next, salient features about the theory and mechanism of flocculation under phase II are given. Lastly, a brief review of the role played by protozoa and algae in sewage purification is furnished in this chapter.

Relative distribution of the quality and quantity of microorganisms involved in substrate utilization

There are quite a few objectives publications dealing with the relative distribution of the types, nature and function of microorganisms themselves which are responsible for purification in the activated sludge and trickling filter systems but practically none dealing with the oxidation pond method. In the first two cases, selective media were employed to evaluate the prevalence of the several individual types. Hotchkiss (1924), for example, used various kinds of media for evaluating the relative abundance of the several physiological groups of bacteria in a trickling filter. She classified the isolates as proteolytic, denitrifying, nitrifying, H_2S - producing, sulphur-oxidising, and cellulose-decomposing. She reported that the proteolytic bacteria increased to a maximum in the upper three feet while the reducing organisms progressively decreased, and the nitrifying organisms increased with the depth of the filter. Bollen (1951) was also able to identify numerous genera and species from trickling filters and polluted waters.Calaway, Carrol and Long (1952) made use of nutrient agar for isolating the heterotrophic zoogloeal and coliform bacteria in the film coating the sand particles in intermittent sand filters. They found 14 species of which Flavobacterium and Bacillus were found throughout the system; and that Bacillus spp were very active in the breakdown of carbohydrates and proteins. Zoogloeal bacteria were found only in the upper layers; and the coliforms decreased with increasing depth in the filters. Butterfield (1935) first observed zoogloeal masses " in every good activated sludge; " isolated from them a zoogloea-forming bacterium in pure culture producing a floc which simulated the activated sludge in synthetic media and in sterilized sewage. The morphological, cultural and biochemical characteristics of this bacterium tentatively identified as Zoogloea ramigera was worked out; and it was also suggested that an adequate knowledge of this bacterium and related organisms might be of considerable significance in sewage purification. The primary characteristic of this organism, besides its oxidising capacity, appeared to be its ability to grow in a liquid medium in a massed floc or colony which bound themselves so tenaciously as to remain intact even under the agitation of the aeration required to maintain aerobic conditions.

Heukelekian and Schulhoff (1938), Heukelekian and Littman (1939), Wattie (1943), Lackey and Smith (1956), Dugan and Lundgreen (1960), Unz and Dondero (1964,1967) Dias and Bhat (1964) and Crabtree, McCoy, Boyle and Rohlich (1965, 1966) have not only confirmed the importance of zoogloea-forming organisms but also stated that the majority of the organisms in activated sludge flocs consist of zoogloea-forming organisms. But McKinney and Horwood(1952) first isolated six different floc-forming bacterial types from activated sludge. Excepting the sixth zoogloea-forming organism, the other five strains had no special zoogloea producing characteristics. Later McKinney and Weichlein(1953) isolated 72 bacteria from various activated sludge sources. Microscopic examination of the flocs produced by all these isolates showed practically no difference in floc formation. All the bacteria clumped together as definite cells. So, they concluded that the special zoogloea-forming bacteria were unnecessary for the formation of activated sludge flocs.

Anderson (1964) isolated 207 strains from samples of activated sludge, out of which 11 strains alone produced floc. So he concluded that non-flocculating bacteria played greater role than the flocculating ones.

The above authors had also isolated several other organisms such as species of <u>Pseudomonas</u>, <u>Flavobacterium</u>, <u>Achromobacter</u>, <u>Bacillus</u>, <u>E.coli</u>. <u>Alcaligenes</u> etc. Most of them have the ability to form flocs, but which are chiefly responsible for purification and which are found by accidental entrapment in the floc is still unknown. However, the consensus has been and still continues to be that a single bacterium, <u>Z.ramigera</u> is the most dominant bacterium in the activated sludge flocs.

<u>Bacterial adaptation</u>

Sewage consists of heterogeneous microbial populations; and its purification is effected by the concomitant removal of the dissolved organic matter. Krebs (1953), Delwiche (1956), Nickerson (1956) and Simpson (1960), have described the common pathways of biological oxidation of the chief constituents. These pathways are mediated by many enzymes systems which do not occur in all bacteria. It is also generally believed that stabilization of sewage and other organic wastes is caused by the combined metabolic activity of the mixed population of heterotrophs acting concurrently on the soluble substrates to which they are best suited. But very little connected information is available by which one can characterise the changes taking place in an eco-system as to its total number of bacteria and their types at different stages of purification. The situation in an activated sludge system is in a state of constant flux, and there is practically little information about the ability of microorganisms to adapt themselves to changing conditions in the system. The well-known adaptive mechanisms are enzyme adaptation, modification of cell structure, changes in the quality and quantity of microbial populations as a result of death, and multiplication or shifts in predominance.

According to Phelps (1939) the reaction in an activated sludge system occurs in distinct stages each associated with its own type of organisms, products of one stage becoming the raw materials for another. Little is known of the chemical changes taking place or of their biological significance.

Engelbrecht and McKinney (1959) found that acclimated activated sludges developed on pure organic compounds were morphologically and biochemically alike. They also found that the type organisms dominating the sludges depended upon the chemical structure of the organic matter. Ludzack and Ettinger (1957) found in their studies on suppression of evaporation in lake waters that the monomolecular layer of cetyl alchol was easily degraded by water bacteria which adapted themselves to the new environment. But Gaudy (1962) reported the occurrence of sequential substrate removal in a synthetic waste containing glucose and sorbitol. In this system glucose blocked the metabolism of sorbitol. Gaudy, Gaudy and Komolrit (1963) reported sequential removal of substrates in systems of non-proliferating bacteria also.

This phenomenon of sequential utilization of substrates was first observed in pure bacterial cultures by Monod(1942) who described the two-stop growth curve associated with it as "diauxie". The plateau in the growth curve following exhaustion of one substrate was attributed to the formation of enzymes which were inducible, but the formation of which was suppressed by the first substrate. Other compounds were also tested by Gaudy (1962), and Komolrit and Gaudy (1964) to substantiate that sequential removal occurred using heterogeneous populations. It was also reported that only young cultures exhibited sequential removal of substrates but not the old cultures. (Gaudy, Komolrit and Bhatla,1963).

Stumm-Zollinger, Busch and Stumm (1964) stated that substrates were removed not sequentially in a glucose-galactose system by a heterogeneous population of micro-organism and so laid stress on the need to depend, not only on specific information on the rate of growth and substrate utilization for each constituent of the medium, but also on a knowledge of the extent of substrate interaction and operation of treatment plants.

According to Pardee (1962) the quantity of enzymes in a system can be adjusted to meet the requirements of bacterial cells by means of induction and repression. So, the former supplies enzymes for the utilization of temporarily available nutrients in metabolic activities. From his studies on metabolism of aromatic compounds by Pseudomonads, Stainer (1947) found that this role of induction can be extended to an entire sequence of reactions by a phenomenon known as sequential induction or simultaneous adaptation. " In such a chain reaction when a nutrient, "A" is fed to bacteria an inducible enzyme "E_a" is formed which attacks its substrate, "A" to form "B" ; then "B" serves as an inducer to the formation of enzyme "E_b" which in turn attacks "B" to form "C" and this chain of events continues until the final products are formed." The simultaneous adaptation theory assumes that each metabolite serves as an inducer for the enzyme, which directly metabolizes it. Another possibility is that one metabolite induces the whole series of enzymes necessary for the degradation of the intermediates. Evidence in favour of this rises from the fact that if an intermediate such as "D" in the metabolic pathways is fed, only the intermediates that follow, namely "E" and "F", are metabolized and not the preceeding substrates "B" and "C". However, exceptions to this were also reported (Kalekar 1955).

Following the simultaneous adaptation technique of Stainer (1947) to heterogeneous populations, McKinney, Tomlinson, and Wilcox (1956) studied the metabolism of various aromatic compounds using activated sludges developed on a synthetic waste containing nutrient broth and glucose, and acclimated to various aromatic compounds. Symons, McKinney, Smith, and Donovan (1961) also developed acclimated sludges and studied the degradation of N-containing aromatic compounds.

Mohanrao and McKinney (1962) used a synthetic waste containing acetic acid and mineral salts to build the sludge for subsequent acclimation to quaternary compounds; and from the acetate-grown sludge acclimated to readily utilizable dimethyl glutaric acid, they isolated 5 species of bacteria belonging to the genera Pseudomonas, Flavobacterium, Bacillus and Nocardia and concluded that a bacterial population common to activated sludge could be developed to metabolise the quaternary carbon compounds. In the above two studies, Stainer's(1947) simultaneous adaptation technique was used to explore the metabolic pathways of various aromatic and nitrogenous compounds. In the works of McKinney and his associates and of Gaudy and his associates, the interpretation that enzyme adaptation was the mechanism operating in the adaptation process, rested on the assumptions that the constitution of the microbial population was similar to that of activated sludge and did not change significantly during the sludge acclimation period. Prakasam (1966) found that an artificially developed activated sludge and a treatment plant activated sludge behaved differently. In the former case where there was no population heterogenity, sequential removal did occur and in the later case it did not occur significantly. The sludge from the synthetic waste differed from treatment plant activated sludges in total numbers of bacteria and in the types of bacteria able to grow on various aromatics. In the process of adapting to utilization of aromatic substrates, the populations of bacteria became differentiated from each other.

Change in bacterial population

There are several references in the literature on the subject to show that the quality and quantity of microorganisms do change depending upon the environment. Jasewicz and Porges (1956) found the dominance of the genera Alcaligenes, Flavobacterium, Pseudomonas and Micrococcus in the endogenous sludge and of the genera Bacillus and Bacterium in the actively assimilating sludge. Wuhrmann (1960), Pasveer (1959) and

McNicholos and Tench (1959) increased the rate of purification by increased rates of aeration. The increased rate of purification was attributed to changes in species composition of the bacterial flora of the activated sludge which became biologically adapted to higher concentration of dissolved oxygen. Most probably the micro-aerophilic species were replaced by aerobic organisms which could tolerate the higher concentration of oxygen. Pankhurst (1959) observed that the biological oxidation of thiocyanate in gas liquor did not take place until the oxidation of phenols was complete; and this was attributed to the fact that the thiocynate-oxidising organisms could not flourish in the presence of the phenol oxidizing organisms.

Theory and mechanism of flocculation of the synthetised cells (Phase II) in activated sludge process

Among the accumulated mass of literature on the theory and mechanism of flocculation of the synthetised cells in activated sludge process of sewage treatment, one can discern atleast seven conflicting schools of thought. The first school is led by Butterfield (1935) and his associates who had advocated the special flocculating bacteria theory. The production of activated sludge by *Zoogloea ramigera* in synthetic sewage which was free from detritus and suspended matter, was positive evidence of the fact that the organism was responsible for the formation of activated sludge. So, they concluded that the primary characteristic of this type of organisms, besides their oxidizing capacity, seemed to be their ability to grow in a liquid medium in a massed floc or colony, which bound themselves together so tenaciously as to remain intact even under the agitation of aeration required to maintain aerobic conditions. These facts tended to strengthen the belief that flocculation was a direct result of *Zoogloea ramigera*.

A second theory that the presence of capsules or slime on bacteria would result in attachment of other bacteria as pre-requisite to flocculation has also been proposed. The activated sludge is stated to consist of gelatinous masses in which bacteria exist, and the floc is formed as a direct result of slime excretion by the Zoogloea organisms.Therefore, flocculation was believed to be principally a result of sticking together of particles by the gelatinous material or capsule secreted by the bacteria; and so these organisms were necessary for the best operation of aerobic biological waste treatment processes. Buswell and Long (1923) were the first to propose this theory. Theriault and McNamee(1936), Heukelekian, and Schulhoff (1938), and Cavel (1931) have supported it.

Edwards (1935), Dienert (1935), McKinney and Horward (1952), McKinney and Weichlein (1953), Crabtree, McCoy,Boyle and Rohlich (1966) have opposed this theory. Some of their important objections are stated below. (1) Slime forming bacteria form viscous slime in cultures and mucoid or butyrous in agar slants or plates but zoogloea colonies are dry and wrinkled. (2) In activated sludge the ratio of capsulated to non-capsulated cells is comparatively small. (3) Even granting the existence of capsulated bacteria in activated sludge, it is not known how they could bind themselves into flocs "in the turbulent environment of the activated sludge process or shaken laboratory cultures." Crabtree, McCoy, Boyle, and Rohlich(1966) failed in their attempt to demonstrate the presence of capsules or slime on zoogloea isolates in sterile sewage or in special media believed to enhance the synthesis of capsules for many bacteria. They argued, therefore, that perhaps Zoogloeal organisms in activated sludge flocs might possess special types of capsules which were not ordinarily stainable by the usual staining techniques. So, they tried to harvest the capsular material by physical means and chemical extraction method respectively by the modified procedure of Juni and Heym (1964) and the hot water extracted procedure of Guex-Holzer and Tomsick (1956) and concluded that capsules were not involved in flocculation.

A third "bio-physical and adsorption theory" has been advocated by Renn (1956) and Katz and Rohlich (1956) for biological oxidation of organic matter taking place in both the activated sludge and trickling filter systems. Renn (1956) states: " If we look at the aeration phase of the activated sludge process as a system generating its own surface by supplying large air-water bubble areas or by tumbling small masses of bacterial stuff until they adhere in larger floc particles, we have built up a useful picture (Fig. 1-66). As soon as we introduce fine bubbles of gas by one method or other to whip up foam emulsion, we change the structure of a water mass to favour biological activity. We may regard activated sludge as an ephemeral type of trickling filter. It contains all the essential filter features; that is large air-water surfaces that adsorb and accumulate organic material and bacteria, and large solid water surfaces that also accumulate bacteria and adsorb organic material. We may waste the sludge or not, as we choose. We want to do the same things that happen in a trickling filter; that is, we wish to remove dissolved organic material from solution and localise it so that the water can be recovered. "

Kats and Rohlich (1956) on the other hand, consider clarification of sewage by the activated sludge process as being due entirely to adsorption phenomenon. But it has not been possible to demonstrate to date the adsorption of dissolved organic matter compounds on sludge flocs(Wuhrmann,1956).

Bio-synthesis of the endogenous metabolite (PHB) and the direct chemical bond theory

Crabtree, McCoy, Boyle and Rohlich (1965) have observed from a study of pure culture floc-forming organisms associated with activated sludge that (a) Zoogloea organisms accumulated rapidly a large amount of sudanophilic granules suggestive of poly-β-hydroxy-butyric acid (PHB), which is an endogenous metabolice peculiar to certain bacteria according to the findings of Lemoigne (1927), Williamson and Wilkinson(1958), Doudoroff and Stainer (1959), Rouf and Stokes (1962), Dias and Bhat (1964), and Dawes and Ribbons (1962) ; (b) that the rapid accumulation of PHB was intimately associated with the flocculation of the organisms since capsules or "gums" polysaccharides were not demonstrable on the cells; (c) these organisms always flocculated when the intra-cellular accumulation of this polymer became visible microscopically by Burdon's (1946) test; and (d) the polymer might suggest " a mechanism involved in the conversion of organic material to the polymer during the initial stages of the activated sludge process and possibly involved in formation of the flocs. " So, they isolated and characterised the sudanophilic granules from these organisms and attempted to establish the role of PHB in floc formation as follows :

They found that the typical Zoogloeal organisms isolated from activated sludge were devoid of enzymes for hydrolysis of complex organic matter such as lignin, protein and cellulose. So, "Flocculation due to formation of relatively specific chemical bonds such as peptide or ester bonds linking the cells or cellular compounds together was thought of to be the most likely cause for zoogloeal isolates. When the cells accumulated a large amount of polyester-linked β-hydroxy butric acid, they not only tended to stick together but also divided incompletely, forming netted lacelike masses of cells (flocs). Likewise, the polymer-rich cells in flocs subjected to mechanical dispersion do not remain dispersed but flocculate rapidly into large irregular masses when agitation is stopped. Thus, the influence of this polymer on flocculation needed to be investigated " (Crabtree, McCoy, Boyle and Rohlich 1966). Further, after carrying out a series of laboratory experiments they showed that flocculation was closely associated with polymer-accumulation and that "a mechanism of cellular flocculation is similar to that of the polymerisation of PHB through esterification. "

Tenney and Stumm (1965) had also proposed that "Biological self-flocculation results from the interaction of naturally produced poly-electrolytes which form bridges between individual microbial particles. " But McKinney(1956b) considered "chemical bonding" to be negligible because of the low reactivity of the polysaccharide capsular layers in cells.

The theory of electrokinetic phenomenon on bacterial surface and the chemical composition of the slimy layer

The principal advocates of the above theory and mechanism are McKinney and his associates (McKinney and Horwood 1952, McKinney and Weichlein, 1953, and McKinney 1956b). Investigations carried out in order to study the nature of the bacterial surfaces revealed the existence of a poly-saccharide slimy layer surrounding the bacterial cell. The continual shearing of the polysaccharide slime, the lack of structural strength in the polysaccharide slime and the presence of polysaccharide in varying quantities in the bacterial flocs indicated according to McKinney and his school that the polysaccharide slime cannot bind the bacterial cells into flocs. So, they examined the colloidal concept and made electro-phoretic measurements and suggested that the surface charge on bacterial cell was not hindering floc formation; that the floc did not result until the bacteria reached the endogenous phase of metabolism and that the bacteria became a part of the floc only when they lacked sufficient energy for mobility to break away from the floc. They concluded from the above that biological flocculation was the result of several factors such as the energy content of the system, mass of active and inactive microorganisms, inorganic and organic colloids, salt concentration and pH. Of all these factors, the energy-microorganism relationship was the most important in floc formation in the activated sludge process. In other words flocculation takes place as a result of auto-oxidation or endogenous metabolism.

Tenney, Quitter and Fiore (1966), on the other hand, have shown by selective staining techniques that bio-flocculation is dependent on the presence of extra-cellular material rather than intracellular material. When the extracellular material is removed by centrifugation and the microorganisms re-suspended, stable dispersions result. This extra-cellular material has been extracted, isolated and reused as a flocculent. Thus, " the mechanism of flocculation is explained in terms of the bacterially produced polymer bonding-bridging theory. "

So, it will be seen that upto the present day " only a few basic facts have been formed; as far as the ecology and metabolism of activated sludge are concerned a few data are available but particularly little is known, about the principles of the formation of bacterial flocs" (Van Gils, 1964)..

CONSUMERS

<u>The Phylum Protozoa</u>

In the following an attempt is made to discuss the parts played by protozoa (consumers) in the overall stabilization of organic wastes.

The phylum protozoa may be divided into three major sub-groups; rhizopoda, mastigophora (or flagellata) and ciliophora, based on their methods of locomotion. The rhizopoda with flexible bodies move and ingest food by allowing the body fluid or protoplasm to flow over the substrate by means of ' pseudopodia '. The mastigophora or the flagellata move by means of their long flagella and ingest solid bodies. Ciliophora, on the other hand, move on account of their cilia and engulf bacteria. The last sub-group which is reported to play a greater role in sewage purification than the other two groups is further sub-divided into : (a) holotrichia (or uniformly ciliated organisms), examples of this class being Paramecium, Colpidium, Lionotus etc. ; (b) heterotrichia (organisms with fine and tougher cilia in bands), e.g. Stentor; (c) hypotrichia (or organisms possessing flattened ciliates having stiffer cilia in bands ventrally as legs), e.g. Aspidisca, Euplotes etc., ; (d) peritrichia (or organisms with belt-shaped bodies borne on stalks). e.g. Vorticella, Carchesium, Opercularia, etc.; and (e) Suctoria(which are not ciliated in adult form but bear suctorial tentacles), e.g. Podophrya, Acineta etc. One or several of these groups or other are reported to occur in the different situations of a sewage disposal works.

A large portion of the protozoa is either holozoic or saprophytic in habit. The holozoic forms obtain their food from sewage by the ingestion of solids (chiefly bacteria which have been formed as a result of synthesis and assimilation of soluble organic material) and the latter assimilate soluble organic nutrients into the protoplasm of their bodies. In this manner large quantities of nutrients are held in their bodies, which are deposited in the sludge in sedimentation or humus tanks. This would mean that they form an important link in the purification system. The exact role of protozoa vis-a-vis bacteria has never been fully defined.

Role of Protozoa in the purification of sewage

The relative roles of protozoa and bacteria have been assessed variously in activated sludge process. Two different views are held on the subject. They are : (1) that bacteria are primarily responsible for purification, the protozoa acting only as polishing agents in the final stage and (2) that protozoa are mainly responsible for the purification, the bacteria being of secondary importance. The claims of the protagonists of each viewpoint may be considered briefly.

Butterfield (1935) found that pure protozoa-free zoogloeal organisms alone, were able to bring about 60% purification in 5 day BOD. Heukelekian and Littman (1939) have confirmed it. Butterfield and Wattie (1941) also found that the percentage of purification increased in the presence of Colpidium, a free-swimming ciliate. This was interpreted as being due to the fact that the physiological activities of the bacteria were stimulated more by the predatory action of the protozoa (Cutler and Bal 1926; Meikle John 1932). McKinney and Gran (1956) concluded from the relatively small amount of oxygen uptake by the protozoa in the absence of bacteria in their Warburgh apparatus experiments that protozoa were not the primary purifying agents, though playing a significant role.

Hawkes (1960) is of the view that the presence of certain protozoa in an efficient sludge does not necessarily mean that they play an important role in the purification process. They may merely reflect the prevailing satisfactory condition. He adds further that in case they play a dominant role, they should act as primary feeders in the ecological system. At the early stages of the activated sludge process, although rhizopoda compete with bacteria for food, the latter are always comparatively predominant.

Pillai (1941); Pillai and Subrahmanian (1942, 1943, 1944); Pillai, Wadhawani, Gurubaxani and Subrahmanian (1947); hold the other view that protozoa are directly responsible for purification in the activated sludge process. Pillai and Subrahmanian (1945) carried out experiments with five genera of protozoa and found Epistylis as being the most efficient among them in oxidation and flocculation. Also, they considered Epistylis as efficient as Vorticella.

In commenting upon the exhaustive researches of Pillai and his associates, Curds (1963a) has stated that :(a) Pillai and Subrahmanian (1942, 1943, 1944, 1945) used " whole activated sludge without attempting to control the action of bacteria associated with the protozoa. " Secondly in their so called pure culture experiments with Epistylis sp, their mode of culture was to wash the mucilagenous clumps of Epistylis free from bacteria and culture them in sterile sewage by bubbling air. " They gave no account of checking the axenic condition with bacteria slants, nor did they describe whether the air bubbled in was sterile, and it is unlikely that the Epistylis cultures remained axenic, and that bacteria introduced via the ciliates and air accounted for the purification processes observed. " They also add that it is very difficult to culture Epistylis plicatilis in aerated sewage in the presence of its associated bacteria and that axenic conditions are much more difficult to achieve. Again, Curds states that the conclusions reached from the experiments carried out by Pillai, Wadhawani, Gurubaxani and Subrahmanian (1947) with Epistylis sp. were hardly warranted in view of the fact that there was no accurate bacterial control; and that Curd's similar experiments with correct bacterial controls had confirmed that the protozoa do not play a direct or major role in purification. He found that the ciliates could account for anything between 2 to 5% of the purification only, while bacteria accounted for 11 to 63%. He also found that the bacteria associated with the different species of ciliates had vastly different efficiencies as determined by the acid $KMnO_4$ test.

Hartman (1963) showed by laboratory experiments that ciliates had no role in the degradation of organic matter and also that they were not responsible for the first stage of purification.

Lloyd (1945) and Sugden and Lloyd (1950) have suggested a minor role for the protozoa. The continuous and powerful lashing of the cilia of the protozoan Carcnesium, and their rapid body movements may help in aerating the water and in the mixing of sewage and sludge. This effect may be appreciable in a large bulk of water.

Inter-relationship between Protozoa and Bacteria

Bactericidal organisms are provided by nature for keeping bacterial populations within bounds and insuring their effective and continuous activity. This role is played by protozoa in sewage purification and was demonstrated by Purdy and Butterfield (1918) using Paramecium caudatum as the protozoan. Butterfield, Purdy and Theriault (1931) also demonstrated the same fact using Colpidium. From these two experiments it would appear that bacteria assimilate the nutrient organic substances present in sewage, concentrate them in their own bodies, and become sufficient food in themselves to stimulate the growth of protozoa. So, the bacteria may be said to be " Concentrators " or " Condensers of the dilute food materials present in sewage. "

Barritt (1940), and Pillai and Subrahmanian (1942) showed that the protozoa disappeared almost completely if aeration was continued without replacement of fresh sewage. This was attributed by them to the flocculated bacteria, being unsuitable as food for the protozoa. This may not be the correct explanation. If fresh sewage is not added and aeration is vigorously continued, a situation is reached when food for the bacteria is practically nil. Under these conditions auto-oxidation or endogenous respiration will take place resulting in the disappearance of bacterial cells. So, bacteria which constitute the food for protozoa are not there and the result is the death of the protozoa.

Barker (1946) has shown the rapid agglutination of bacteria by Paramecium caudatum. Pillai and Subrahmanian (1942) have demonstrated that both Epistylis and Vorticella are effective in a similar way. Watson (1945) has shown how the soil ciliate Balantrophorus minutus is capable of agglutinating mixed cultures of bacteria. That the protozoan Oikomonas termo is capable of flocculating bacteria was shown by Hardin (1943).

Cutler and Bal (1926) and Meiklejohn (1932) have stated that the protozoa increase the bacterial activity on account of their predatory behaviour. BarKer (1946) found the protozoans to clarify a sewage either by eating away bacteria or by instituting or increasing flocculation.

Heukelekian and Gurbaxani (1949) have examined the relative roles played by zoogloeal bacteria and protozoa in activated sludge process and concluded that protozoa assist in clarification of the effluent giving a more highly polished effluent and therefore are of secondary importance in sewage purification.

Curds (1963) has found that the bacteria associated with different species of ciliated protozoa in his cultures have different purifying efficiencies. He found their efficiencies as follows :

Activated sludge	62.8%
Vorticella similis	47.3%
Epistylis plicatilis	45.6%
Vorticella microstoma	28.8%
Aspidisca costata	22.0%
Paramecium caudatum	16.8%
Zoothamnium altertans	11.5%

The above table shows that the bacteria associated with Epistylis plicatilis and Vorticella similis are more efficient than others.

Protozoa as indicators of the degree of pollution and purification

Every modern aerobic biological waste treatment system is similar to self-purification of stream with the difference that the purification activity here is intensified. Many of the reactions that slowly proceed in bodies of water receiving sewage are accomplished more quickly in modern sewage treatment plants. The organisms which are found in different stages of sewage treatment are the same as or very similar to those met within the polluted and purified stretches of a stream.So one of the uses of biological studies has, therefore, been to identify indicator organisms for specific environments. Kolkwitz and Marsson (1909) have listed the status of these organisms as parameters of self-purification. They have called them "saprobien" (or saprobic organisms) - those referred to as biological indicators.

This term has also been applied to the properties of water. "Saprobity" or "Saprobicity" was defined by Sladecek (1961) as the characteristic feature of an eco-system determining the composition and development of saprobic communities. According to Elster (cited by Sladecek 1966) it is the amount and intensity of decomposition of soluble organic matter, so that it is equivalent to the rate of decomposition. Caspers and Karve (1966a,b) define it also as the intensity of decomposition of dead organic matter. In the Prague Colloquium in 1966, it was defined as the sum of those metabolic processes which are the anti-thesis of primary production; and therefore the expression of all the processes leading to the loss of potential energy. The degree of saprobicity can be estimated either by measuring the dynamics of metabolism by the analysis of communities of aquatic organisms present (Sladecek 1966). So, degradation of organic matter is the essential component of the concept of saprobicity; and the assessment of the bio-mass of the groups of organisms participating in the metabolic-dynamic processes of sewage purification can yield information relating to saprobic conditions. This approach has been made in the case of modern sewage treatment systems.

One of the three important modern sewage treatment processes is the activated sludge which was invented by Arden and Lockett in 1914. Since then many workers have published voluminous data on two aspects : (a) protozoan succession during sludge formation and (b) indicator organisms for sludge conditions. These two aspects are dealt with below.

Protozoan succession during sludge formation

Buswell and Long (1923) were, perhaps, the first to point out that there was a definite succession of different species of protozoa from the time sewage enters an activated sludge tank to the time that the mixed liquor is discharged into the sedimentation tank. Kolkwitz (1926) found that there was a retrogression of hypotrichs and peritrichs in over-ripe sludge. Experiments conducted by Agersborg and Hatfield (1929) revealed that increased aeration resulted in a succession of flagellates, hypotrichs and peritrichs. Barker (1949) concluded from his studies on trickling filters that there was a succession of protozoans. Barker (1946) recorded three trophic levels (Fig. 2-1 to 2-3) in a developing sludge. During the first ten days of aeration when fresh sewage is daily added, all the three groups of protozoa are represented by increase in species variety. During the next twenty days of aeration when the time required for purification is less than three hours, the mixed liquor is characterised by an increase in numbers of ciliates with much less of rhizopoda and mastigophora. Within the ciliates, there is a succession of species, the attached peritrichs replacing the dominant free-swimming ciliates. Further stages of purification which are not reached in the activated sludge process, would result in the development of autotrophic diatoms, which in turn would support the larger species of holozoic ciliates.

Hawkes (1960), on the other hand, found no such successions in activated sludge process on account of recirculation of sludge.

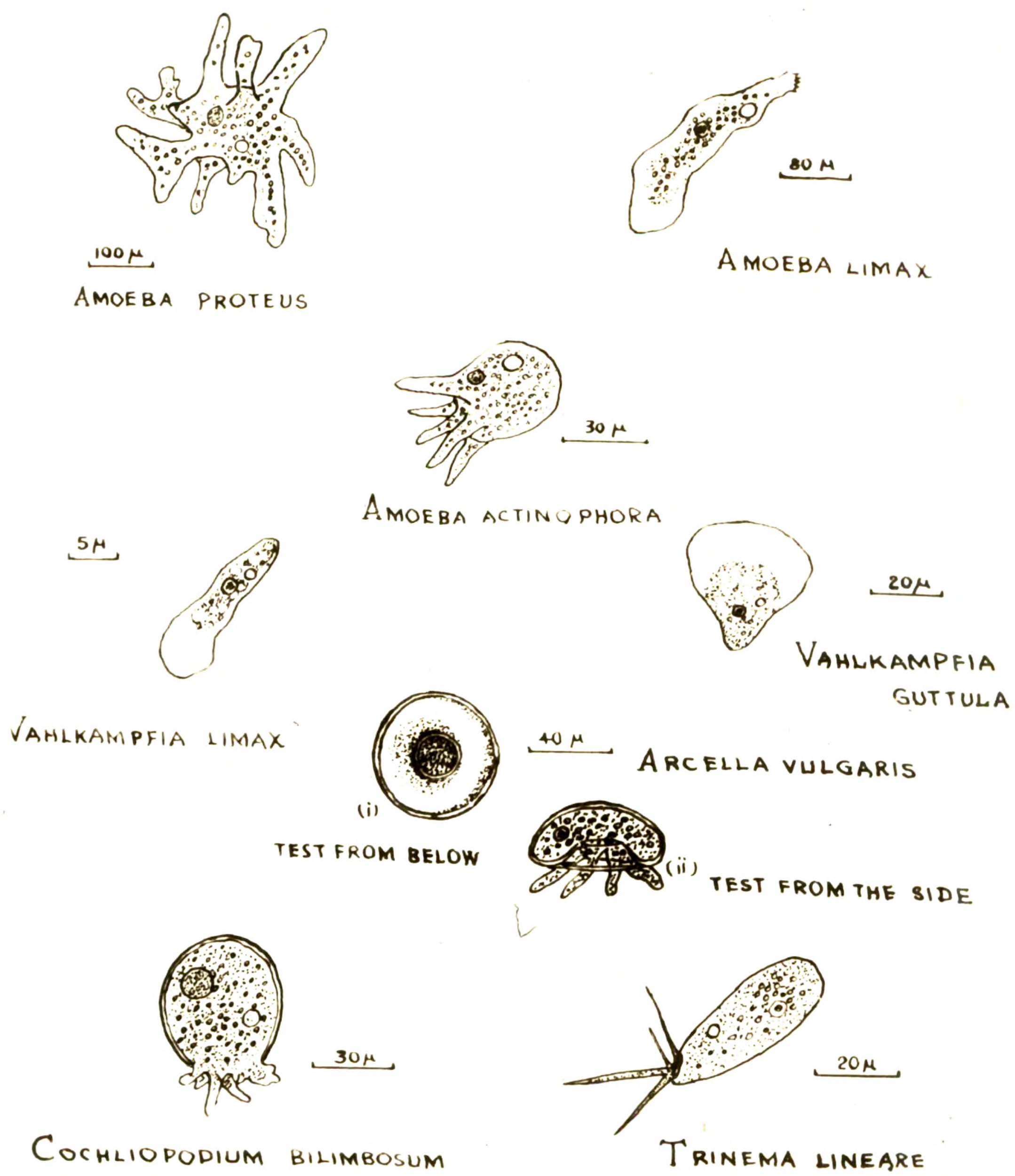

Fig. 2-1: Rhizopods commonly found during development of activated sludge (After A. N. Barker, 1949)

But Curds (1966) found that the sludge proceeded from a condition similar to the polysaprobic conditions of a heavily polluted river towards oligo-saprobic conditions along with a definite sequence of protozoa such as flagellates, free-summing ciliates, crawling, hypotrichs, and attached hypotrichs. He also found an inverse correlation between the crawling ciliates and the peritrichous ciliates. He thought that nutrition of the protozoa was the factor-controlling the successions of protozoa. He also fed different protozoa on different species of bacteria and found that ciliates followed the successions of bacteria as Allen (1944) had indicated.

Indicator organisms

Ardern and Lockett (1936) stated that good activated sludges were characterised by (a) a preponderance of the ciliate protozoa such as Carchesium, Vorticella, Euplotes, Epistylis, Loxophyllum, Choenia, Chilodon, Colpoda, Colpidium, and Aspidisca; (b) very few flagellates, amoeba and other rhizopods; (c) very little of filamentous growth; and (d) the presence of rotifer fauna. Bad or unsatisfactory sludges were characterised by a preponderance of flagellate protozoa, very few ciliates, and a large amount of filamentous growths. They have also stated that the presence of Vorticella can be taken as an indication of a good sludge.

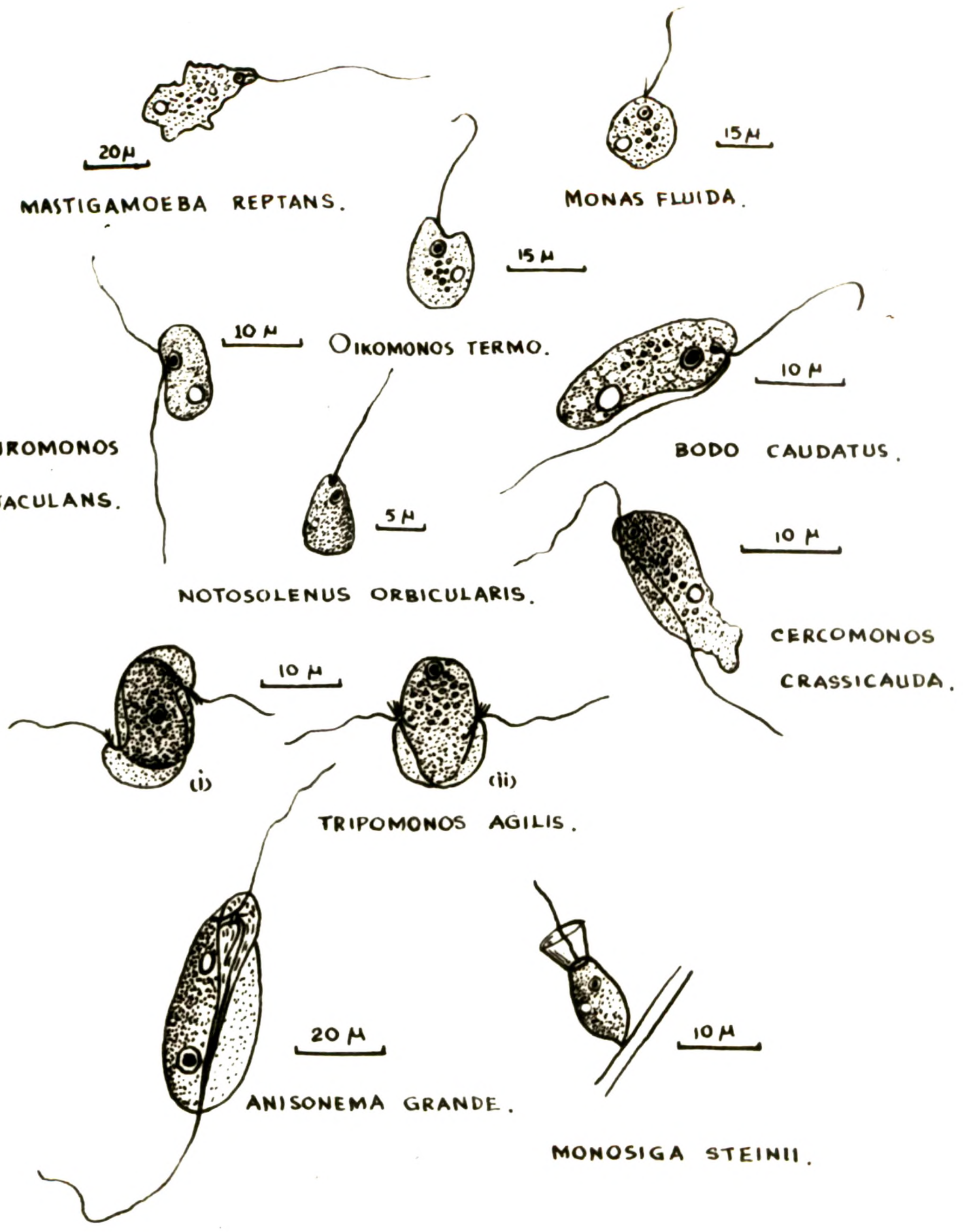

Fig. 2-2: Flagellates commonly found during development of activated sludge (After A.N.Barker, 1949)

Reynoldson (1942) found that the better the quality of the effluent, the higher the vorticella count. Baines, Hawkes,Hewitt, and Jenkins (1953) have stated that Aspidisca with peritrichous ciliates is an indicator of a good sludge; Lionotus with Oxytrichia and stylonchia as representing intermediate sludge types; and Paramecium and Colpidium as indicative of a poor sludge.

Mckinney (1957) stated that the relative types of protozoa and their numbers can be used for any particular system to estimate roughly the efficiency of the purification process. According to Mckinney and Gran (1956) a good activated sludge will contain a relatively high active population of stalked ciliated protozoa such as Vorticella, and an occasional rotifer or the free-summing ciliate Stylonchia. Hawkes (1960) found several species of Vorticella occuring along with Opercularia, Aspidisca, and Lionotus sp. when the effluent is excellent. But one species i.e. Vorticella microstoma alone has been found when the effluent quality is poor. Paramecium Caudatum (Fig. 2-4, and 2-5) is ordinarily found with a less efficient sludge but, at times it is seen when the effluent also is good.

Curds (1966) has shown that certain Ciliates are associated with definite ranges of saprobity. The peritrichs usually indicates β - mesosaprobic to oligosaprobic conditions. But Vorticella microstoma (Fig. 2-4 and Fig.2-9)

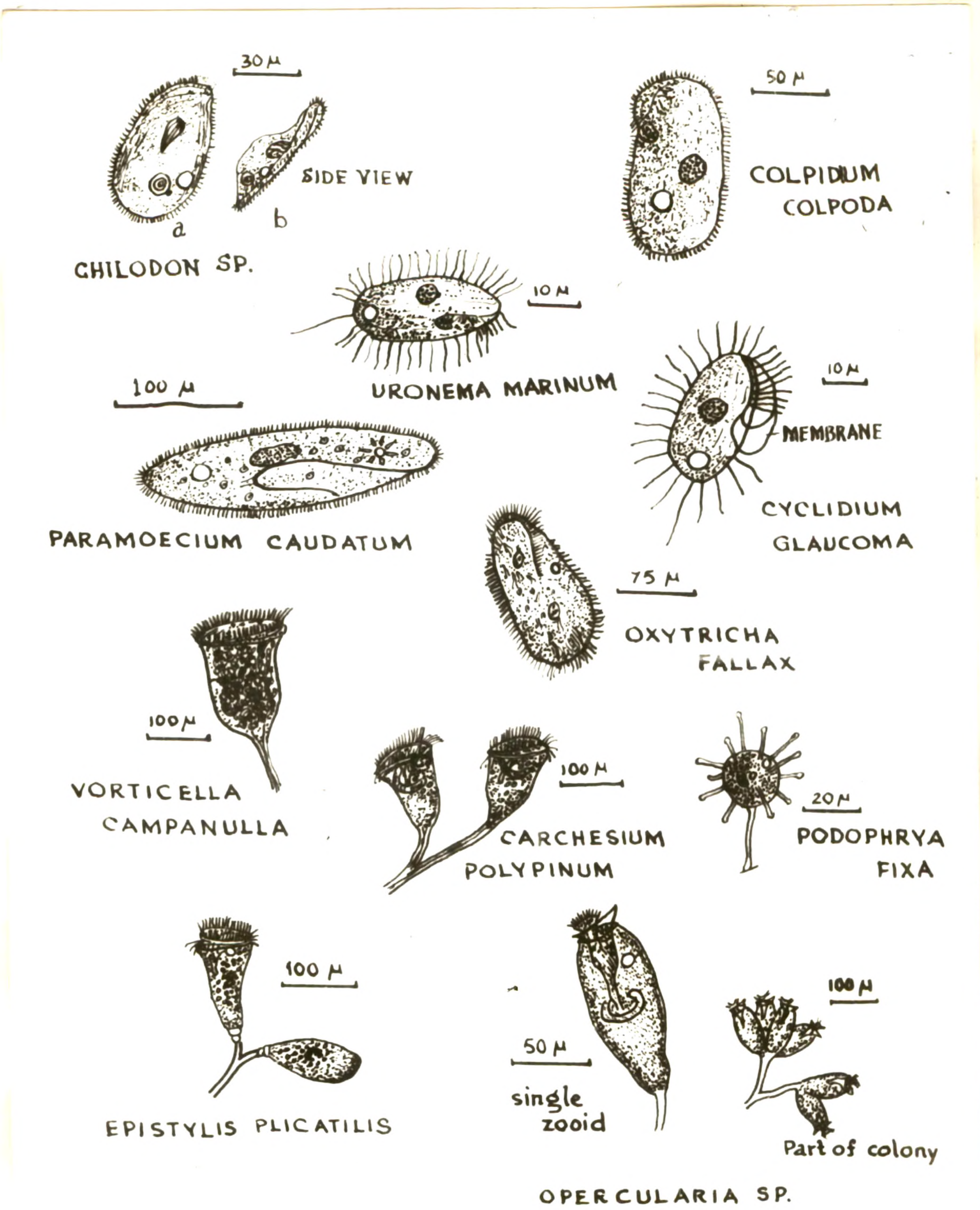

Fig. 2-3: Ciliates commonly found during development of activated sludge (After A.N.Barker, 1949)

or Vorticella putrina is usually associated with poly-saprobic conditions. He also adds that there is a wide variety of ciliates which are indicators of various saprobic conditions so that it is very difficult to state as to the quality of the sludge at any one time.

Curds (1963), has summed up the views of different workers regarding the occurrence of protozoa as indicators of the degree of pollution and purification in activated sludge process in Table 31 of his thesis as follows :

Protozoa	Expected Sludge or effluent condition	Author
1. Paramecium Caudatum	Inefficient sludge	Hawkes (1960)
	Good effluent	Hawkes(1960)
	Capable of flocculating sewage bacteria	Barker (1946)
2. Paramecia	Clear effluent of unusual clarity	Johnson(1922)
3. Paramecium sp	Unsatisfactory conditions	Ardern and Lockett(1928)
4. Arcella sp.	Inefficient sludge	Hawkes(1960)
	Good, highly nitrified effluent	Hawkes(1960)
5. Amoeba sp.	Inefficient sludge	Lockett(1928)
6. Flagellates	Inefficient sludge	Lockett(1928)
7. Oikomonas termo	Capable of Bacterial flocculation	Hardin (1943)
8. Ballantrophorus minutes	Capable of bacterial flocculation	Watson (1945)

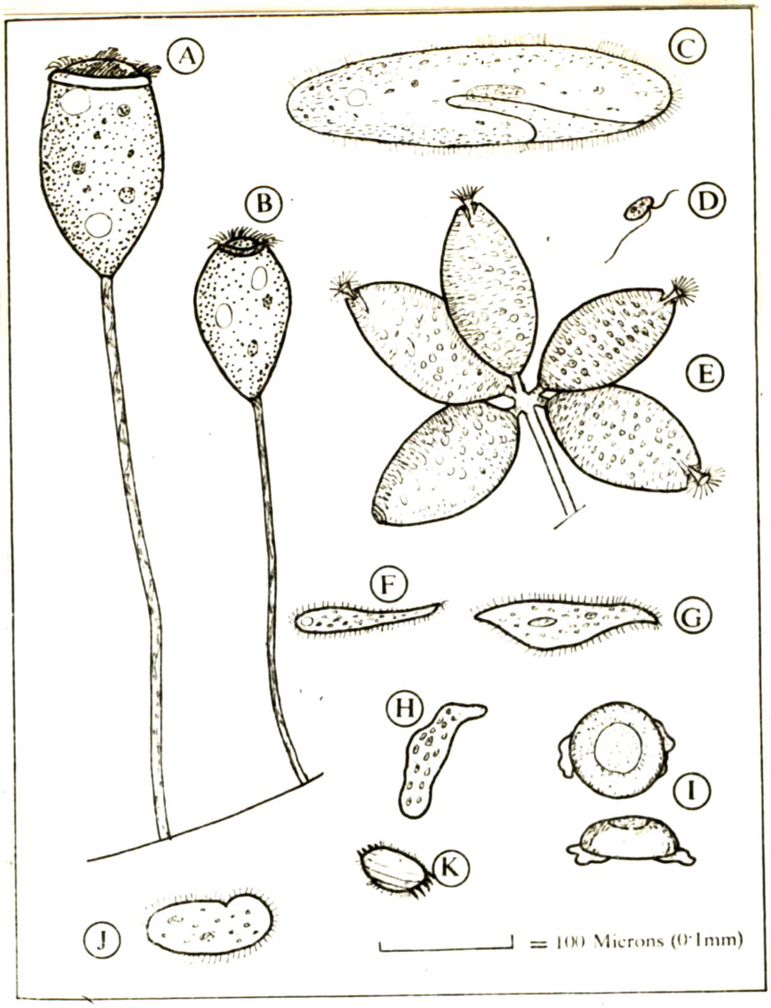

Fig. 3.3. Some protozoa common in activated sludge and bacteria beds: (A) *Vorticella* sp., (B) *Vorticella microstoma*, (C) *Paramoecium caudatum*, (D) *Bodo caudatus*, (E) *Opercularia* sp., (F) *Lionotus fasciola*, (G) *Amphileptus* sp., (H) *Amoeba limax*, (I) *Arcella vulgaris* (surface and side views), (J) *C pidium colpoda*, (K) *Aspidisca polystyla*.

Fig.2-4: Some Protozoa common in activated sludge and bacteria beds (After H.A.Hawkes)

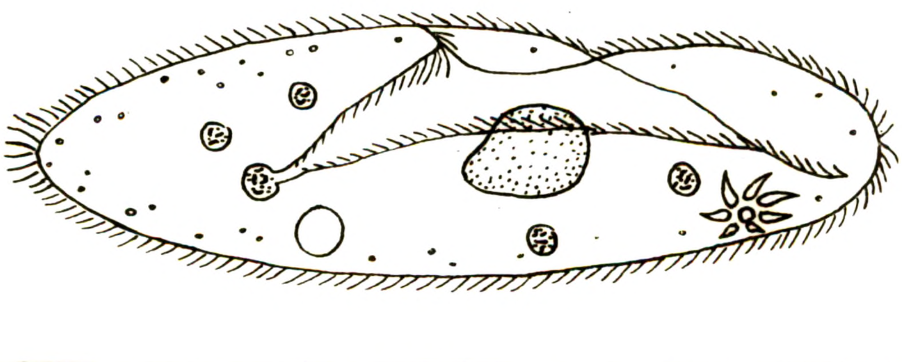

Fig.2-5: A view of Paramecium caudatum (After W.T.Callaway)

Protozoa	Expected Sludge or effluent condition	Author
9. Lionotus sp.	Efficient sludge	Hawkes (1960)
10. Aspidisca sp.	Efficient sludge satisfactory condition	Ardern & Lockett (1928)
11. Choenia sp.	Good condition	Lockett (1928)
12. Vorticella sp.	Satisfactory or good effluent	Ardern & Lockett (1928)
	Good effluent	Reynoldson (1942)
	Efficient sludge	Hawkes (1960)
13. Vorticella microstoma	Inefficient sludge	Baines et al (1953)
14. Opercularia sp.	Efficient sludge	Hawkes (1960)
15. Epistylis sp.	Capable of flocculation, nitrification and will lower $KMnO_4$ value	Pillai and Subrahmanian (1944)
16. Carchesium sp.	Capable of clarification and will oxidise	Pillai et al (1960)
17. Carchesium and Stentor Sp.	Associated with bulking	Arden and Lockett (1928)
18. Suctoria	Satisfactory or good	Lockett (1928)

Krishnamoorthi and Bick (1966a,b) studied the succession of the dominant species of ciliate protozoa for improving the biological monitoring of water pollution by adding varying quantities of peptone to stored water. They found that Colpidium campylum, Glaucoma pyriformis, Paramecium caudatum, Tetrahymena pyriformis, and Vorticella

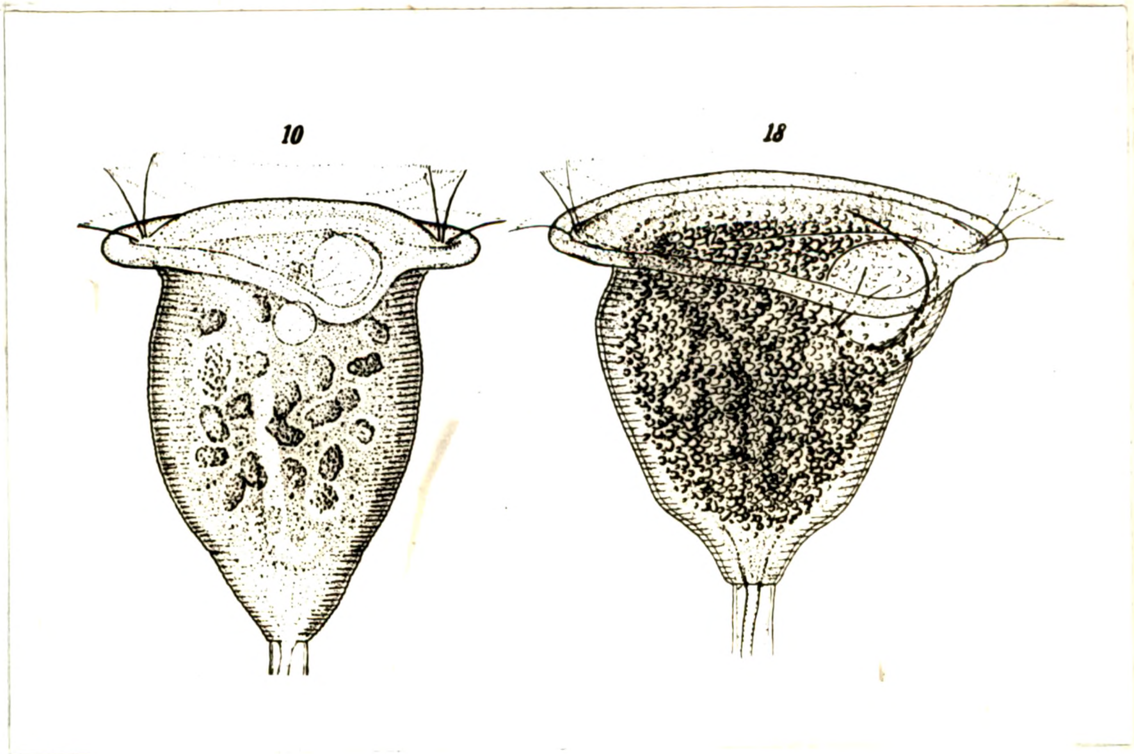

Fig.2-6: A view of Vorticella convellaria x 600 (After Noland and Finley, 1931)

Fig.2-7: A view of Vorticella campanula x 600 (After Noland and Finley, 1931) microstoma tolerated high ammonia content and low dissolved oxygen tension. They found Colpidium campylum, Glaucoma scintillans, and Tetrahymena pyriformis in the initial stages of pollution; and Chilodonella cucullulus, Chilodonella uncinata, Paramecium bursaria, P. caudatum, P.trichium and Vorticella microstoma in the later stages. An increase in the amount of ammonical nitrogen following the addition of peptone prohibited the development of ciliates. Species representation is very limited but individual counts are high. The dominant species were polysaprobic. They concluded that Colpidium campylum and Glaucoma scintillans were good indicators of polysaprobic conditions and that Tetrahymena pyriformis and Vorticella microstoma were hampered by high ammonia content.

Curds (1963) has also concluded that for several reasons there is some confusion among workers on the exact role of protozoans in sewage purification; (a) lack of accurate information about the protozoans themselves as to how they bring about purification; (b) incorrect identification upto the species level, and (c) ignorance about the bionomics of the species concerned.

PRODUCERS

As regards algae which are the primary producers; they are distinguished from fungi by their chlorophyll content and autotrophic mode of nutrition. The colouration of their pigments formed the basis of their broad classification into six groups until the beginning of the 20th century, but now

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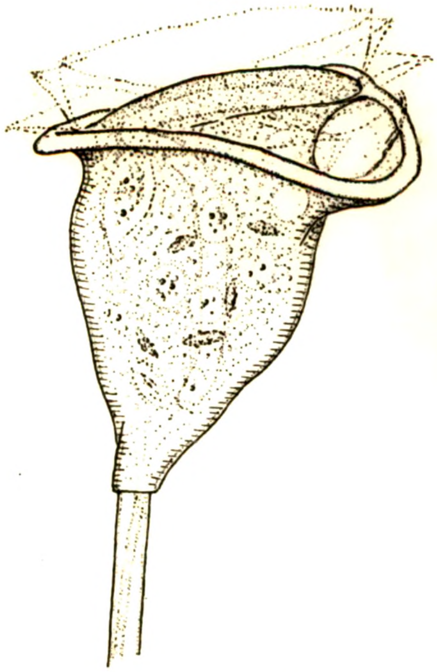

Fig.2-8: A view of Vorticella nebulifera x 600 (After Noland and Finley, 1931)

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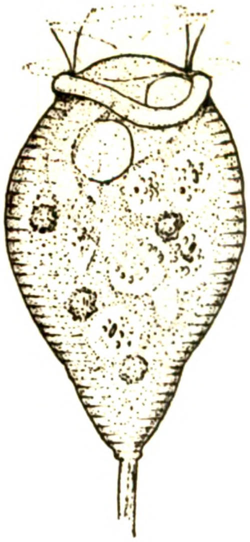

Fig.2-9: A view of Vorticella microstoma x 600 (After Noland and Finley, 1931)

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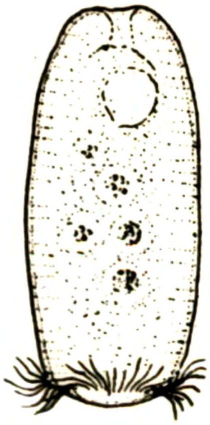

Fig.2-10: A telotroch of Vorticella x 600 (After Noland and Finley, 1931)

it is possible to distinguish clearly eleven groups. The former six algal groups are : Chlorophyccae, Cyanophyceae, Diatomaceae, Phaeophyceae and Rhodophyceae. The first two groups are mostly found in fresh water and sewage ponds. The former contains Chlorophyll type pigment, starch grains and cellulosic cell wall. The latter contains in addition to Chlorophyll other pigments such as Carotenes, Xanthophylls and phycobilins.

The algae can also be broadly classified into two groups as fat producers and starch producers.

The food requirements of algae are essentially mineralised product of decomposition of organic matter such as carbon dioxide, nitrogen compounds, phosphates, silicates, salts and alkali and alkaline earths and trace elements.

OXIDATION POND

Historical

The conventional oxidation pond method of sewage purification and its various modifications as we know them now did not develop from a well planned research programme but was evolved out of the time-honoured emperical practice of lagooning organic liquid wastes in basins or depressions or ditches used chiefly for seepage, settling or holding. Soon after, such accidental or designed use of ponds was reported as a means of treating municipal wastes. The earliest records of the existence of one such oxidation pond is in Santa Rosa in the U.S.A. since 1924; and the first paper giving real importance to oxidation pond as a distinct method of sewage treatment is by Caldwell (1946). Since then numerous publications have appeared, all of which deal with either design, operation and performance or all the three. This treatment system depends on the effective use of bacteria for degradation of the soluble organic constituents and ordinarily of green algae for oxygenation. Aerobic conditions are maintained near the surface and sometimes throughout most of the depth of the pond.

Fitzgerald and Rohlich (1958) have evaluated the existing literature on the subject under several heads such as the history of the use of oxidation ponds; their effectiveness in lowering BOD; relationship between algae and standard BOD measurements and its removal; organic loading and purification; their effectiveness in lowering coliforms, pathogenic bacteria and nutrients; algae, their yield, seasonal variations and limiting factors; their economics etc. Recently Gloyna (1965) has furnished an account of the experience and concepts pertaining to waste stabilization ponds.

Fundamehtal quantitative data for their economical and efficient design have been furnished by the California University Engineering Research Group led by Gotaas and Oswald. Gotaas and Oswald (1955), and Oswald and Gotaas (1957) have developed design criteria for oxidation ponds taking into account both controllable and uncontrollable factors.

Suwannakaran and Gloyana (1963) have evaluated under laboratory conditions the effects of temperature and organic loading on the performance with a view to establish better design criteria. They found that within limits the BOD removal increased with the increase in temperature; changes in biological activity due to temperature fluctuations influenced the pH, MPN of coliforms, suspended solids, light transmission, predominent algal species etc. They claimed that it was possible to formulate a design equation taking into account both temperature and pond loading. Van R.Marais (1963) has also presented a rational theory for designing oxidation ponds in tropical and sub-tropical areas of Africa based on the correlations of the kinetics of BOD and faecal bacteria reductions in a series system of oxidation ponds. Wachs, Rebhun, Meron, Kott and Sless (1961) have studied the oxidation ponds in Israel and stressed the need for investigations regarding the chemical and biological processes involved in them. Neel, Dermott and Monday (Jr.)(1961) studied five identically sized oxidation ponds loaded at 40, 50, 60, 80 and 100 lbs/acre/day and showed that oxidation ponds were very efficient in reducing BOD, phosphate and nitrogen compounds. Parker (1962) has furnished data on eight oxidation ponds working in series in Australia. The BOD reduction was reported to be excellent throughout the ponds. Only the eighth pond showed a definite reduction in nitrogen content. Bogan, Alberton and Plunze (1960) studied the removal of phosphates by algae and found that these were removed better by chemical purification rather than by algal metabolism. Bush, Ishwerwood and Rodgi (1961) attempted to remove the nutrient substances from an activated sludge effluent by treating it with a continuous supply of carbon dioxide for increased algal growth and to maintain a pH between 7.0 and 8.5. Seventy six percent of the phosphates and 100% of the nitrogen were removed. Ganapati, Prasada Rao, Godbole, Kothandaraman and Koshy (1965) have studied the bio-ecology of solar sewage drying beds (which are nothing but small-sized oxidation ponds) in the Perana Sewage Farm at Ahmedabad. Gann, Collier and Lawrence (1968) state that the bacteriology of stabilization ponds is conspicuous by its absence from literature.

From the foregoing review of the literature it will be seen that most of the studies deal either with the non-pathogenic coliforms which are indicators of sewage pollution or with pathogenic bacteria of the Salmonella and Shigella groups; and practically none about the relative distribution of microbial populations involved in the so called "algal-bacterial symbiosis" as they undergo environmental stress as a result of long storage or retention period of 20-30 days.

Role of algae in the purification of sewage

In the two conventional methods of sewage purification - the activated sludge process and the trickling filter, -

oxygen which is essential for oxidation of the putrid and decomposing organic matter is obtained by mechanical means. But in the oxidation pond method, oxygen is released into the water medium by fresh algal cells which split water molecules as a part of their photosynthetic activity. Thus natural light energy is used to produce oxygen, when two basic types of reactions are taking place together: Oxygenation by algal photosynthesis and bacterial oxidation of the decomposing organic matter. The rates of these reactions is governed by the rate of growth of algal cells, which is believed to depend primarily upon the availability of CO_2 and the amount of light. So, sun's energy is trapped through photosynthesis as the principal synthetic force for purifying sewage in oxidation ponds.

Algae may release twenty times as much oxygen in photosynthesis as they utilize in metabolism (Palmer 1956). The significance of this in the biological aerobic purification of organic wastes is obvious, since rapid decomposition of wastes depends primarily on aerobic bacteria. So, intensive cultivation of algae in fresh sewage is a highly effective means both for supplying oxygen for aerobic decomposition of organic matter and for reclaiming nutrients from the wastes in the form of algal cells.

During the first stage of oxidation of organic matter, heterotrophic bacteria produce CO_2 and liberate ammonia. Green algae utilizing energy from the sun produce carbohydrates from CO_2 and H_2O ; and assimilate the same together with the liberated ammonia and other products of biological significance for synthetising fresh algal cells each of which is capable of fixing solar energy.

One gram of algae produced is usually accompanied by 1.6 gm. of molecular oxygen. The heat combustion of the algal cell is estimated at 6 k.cal per gram, so that about 3.6 k.cal of solar energy is fixed in the production of one gram of oxygen. Since the amount of oxygen required by bacteria to oxidise the waste can be readily determined (i.e. BOD), the weight of the algae that must be grown, and the quantity of solar energy that must be fixed in order to produce the required quantity of oxygen by photosynthesis may also be determined.

When an alga synthetises fresh cells, it is most likely that a certain amount of organic substances formed within it, escapes into the surrounding medium (Fogg and Westlake 1955). Such liberation takes place not only from various species of Chlorophyceae but also in growing cultures of Myxophyceae (Fogg 1953). So, the resulting substances may be biologically active even in low concentrations and according to Lucas (1947) their ecological effects may be considerable.

These substances may be either growth promoting for other organisms (Krogh, 1931) or inhibiting according to

Lefevre and Jacob (1949). Such substances have neither been isolated nor their mode of action investigated.

Fogg and Westlake (1955) have shown that the extra-cellular organic substances produced by algae may exert ecological effects indirectly by forming chemical complexes with other dissolved substances. The material liberated by Anabaena cylindrica also shows this property (Fogg and Westlake 1955).

In cultures of blue-green algae as much as 50% of the nitrogen fixed appears in the medium in soluble form (De 1939). Watanabe (1951) found little free amino-nitrogen generally in such cultures, although Calothrix brevissima liberated appreciable quantities of aspartic acid, glutamic acid, and alanine. Fogg (1952) concluded that the extra-cellular nitrogen is in the form of a polypeptide. Lewin (1956) found that all of the 18 species of Chlamydomonas which he studied liberated polysaccharides into the medium and that these constituted 25% of the total carbon produced. Galactose and arabinose were the main components of these polysaccharides but smaller amounts of fucose, rhamnose, uronic acids and unidentified compounds were found.

Fogg and Westlake (1955) have isolated appreciable quantities of dissolved peptide nitrogen in English lakes similar to that found in culture filtrates. Using radio isotopes in lake waters, Fogg (1958) found that between

1 and 11% of the material synthesised by phytoplankton is liberated into the surrounding medium under normal conditions.

Again, according to Fogg (1960) if the turnover of simple organic acids and polysaccharides were high, they would constitute the means for the transference of considerable quantities of material. So, he concludes that if the phenomenon of extracellular enzyme production is at all widespread, the algal organisms must play quite a considerable part in the breakdown of organic matter present in water and that is of particular interest in view of the growing use of algae in sewage purification and deserves further investigation. He also thinks that the biologically potent substances released by algae may play a part in determining what species be present in a given situation. He would try to explain the periodicity of phytoplankton in terms of growth substances and antibiotics which, being released by one dominant species determined the species which is to succeed it.

According to El-Baroudi and Moawad (1967) the phenomenon of secreting extracellular organic substances of biological significance, in considerable amounts would add to the BOD load in an oxidation pond and thus reduce the stabilization capacity achieved by algae synthesis.

Several investigators have found indications of antibiotics being liberated by certain species of algae. Oswald, Gotaas, Ludwig and Lynch (1953b) have found a low bacterial population in presence of Chlorella compared to bacterial population attained in systems in the absence of the algae and in systems containing Euglena gracilis. In systems containing Chlorella, despite the availability of oxygen in the culture they found that the BOD of the clear supernatant was higher as compared to that obtained for Euglena supernatants. The BOD of the whole effluent was similarly higher than that of the influent sewage for all but the shortest retention periods. Similar findings have been reported earlier by Caldwell (1946) and Gotaas, Oswald, Ludwig and Lynch (1951). So, they concluded that there was some factor inhibiting bacterial action upon the sewage substrate when Chlorella are present.

Again Oswald, Gotaas, Ludwig and Lynch (1953a) have stated that Chlorella inhibited bacterial decomposition of the sewage, thereby imposing a self-limiting factor upon its own growth. They add that utilisation of carbon from alkalinity of sewage or from the air that they used for bubbling does not contribute to sewage treatment. They considered it most likely that Chlorella utilized other carbon sources such as sugars than CO_2. They concluded that the direct use of carbon sources (extra cellular products or fatty acids) may partially account for the fair degree of sewage treatment obtained even though bacterial growth was inhibited. Spoehr et al (1949) has determined the nature of the antibiotic liberated by *Chlorella pyrenoidosa* as fatty acids.

Algal-Bacterial symbiosis

Since algae obtain their energy for synthesis from sunlight, there is no necessity for them to metabolise organic compounds like the bacteria and the fungi.

The mode of nutrition of algae is autotrophic;bacteria and algae can work together one helping the other in commensal relationship. In other words, the bacteria metabolize the organic components of the waste and release some substances utilizable by algae. During synthesis of fresh algae cells, algae release oxygen, which is utilized by the bacteria for stabilization of organic matter.

In the absence of sunlight, algae obtain the energy to remain alive from metabolism of organic matter just like bacteria and fungi. This organic matter normally comes from stored food within their cells but in some cases, it can come from the organic matter in the wastes.

The treatment effected by an oxidation pond results from a complete symbiosis between bacteria and algae(Ludwig, Oswald, Gotaas and Lynch,1951). Symbiotic activity is reduced because of bacterial inhibition in the case of *Chlorella* only, as far as we know at the moment. Other algae are not reported to produce such an effect.

EI-Baroude and Moaward (1967) found throughout their experimental studies on a laboratory model oxidation pond, consistently high and low rates of B.O.D. reduction, which they attributed to alternative reactions of bacterial degradation and algae synthesis not always achieving a dynamic equilibrium, when alone a stable degree of B.O.D. removal is possible. The symbiotic relations between algae and bacteria are not so simple as postulated by Gotaas, Oswald and others, for their is no steady dynamic equilibrium between synthesis and breakdown of organic load. In certain cases, they stated that the conditions might be such that instead of mutual inter-dependence, the development of one partner might proceed at the expense of the other leading to the disturbance of the acquired balance. In other words the commensal relationship might be disturbed by the failure of one partner(the bacteria) when the other (the algae) existed in a highly developed stage. A rapid inactivation and death of algae cells might inevitably follow since they would be deprived of the nutrients resulting from bacterial action.

Also, the region of photosynthesis might be reduced due to voluminous algal growth; and this situation would inevitably result in the death and disintegration of algae which might add to the already existing B.O.D. load.Finally, there will be a remarkable decrease in the B.O.D. reduction efficiency of the pond. A lag period will be needed before bacterial activity is re-established with the gradual recovery of the pond efficiency. In this way, they state, that the stabilization of organic matter in the oxidation pond will be associated with a cyclical fluctuation which temporarily amplifies the stabilization efficiency or reduces it to such an extent that an effluent B.O.D. higher than that of the influent may be observed as Amaramy (1960) found.

Algae-Protozoan relationship

Rotifers, Cyclops and Cladocerans are known to eat away algae in oxidation ponds, thereby reducing the high algae concentration necessary for the ponds' efficiency. Shallow depth and too long detention periods are reported to be favourable for predatorship in the Dakotas. (U.S.D.H.E. & W. 1957). In a series of three ponds, the first pond was found to be green, while the second and the third were colourless and clear especially during sunny months on account of predatory organisms, which were present in the last two ponds. Krishnamoorthi (1965), Parabrahman,Khan and Laxminarayana (1965) found that a dephnid was responsible for eating away the Chlorella bloom (40,000 per litre) in an oxidation pond at Nagpur. Oswald (1960) found rotifers and Cladocera occasionally in their pond, eating away the algae population. Jayangoudar (1967) found a dephnid in large numbers in the oxidation ponds of Ahmedabad, which were dominant. Gummert, Meffert, Stratman (1953) found that Chlorella cultures were more readily attacked by protozoa than Scenedesmus which showed a greater resistance. So, it would appear that protozoa can eat away small sized algal organisms like Chlorella and not filamentous forms like Arthrospira sp or Oscillatoria spp. (Jayangoudar 1967).
