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IX

# GENERAL SUMMARY

## MECHANISMS OF SEWAGE PURIFICATION IN THE OXIDATION POND METHOD

Composition of Sewage

On the average sewage consists of 99.9% water and 0.1% solids (Veatch, (1938). The solids, in turn, consist of coarse suspended matter, colloidal and of soluble organic matter. Most of the non-settleable insoluble material in sewage is in a state of colloidal aggregation and this might be taken to indicate that " the colloidal matter in sewage has a natural tendency to form aggregates or that it is a transitory stage between larger particles and material in solution " (Heukelekian 1941). According to Rickert and Hunter (1967) sewage solids are 14% settleable, 11% supra-colloidal, 6% colloidal, and 69% soluble. The soluble matter constitutes the largest amount, and covers a wide range of compounds; carbohydrates (xylose, glucose, maltose, lactose, sucrose, dextrin and soluble starch); alcohols (methyl and ethyl alcohols, ethylene glycol, glycerol); organic acids (formic, acetic, tartaric, citric, lactic and oxalic acids), amino acids (glycine, alanine, glutamic acid, tyrosine, cystine etc.); proteins (peptone, gelatin) and miscellaneous compounds such as formaldehyde, ammonium acetate, calcium gluconate, olive oil, soap, mineral oil, acetonitrite and thioglycolic acid (Placak and Ruchhoft, 1947). Generally speaking, raw sewage is stated to contain carbohydrates, proteins, amino acids and fat both in suspension and in solution.

Organic wastes vary greatly in composition and intensity of the above constituents. For example Baroda raw sewage differs from English sewage in containing comparatively more fat and protein. So, any or even a majority of the above mentioned organic substances may be found as constituents of any given sewage. The removal of the colloidal and soluble material by aerobic heterotrophic bacteria present in sewage is the chief principle involved in all the three-well-known secondary sewage treatment devices such as the activated sludge process, the trickling filter method and the oxidation pond system. But the exact mechanisms of purification may be expected to be different, as the time taken for purification in each case is strikingly different. In the case of the activated sludge system, the contact period is about 4-6 hours between the organic wastes and the activated sludge when stabilization is brought about. If molecular oxygen replaces air in the above process, purification is effected in less than one hour. Trickling filter, on the other hand, does not remove as much organic matter as activated sludge or oxidation pond, as the purifying films on the filter media are stationary with the sewage momentarily flowing over them; and the contact period between the wastes passing through the trickling filter and the microbial surfaces on the filter stones is about thirty seconds only (McKinney and Pfeffer, 1965). But in the case of the conventional oxidation pond, neither of the above phenomenon takes place. The time required for organic wastes to be purified by this comparatively sluggish process is 20-30 days which is lessened to 2-5 days in the high-rate oxidation pond. (McGauhey, 1960). So, the exact principle or pattern of purification may be expected to be different in each of the three cases.

As there are no references in literature regarding the mechanisms of purification (barring vague and generalised statements such as "algal-bacterial symbiosis") in the oxidation pond method; and as hundreds of references are available on the activated sludge system, it was considered desirable to examine the latter data first for a proper understanding of the mechanisms involved in the oxidation pond method of sewage treatment about which this thesis deals.

Mechanism of Sewage Purification in the Activated Sludge Process

The activated sludge process has been succintly described by Phelps (1938) and it cannot be bettered. He says: "suitable exposures of sewage to the action of living forms which it normally contains and in the presence of an excess of oxygen results, in time, in the development of an active biological material, the basis of which is a bacterial Zoogloea. When fully developed this is a mixture of plant and animal forms including protozoa. Whether it is characterised and dominated by one organism or a few specific types of organisms is not known. This active mass has a highly adsorptive property for the dissolved organic matters of sewage including ammonia and also an agglutinating or flocculating property, so that sewage, intimately exposed to it, is rapidly freed from its dissolved and suspended impurities."

The activated sludge floc in plants scattered far and wide is surprisingly enough constant in appearance and properties. There must, therefore, be some fundamental mechanism which will explain the method of formation and mode of action of the floc. Fundamental studies in this regard have been in progress since the discovery of the process by Ardern and Lockett in Manchester in England in 1913 without formulating as yet any definite and commonly accepted theory relating to its accelerating influences. Attempts have been made to study the nature of the floc from two angles: its biological character and its response to various organic substances and they are described below.

The Biological Character of the Activated Sludge Floc

The biological character of the floc is evident from the research papers of Butterfield (1935) and Butterfield and Wattie (1941). They stated that the predominant zoogloeal bacteria of trickling filters and activated sludge were the active agents in purification by biological processes; that these bacteria were present atleast to the extent of 300 millions per ml of the filter growth; and that pure cultures of these bacteria have the ability to produce adherent growths on the stones of a filter or produce a floc of the same general appearance as of activated sludge. These bacteria, when aerated in pure culture in a clear synthetic medium or in sterilized sewage, produce a growth which exhibits the characteristic properties of activated sludge such as flocculation, rapid settling, and clear supernatant with high rates of oxidation and total purification of the contained soluble organic material (Butterfield, Ruchhoft and McNamee,1937).

So, the most commonly accepted view has been that a single bacterial species namely, Zoogloea ramigera is the predominant bacterium in the activated sludge flocs as stated by Butterfield (1935), Calvert (1932), Butterfield and Wattie (1941),Heukelekian and Schulhoff (1938), Falk and Rudolfs (1947), Helmers et al (1951), Varma and Reid (1964), Dugen and Lundgren (1960), Unz and Dondero (1967), Crabtree, McCoy, Boyle, and Rohlich (1965, 1966) and Friedman and Dugan (1968).

But the studies by Allen (1944), McKinney and Horwood(1952) and McKinney and Weichlein (1953) have shown that there are many bacteria having the ability to flocculate like Zoogloea ramigera. It has also been postulated that all the bacteria possess the ability to flocculate under certain environmental conditions (McKinney 1956b). Very recently Dias and Bhat(1964) and Unz and Dondero (1967) have also shown that Zoogloea ramigera is not the only organism responsible for purifying sewage by the activated sludge process. The latest position has been explained by Crabtree, McCoy, Boyle and Rohlich (1966) regarding the role of Zoogloea ramigera in the activated sludge process to which we will revert later.

Removal of Finely Dispersed or Colloidal Matter in Sewage

According to Heukelekian (1941) finely dispersed matter (i.e. colloids in sewage) is amenable to flocculation which is brought about both by physical and biological agencies working either independently or simultaneously. This observation is based on the fact that the removal of dispersed matter from sewage can be accomplished under sterile conditions. The removals are appreciable with short periods of flocculation which in the absence of a mass of active biological slime is an inadequate period for biological action to take place. He states further that flocculation takes place even under quiescent conditions. Increasing the internal surface accelerates flocculation. The internal surface may be increased by (a) adding clean solids materials, (b) agitation and (c) aeration. When the internal surfaces become coated with an active biological slime, the rate of removal is further accelerated.

Mechanical flocculation and bioflocculation proceed simultaneously. The former plays a more important role during short periods. As the period is increased bio-flocculation plays an increasingly important role. When the surfaces become coated with biological growth, the rate of removal of both dispersed and soluble material is increased.

In the case of the activated sludge and trickling filter methods, the success of the purification depends upon (a) nutrient for the bacteria, (b) continuous supply of oxygen, (c) bacterial mass or flocs and (d) upon the physical methods adopted to keep (a), (b) and (c) dispersed and continuously in contact with each other. In the case of the activated sludge process, the sludge sweeps through the liquid kept continuously in motion by agitation with compressed air which also provides a continuous source of oxygen for the bacteria to act. In the trickling filter on the other hand, the sludge mass is held dispersed on a frame work of stones or other material while the sewage trickles over the surfaces of the sludge. In this manner the finely dispersed or colloidal matter of sewage is removed in these two systems.

Response of Activated Sludge to Various Soluble Organic Substrates

Placak and Ruchhoft (1947) studied seven carbohydrates (l-Xylose, glucose, maltose, lactose, sucrose, soluble starch and dextrin ) and found always an increase in sludge solids and a transient deposition of material within the cell confirming the findings of Gale (1943). Alcohols, organic acids and others such as methyl and ethyl alcohols, ethylene glycol, glycerine, formaldehyde, ammonium acetate, calcium gluconate and formic, tartaric, citric, lactic and oxalic acids were also studied. It was found that alcohols and organic acids were quite readily removed from solution with the exception of methyl alcohol and oxalic acid. They also studied thirteen substances like amino acids, proteins and miscellaneous compounds and found that amino acids were quite readily removed from solution. Urea was simply hydrolysed resulting in the production of free ammonia, which accumulated to raise the pH. Soap which is a normal constituent of any sewage was not too readily oxidised in the low concentrations found.

So, from the results of the 36 pure organic substances investigated, - representing a wide range of compounds(namely sugars, alcohols, aldehydes, organic acids, amino acids and miscellaneous compounds) and the response of activated sludge flocs to the above substances, Placak and Ruchhoft (1947) came to the conclusion that : (a) alcohols, amino acids, and organic acids were readily removed from solution with the exception of methyl alcohol, tyrosine and oxalic acid; and (b) all of these substances were capable of being oxidized by activated sludge although in varying degrees. Oxidation took place readily with all amino acids with the exception of cystine with which no oxidation occured. Peptones and meat extracts were shown to be more completely and easily oxidised, whereas soaps and oils were oxidized only to a minor extent. The above authors evolved the general principle that after 24 hours of aeration with activated sludge, 90 to 99% of the compound would be removed from solution and disposed off for the classes of substances mentioned below :

| Class | Percentage oxidized | | Percentage converted to protoplasm (Organized sludge) |
|---|---|---|---|
| | Range | Mean | |
| Carbohydrates | 5 to 25 | 13 | 65 to 85 |
| Alcohols | 24 to 38 | 30 | 52 to 66 |
| Amino acids | 22 to 58 | 42 | 32 to 68 |
| Organic acids | 30 to 80 | 50 | 10 to 60 |

So, they stated that, " in general, organic acids produced the smallest yield of activated sludge and carbohydrates the largest, with the alcohols and amino acids intermediate in sludge production. "

Another striking observation made by them was that " while larger quantities of glucose other than peptone were removed from solution by activated sludge in this experiment, much less oxygen was used during the glucose removal than during peptone removal. Thus, while only about 12% of the glucose removed from solution (out of 1039 ppm glucose added) was oxidized in 22 hours by activated sludge, over 50% of the peptone removed was oxidized during the same aeration time ' (Ruchhoft, Kachmar and Placak, 1940). This shows that oxygen needed for oxidation of glucose was less or rather much less of glucose was oxidized than peptone. The remaining portion of glucose was evidently used up in the building up of the protoplasm and intracellular material, probably PHB. (This observation is very important in regard to similar conclusions arrived at by Crabtree, Boyle, McCoy and Rohlich(1965,1966) nearly thirty years later on the same subject, of glucose metabolism with pure cultures of Zoogloea ramigera). Ruchhoft, Kachmar and Placak (1940) found that large part of the glucose removed from solution was not simply adsorbed upon the surfaces of the sludge floc but was "quickly transformed in the bacterial cell to other materials probably higher carbohydrates or fats."

So, from 1914 to 1950, it was generally considered that the majority of bacteria in activated sludge flocs was a single bacterial species i.e., Zoogloea ramigera, which was considered very important in all aerobic waste treatment processes. Heukelekian and Littman (1939) reported complete removal of 1600 ppm of glucose in five hours by an aerated suspension of this organism, which easily formed settleable flocculent masses. So the organism, Zoogloea ramigera was considered very helpful in clarification of sewage. Also, the glucose metabolism of this organism was exactly similar to the response of activated sludge flocs when fed with glucose, as will be seen later.

In carbohydrate metabolism of Zoogloea ramigera cultures, Crabtree, Boyle, McCoy and Rohlich (1965) found that they accumulated a large amount of sudanophilic granules, suggestive of PHB which is an endogenous metabolite unique to certain bacteria like Bacillus, Pseudomonas, Sphaerotilus and Nocardia. They found the intracellular accumulation of this polymer interesting because the organisms always flocculated when the polymer became visible as observed microscopically by the Burdon's stain. Therefore, they thought that the accumulation of this polymer in these organisms which number more than 300 millions per ml of growth, 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 the formation of the flocs.

So, they isolated the sudanophilic granules from pure cultures of Zoogloea ramigera and confirmed them as PHB. Next, they studied the role of PHB in floc formation as follows. They cultured pure zoogloeal organisms in a chemically defined basal medium without carbohydrates and supplemented with growth factors and they termed the cultures of the organism as carbon "starved" cells, which showed the apparent absence of "reserved food" other than PHB; and also were turbid. Neither Anthony's nor Hiss's staining method was able to demonstrate the presence of any extracellular polysaccharids. Intracellular reserve foods like volutin and lipids (PHB) included were not demonstrated by Alberts and Burdon's staining techniques. So, the cultures were considered as "starved" cells but viable. When glucose or other carbohydrates (0.005 to 0.5%) were added to this medium at the beginning of inoculation, flocs were formed as growth proceeded.

But if carbohydrates were added later to the growing dispersed population, the cells flocculated rapidly within half an hour.

Small but visible sudanophilic granules were observed about half-an hour after the addition of glucose when the cultures began to flocculate. Later the granules coalesced into a mass which varied in size. Capsule, volutin or significant increase in population was not seen. When the C/N ratio exceeded 20/1, the accumulation of the granules by the culture was increased, but when it was less than 20/1, the cells multiplied.

They also proved glucose as a precursor substrate for the polymer PHB by uniformly tagging radio-active glucose for the purpose. They found that six hours after addition of glucose, nearly 50% of the activity was found in the polymer and 38% in the form of $C^{14}O_2$. More than 30 sugars and related substrates and various peptides supplied in sufficient amounts were converted oxidatively to the polymer and caused the organism to flocculate. These results were similar to those of Doudoroff and Stainer (1959) who have examined the products of oxidative assimilation from glucose, acetate, and butyrate in <u>Pseudomonas saccharophilia</u> and of photosynthetic assimilation from acetate and butyrate in <u>Rhodospirillum</u> <u>rubrum</u>. They found that in all cases, major portion of the assimilated carbon (60 to 90%) initially accumulated within the cells as PHB.So, they suggested that an important physiological role of PHB was as an endogenous metabolite. Other carbonaceous substrates such as fructose, galactose, mannitol, glycerol, arabinose and xylose and an abundant supply of non-carbohydrate nutrients such as yeast extract, can be metabolised for synthesis of the polymer. However, hexoses in general and sugar alcohols were much more readily metabolised for synthesis of the polymer than pentoses or non-carbohydrate substrates as found from the rate of accumulation and final concentration of the polymer formed within the cells.

PHB is insoluble in water and therefore is an ideal metabolite for the survival of some of the bacteria. So, it can be accumulated in large quantities as intra-cellular granules. Since capsules or gums polysaccharides were not demonstrable on the cells, the flocculation would seem to be closely associated with the intra-cellular accumulation of PHB.

As endogenous dissimilation of PHB resulted in deflocculation, the influence of the polymer on flocculation was next examined. When the cells accumulated a large amount of the polyester linked β-hydroxy butyric acid, cells not only tended to stick together but also divided incompletely forming settled, lace-like masses of cells (flocs). Likewise, the polymer-rich cells in flocs subjected to mechanical dispersion did not remain dispersed but flocculated rapidly into large irregular masses when agitation was stopped. For this purpose the above authors added 0.5% of glucose to dispersed cultures of Zoogloea ramigera grown in the arginine medium in triplicate and studied the morphological changes occurring in the cells for ten hours. They found striking pleomorphism. They found that the cells at the beginning of the experiment were uniform in size, motile, well dispersed and devoid of granules. An hour after the addition of glucose the cells began to divide and also accumulated small but visible granules. After two hours the culture began to aggregate into small but visible clumps. About three hours later, the small discrete clumps became definite star-shaped flocs, and the granules inside had coalesced into a large granules, which occupied nearly 50% of the cell volume. At the end of ten hours striking pleomorphism was seen. The entire cell volume, including those cells which failed to separate, was distended and sudanophilic. The polymer-rich cells appeared two to five times larger than the dividing cells. The polymer partially or completely freed from the cell wall, either aggregated with other free polymer or with polymer rich cells. PHB rich cells in clumps appeared to be fused together. They also found that the visible counts before the addition of glucose were $2 \times 10^9$ cells/ml, at 5 hours $9.1 \times 10^8$ cells/ml and in 10 hours about $2 \times 10^8$ cells/ml. The amount of polymer recovered from several Zoogloea species ranged from 12.0% to 50.5% of the dry weight of the cells.

So, they concluded, " The biological mechanisms involved in the rapid removal of BOD at the initial stage of aerobic waste treatment processes may be analogous to the very rapid bio-synthesis of the polymer by the carbon-starved culture, when provided with an excess of external carbon and energy source. Flocs may originate from one or more polymer - rich cells of the Zoogloea sp. The flocculent growth may be caused by initiation of the rapid polymer synthesis, which disrupts the orderly biochemical process of cell division causing cells to divide incompletely, leaving the two cells attached. This incomplete cell division process will repeat continuously as long as favourable nutritional condition is maintained. Then, the cells form lacy tape like flocs. But, in the activated sludge process collision of the polymer accumulating, incompletely divided cell clumps is inevitable. Adhesion of these small cell clumps may be by mechanical entanglement at first, but as these cells respire and accumulate more polymer cells (probably dead) may be linked firmly by specific ester bonds which behave as poly-ester linked β-hydroxy butyric acid. Once the cells accumulate a sufficient quantity of the polymer, washed, alive, dead or even killed cells will proceed to flocculate."

"The exact nature of the ester-linkage between PHB molecules in adjacent cells is not completely clear now.

It is proposed that inflation of the cell as it accumulates the polymer results in a direct contact of the polymer with the stretch wall membrane. The exact phenomena involved in the fusion or adhesion of two protruding membranes, with PHB backing each, will have to wait for further investigations."

" So, the present observations provide strong evidence that a mechanism of cellular flocculation is similar to that of the polymerisation of PHB through esterification."

Mechanisms of Purification in the Oxidation Pond Method

The conventional oxidation pond method of secondary sewage treatment differs from the activated sludge process in several important respects: in the physical structure of the pond, time taken for final purification, surface area, oxygenation process, and in sludge formation. The oxidation pond is usually constructed of earthen embankments with a comparatively larger surface area for reaeration and for exposure to bright sunshine for trapping the energy of the sun through photosynthesis as the principal synthetic force. The utilization of this solar energy by algae developing naturally in about 7-10 days in the presence of nutrients available in sewage, results in the proliferation of new algal cells and in the concomitant release of molecular oxygen in the water during the algal phase II. Also, there is practically no problem of sludge formation and disposal, as in the case of the activated sludge process. No doubt, the time taken for purification of sewage by this process is comparatively much greater, being 20-30 days. So, the metabolic processes taking place in the conventional oxidation pond can naturally be expected to be different from those of the classical activated sludge process; and it appears to be so as the following will show.

When raw sewage freed from easily settleable matter is stored for about 20-30 days for the natural growth and development of algal organisms, we notice first, within a few days of storage a viscous scum of discrete particles developing on the surface (vide chapter 5 and 6) and sides of the vessel and also something settling down at the bottom. The surface viscous scum is also found to disintegrate and to disappear within a week or so. Simultaneously several changes ( vide chapter 4 ) are also observed in the body of the stagnant liquid in respect of physico-chemical, biochemical, biological and bacteriological conditions. A detailed consideration of the modus operandi in the two cases is attempted below.

Formation and Role of the Viscous Scum

Characteristics of the Viscous Scum. When settled sewage was stored, a viscous scum was gradually formed at the surface within 24-48 hours. The floating viscous scum was leather-like or rubber-like and could be easily removed from the surface by a spatula or even a long needle. The viscous scum as a whole, also, appeared to resemble "the netted, lack-like, masses of cells (flocs)" of Crabtree, Boyle, McCoy and Rohlich (1965). Microscopic examination of a small portion of the scum revealed it to consist of hundreds of zoogloea colonies of different shapes and sizes, very long thread-like bacteria, long wavy rods, stout comma bacillus, long thin spirilla, irregular egg-shaped cocci in twos and fours, short thin and stout rods, cocci and cocco-bacilli. The zoogloeal cells were embedded in a tough hayline matrix. In the dried, fixed stained smears the zoogloea colonies were found packed with small rods. With Burdon's stain either all the cells or only a part of the cells, or the entire colony or only a few brahches or a portion of a few branches showed sudanophilic granules. Also, long thread-like bacteria, long wavy rods, long thin spirilla and short rods showed small but visible granules of varied sizes. Sometimes, the entire long wavy rods, short rods and the egg-shaped cocci became almost entirely sudanophilic. On one occassion the entire slide consisting of long wavy rods and short rods was found to be entirely sudanophilic. There were also other numerous short rods and cocci without the sudanophilic granules.

On analysis, the viscous scum was found to consist of the following: free sugar, 0.03% ; total sugar, 0.07% ; protein, 0.44%;

amino acid nitrogen, 0.03% ; total fat, 7.61% ; and PHB 0.15%. Lipase activity was found to be highest followed by protease activity.

How the viscous scum is formed. Jones and Travis (1926) first observed the appearance of discrete particles either floating in the liquid, attached to the sides of the vessel, or settled to the bottom of a beaker when sewage from which all settleable matter had been carefully strained, was stored for a day or so. This phenomenon has been named bio-flocculation by Heukelekian (1941).

The same phenomenon takes place in oxidation ponds primarily during bacterial phase I as a result of biophysical forces also as described by Renn (1956). He says : " When water containing low concentrations of organic matter stands undisturbed, the molecules of dissolved organic stuffs reorient themselves in the water mass so that energy is lost. This energy loss is effected by the trans-location and concentration of organic materials at the air-water interface and at the containing solid-water interfaces. In effect, work is done through decrease in the interfacial tentions existing at the air and contained interfaces. "

He states further : " If the solution is very weak, a large proportion of the total organic material will be found at the air-water interface and at the solid-water interface and little will exist in solution. "

" The migration of organic substances and other materials that tend to decrease interfacial tension to surfaces is inevitable. The process goes on in sterile systems quite as well as in the biologically active waters."

He also adds that it is a common observation that " if a shallow tray of swamp water is allowed to stand overnight in a very quiet room the surface will become evenly coated with a continuous sheet of bacteria. " So, in biologically active ecosystems as in an oxidation pond, the migration of organic matter goes on in the same manner and the growing bacterial population also seem to concentrate at the air-water and water-solid interfaces. " The mechanisms of bacterial concentration are doubtless mixed but must involve both interfacial trapping of moving cells and preferential growth on the surfaces themselves."

Long periods of exposure to air are helpful for the removal of soluble and dispersed materials in sewage (Heukelekian 1941). So, biological flocculation rather than mechanical flocculation plays an important role when the period of exposure to air is long as in an oxidation pond. But mere flocculation and/or bioflocculation alone cannot purify sewage to the required standard of purity. Other forces also must be at work.

The importance of surface area in helping the development of bacteria is stressed by Zobell and Anderson (1936) who stated that the greater the area exposed, the greater the bacterial development in stored sea water, Stark, Stadler and McCoy(1938) showed that accumulation of organic matter is also possible independently of bacterial growth. Heukelekian (1941) has interpreted the above statements to correspond with the transformations of the available soluble food material by the growth of bacteria directly to "insoluble colloidal aggregates of protoplasm."

Importance and Role of the Viscous Scum in Sewage Purification

The biological growth, like the viscous scum, was admitted to be important by Biltz and Krohnke (1904, cited by Heukelekian 1941) only in furnishing adsorptive surfaces, where the adsorption compound resulting from the colloidal matter of sewage and the gelatinous coating was oxidised directly by the oxygen of the atmosphere. But Whitehead and O'Shanghnessy(1936) have observed that the "gelatinous matrix" produced when sewage was allowed to stand furnished the basis for sewage purification and there was practically no difference microscopically between the "gelatinous matrix" or the viscous scum formed at the surface of stored sewage, the slimy growths found in a stream (or at different situations of a Sewage Disposal Works) and the activated sludge flocs about which we have discussed in detail in chapter 5,6 and 7 of this thesis. We have shown on pages 35-42 of chapter 5, and on pages 13-14 of chapter 7, that there are greater points of similarity between the viscous scum and the activated sludge flocs than between the viscous scum and the slimy growths found in different situations of a sewage Disposal Works or a mildly polluted stream.

There is also another way by which purification of sewage may be effected by the bacteria present in the viscous scum about which also we have discussed earlier in chapter 5 of this thesis. There are hundreds of zoogloeal colonies each with a gelatinous matrix, and containing billions of bacilli. Majority of the organisms in the zoogloea colonies has been found to be Zoogloea spp.Also many bacteria are surrounded by capsules. The zoogloeal matrix as well as the capsulated and non-capsulated bacteria embedded in the jelly-like matrix tend to adsorb specific substrates into the matrix, and in consequence a stock of nutrients is established in these zoogloeal colonies for eventual metabolism by the cells embedded in them. In this way soluble organic matter and/or colloidal matter will be oxidised. So, bacterial slimes when they develop, as in activated sludge, or when they adhere and grow on filter stone surfaces or on the surfaces of stored sewage, form films or growths of biologically active materials. This is also indicated by the formation of sudanophilic granules in the bacteria embedded in the zoogloeal matrix, in the long wavy rods, in the thin long spirilla, egg-shaped cocci and other bacteria. The formation of fat droplets in the several types of bacteria mentioned above is the result of biochemical activity of these bacteria on the carbohydrates present in solution in the sewage. This is evident from the carbohydrate metabolism of Zoogloea ramigera where the organism accumulated a large amount of the sudanophilic granules, suggestive of PHB which is an endogenous metabolite (Crabtree et al 1965, 1966). These organisms seem to disappear after about a week most probably due to auto-digestion or endogenous respiration. Otherwise, we should have seen more of sludge formation as is in the case of activated sludge process (Vide page 5-11 of this chapter). Thus a fair amount of the carbohydrates may be used up and removed from solution during the formation of the viscous scum. In this important respect, the metabolic processes in the oxidation pond differ from that of the activated sludge process.

In short, the viscous scum formed at the surface seems to act as the active centre first for the concentration and later for the conversion of the insoluble colloidal and pseudo-colloidal matter of sewage into diffusion forms for the bacteria present by hydrolysis and oxidation. Also, it may be acting as the reservoir for the growth and development of zoogloea and other types of organisms found in it; and thus may be accelerating the rate of flocculation, bio-flocculation and clarification (Heukelekian 1941).

Changes Taking Place in the Liquid Medium

The data presented in chapter 4 of this thesis, does not clearly indicate just what does happen, whether a selective change in organisms or an enzymatic reaction or both as in all biochemical studies of pure cultures. All the same, it is possible to construct a broad picture of the likely pathways by which purification of sewage is brought about by this sluggish process.

The conditions existing in the medium may be briefly portrayed before we describe the likely metabolic pathways. There was no production at all or production in sufficient quantities of $CO_2$ or organic acids to affect perceptibly the hydrogen-ion concentration of the medium, which was found to be alkaline throughout. There was, also, no appreciable production of sludge as in the case of the activated sludge process indicating that the metabolic pathways in this case was different. Besides, there was an increase of fatty materials in the liquid medium. Large scale death and lysis of the coliform group of organisms appear to have released a large amount of the intracellular products like protein and amino acids etc. so that free and total sugar, protein and amino acids registered an increase on the fourth day but decreased gradually later. Free and total sugar, however, almost completely disappeared later from the medium. Again, from the biochemical reactions of the bacteria isolated on different days, it was evident that there were several physiological groups of bacteria at work for the removal of the soluble organic constituents. The percentage of organisms showing sudanophilic granules for example was found to be reduced considerably in the algal phase. Chlorella the dominant algal organism has been reported to release anti-bacterial fatty acids which may have played a significant role in reducing the number of bacteria as well as in contributing to the increase in the soluble fatty substances.

Now, it is possible to explain from the above data how purification of sewage is brought about in oxidation ponds. From the researches of Placak and Ruchhoft (1947) and Crabtree, Boyle, McCoy and Rohlich (1965, 1966) it will be evident that the process of oxidative assimilation is the chief process taking place in the activated sludge process. As a result, a third of the carbohydrates is oxidised for energy and the rest two-thirds are utilized for synthesis and assimilations of new cells. Activated sludge is nothing but the synthetised bacterial cells from the soluble organic constituents of sewage. Also, all these cells have an intracellular reserve material PHB stored in them. How these cells are precipitated as sludge flocs has been explained by Crabtree, Boyle, McCoy and Rohlich (1966). So, it will be evident that almost all the carbohydrates are utilized for the cellular synthesis in the case of the activated sludge process.

But this is not the case in the oxidation pond, where the carbohydrate metabolism appears to be different. In this case, a part of the carbohydrates may have been used for energy purposes, another part for conversion into the reserve storage

Table :9-1: Effluents from the Laboratory Models of Activated Sludge and Oxidation Pond Compared

| Tests | Activated sludge | Oxidation Pond (28th day) |
|---|---|---|
| A. Physical | | |
| Colour | Yellowish | Pale Green |
| Turbidity (Klett units) | 4 | 11 |
| pH | 8.6 | 8.6 |
| B. Chemical | | |
| Phenolphthalein alkalinity (mg/l) | 30 | 10 |
| Total alkalinity (mg/l) | 450 | 975 |
| Dissolved oxygen (mg/l) | 19.2 | 3.6 |
| 5 Day B.O.D. at 20°C (mg/l) | 7.0 | 12.5 |
| Acid $KMnO_4$ (4 hrs.) value (mg/l) | 2.6 | 6.0 |
| Orthophosphate (mg/l) | 3.6 | 18.7 |
| Ammonia-nitrogen (mg/l) | 7.8 | 19.6 |
| Nitrite nitrogen (mg/l) | 31.8 | 0.24 |
| Nitrate nitrogen (mg/l) | 2.3 | Nil |
| C. Biochemical | | |
| Free sugar (mg/l) | Nil | 0.09 |
| Total sugar (mg/l) | Nil | 0.60 |
| Amino-acid nitrogen (mg/l) | 0.17 | 0.22 |
| Protein (mg/l) | 3.5 | 11.25 |
| Fat (mg/l) | 6.85 | 44.20 |
| Chlorophyll-a (mg/l) | Nil | 11.90 |
| D. Bacteriological | | |
| Coliforms at 37°C (MPN/100 ml) | $4.5 \times 10^3$ | 780 |
| E.Coli type I (MPN/100 ml) | 330 | Nil |
| Faecal Strloptococci (MPN/100 ml) | 130 | Nil |
| Citrate Utilizers (MPN/100 ml) | $23 \times 10^3$ | $24 \times 10^3$ |
| Total colonies count (Nos/ml) | $6 \times 10^8$ | $7.2 \times 10^9$ |

product like the PHB, and the rest released into the medium as extra cellular fatty material (or fatty acids). How much of the carbohydrates is used in each case cannot be stated without carrying out further experiments using uniformly tagged radio-active glucose. Microorganisms are known to synthetise fatty substances and some of them are also known to store them as reserve material inside their cells. The alga, Chlorella, also has released extracellular anti-bacterial fatty substances during its synthesis. Thus, there is an increase in fatty materials both during the bacterial and algal phases.

The bacteria with PHB as reserve material and others are oxidised by auto-digestion or endogenous respiration releasing further $CO_2$, $NH_3$ and $H_2O$ into the medium. In the final analysis, as there is no appreciable sludge formation excepting for some dead algal cells the sewage becomes clearer and purified.

It also appears from these studies that the effluent from the oxidation pond method of purification contains much less of total inorganic nitrogen content, but more of phosphates and fat material and considerably low coliforms than the effluent from the activated sludge process. (Vide table 9-1).

## FUTURE LINES OF WORK ARISING FROM THIS THESIS

1. The mechanisms of purification in the high-rate Oxidation Ponds inoculated with different green and blue-green algal cultures.

2. Aeration of soluble organic wastes along with the viscous scum.

3. A comparative study of the viscous scum and activated sludge flocs.

4. Studies on the formation of the zoogloea matrix in zoogloea colonies formed in the viscous scum.

5. Biochemistry of the viscous scums formed on different days in stored sewage.

6. Biocehmical studies of the 4 types of Zoogloea cultures isolated from the viscous scum.

7. Respiratory studies of the viscous scum.

8. Fatty substances produced in stored sewage and their bactericidal effect.

9. A comparative study of the organic constituents present in sewage and sewage effluents of the activated sludge process, the trickling filter and the oxidation pond.

10. Nature of the soluble organic constituents in samples of sewage and effluents taken from different situations of a sewage disposal works.

11. Efficiency of the different methods of sewage treatment in the removal of fatty and nitrogenous matter.

12. Studies on the effect of bacteria on different algae in oxidation ponds.