

A microscopic view of wastewater treatment sludge, showing numerous yellowish-brown flocs of varying sizes and shapes, surrounded by a dense network of fine, dark, thread-like structures, likely filamentous bacteria or other microorganisms. The background is a dark, muted greenish-brown color.

The Biological Basis of Wastewater Treatment

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Introduction

This booklet was written to fulfil the need for a simple explanation of the biological processes that underpin wastewater treatment. It attempts to show how the bacteria involved deal with the organic carbon in the sewage. Remarkably, there are just 3 major processes involved, and these mirror exactly the 3 major processes at work in the plant viz: biodegradation, oxygen removal from the water, and the production of sludge.

The article is divided into two parts. The first section deals with the biology of the bacteria. In the second section, the ways in which these processes underpin the management of a wastewater treatment plant are explained. Inevitably in a brief overview, such as this, much has had to be left out. However, it is to be hoped that this will be justified by the clarification and simplification of the underlying principles.

For an in depth treatment, the 'Biology of Wastewater Treatment' by N.F Gray (Imperial College Press, 2004) gives an excellent and readable review of the subject.

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Biological Processes

Biological treatment by activated sludge

Wastewater comes from two major sources: as human sewage and as process waste from manufacturing industries. In the UK, the total volume of wastewater from industry is about 7 times that of domestic sewage. If untreated, and discharged directly to the environment, the receiving waters would become polluted and water-borne diseases would be widely distributed. In the early years of the twentieth century the method of biological treatment was devised, and now forms the basis of wastewater treatment worldwide. It simply involves confining naturally occurring bacteria at very much higher concentrations in tanks. These bacteria, together with some protozoa and other microbes, are collectively referred to as activated sludge. The concept of treatment is very simple. The bacteria remove small organic carbon molecules by 'eating' them. As a result, the bacteria grow, and the wastewater is cleansed. The treated wastewater or effluent can then be discharged to receiving waters – normally a river or the sea.

Whilst the concept is very simple, the control of the treatment process is very complex, because of the large number of variables that can affect it. These include changes in the composition of the bacterial flora of the treatment tanks, and changes in the sewage passing into the plant. The influent can show variations in flow rate, in chemical composition and pH, and temperature. Many municipal plants also have to contend with surge flows of rainwater following storms. Those plants receiving industrial wastewater have to cope with recalcitrant chemicals that the bacteria can degrade only very slowly, and with toxic chemicals that inhibit the functioning of the activated sludge bacteria. High concentrations of toxic chemicals can produce a toxic shock that kills the bacteria. When this happens the plant may pass untreated effluent direct to the environment, until the dead bacteria have been removed from the tanks and new bacterial 'seed' introduced.

Globally, the composition of effluents discharged to receiving waters is regulated by the national environment agencies. In Europe the regulatory legislation is the Urban Waste Water Treatment Directive (1991) and the more recent Water Framework Directive (2000). In the USA, the Environmental Protection Agency (EPA) ensures compliance with the Clean Water Act (1977). The legislation is concerned with the prevention of pollution, and therefore sets concentration limits on dissolved organic carbon (as BOD or COD), nitrogen and phosphates – which cause eutrophication in receiving waters. It also attempts to limit the discharge of known toxic chemicals by setting allowable concentration limits in the effluent. Recently, in recognition that effluents contain unknown toxic chemicals, a more pragmatic approach to regulation is being introduced in Europe, using Direct Toxicity Assessment (DTA) tests. In the US these have been in use for many years and are known as Whole Effluent Toxicity (WET) tests. These tests are used to measure the toxic effects of effluents on representative organisms from the receiving waters. Any toxicity detected in the effluents will obviously have been present in the sewage entering the plant. Surprisingly, direct toxicity assessment of *influent*s to wastewater treatment plants that could impact on the functioning of the bioprocesses is not yet included in legislation.

The nature and composition of wastewater

Domestic sewage is made up largely of organic carbon, either in solution or as particulate matter. About 60% is in particulate form, and of this, slightly under a half is large enough to settle out of suspension. Particles of 1nm to 100µm remain in colloidal suspension and during treatment become adsorbed on to the flocs of the activated sludge.

The bulk of the organic matter is easily biodegradable, consisting of proteins, amino acids, peptides, carbohydrate, fats and fatty acids. The average carbon to nitrogen to phosphorus ratio (or C : N : P ratio) is variously stated as approx 100 : 17 : 5 or 100 : 19 : 6. This is close to the ideal for the growth of the activated sludge bacteria. However, industrial wastewaters are very much more variable in composition. Those produced by the brewing, and pulp and paper industries, for example, are deficient in nitrogen and phosphate. These nutrients need to be added therefore to achieve the correct ratio for microbial growth, and to allow treatment to proceed optimally.

Degradable and non-degradable carbon

For control of the biological processes in a treatment plant, it is necessary to have some knowledge of the organic strength, or organic load, of the influent wastewater. Three different measures of this are available, and they each have their merits and weaknesses. The Total Organic Carbon (TOC) is analytically straightforward to measure. It involves oxidation by combustion at very high temperatures and measurement of the resultant CO₂. However, TOC values include those stable organic carbon compounds that cannot be broken down biologically.

Organic carbon can also be measured by chemical oxidation. The sample is heated in strong sulphuric acid containing potassium dichromate, and the carbon oxidised is determined by the amount of dichromate used up in the reaction. The result is expressed in units of oxygen, rather than carbon, and the procedure is referred to as the Chemical Oxygen Demand (COD). Again it is an analytically simple method. However, its weakness is that a number of recalcitrant organic carbon compounds that are not biologically oxidisable, are included in the value obtained. Conversely, some aromatic compounds, including benzene, toluene, and some pyridines, which can be broken down by bacteria, are only partly oxidised in the COD procedure. Overall however, COD will overestimate the carbon that can be removed by the activated sludge.

The current method used to determine the biodegradable carbon, is the 5-day Biological Oxygen Demand (BOD₅). This is a measure of the oxygen uptake over a 5-day period by a small 'seed' of bacteria when confined, in the dark, in a bottle containing the wastewater. During this time the biodegradable organic carbon is taken up, and there is a corresponding

decrease in the dissolved oxygen, as some of the carbon is used for the respiration of the bacteria. Respiration is a form of biological oxidation, and will be explained later. Rather unhelpfully, the biodegradable carbon, as in the COD test, is expressed in oxygen units. This is because the test was originally introduced to measure the oxygen depletion in receiving waters caused by the residual degradable carbon in the effluent. Its main value is in regulating the composition of effluents from the treatment water. For process management, where knowledge of the organic loading of the influent is required, BOD_5 is of limited value, because of the 5 days required to make the measurement. There are now moves afoot to replace the use of BOD_5 as a measure of influent strength, with a short-term test (BOD_{ST}), which can be carried out over a timescale of 30 minutes to several hours.

The values obtained for BOD_5 are always lower than those for COD, for 2 reasons:

- Activated sludge bacteria cannot degrade some of the compounds oxidized chemically in the COD test.
- Some of the carbon removed during the BOD test is not oxidised, but ends up in new bacterial biomass. So the BOD is only measuring the biodegradable carbon that is actually oxidised by the bacteria.

The ratio of BOD_5/COD will depend on the composition of the wastewater. For domestic sewage, and also the wastewaters from the slaughterhouse, dairy, distillery and rubber industries, the ratio is about 0.5 - 0.6. However, for effluent leaving the treatment plant, it is closer to 0.2. This is because the readily biodegradable organic carbon has been removed during treatment, leaving behind the compounds that are not readily broken down by the bacteria – ‘hard’ BOD. These will be readily measured by chemical oxidation, but will not be readily degraded and removed by the bacteria in the BOD bottle.

‘Soft’ and ‘Hard’ BOD

The time-course for the removal of the organic carbon varies with the ability of the activated sludge bacteria to ingest it. Small molecular weight compounds will start to be removed from the sewage immediately after it has entered the activated sludge tanks. Their removal may be completed in 1 – 2 hours. This group of compounds is often referred to as the readily biodegradable or ‘Soft’ BOD. Other, higher molecular weight compounds will take several hours to be degraded and removed. Yet other compounds are more recalcitrant, and may still be present after several days. This less readily biodegradable BOD is often referred to as ‘Hard’ BOD. The mechanism of their degradation and removal by the bacteria will be dealt with later.

The net result is that larger, complex organic carbon molecules may be not be degraded because the treatment time available (the hydraulic retention time) is not sufficiently long, and they will therefore pass out in the effluent.

To summarise, the organic carbon in wastewater may be represented as below:

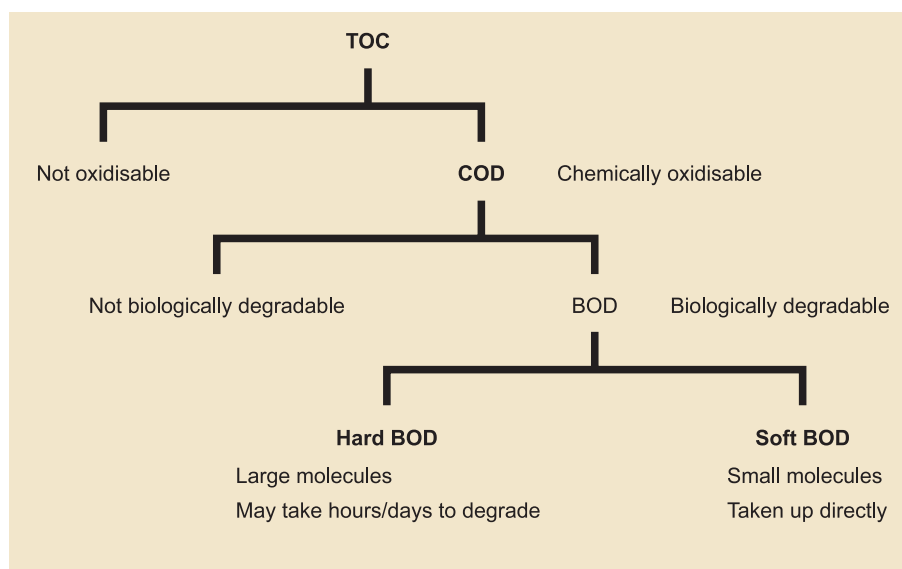


Figure 1 The relationship between the organic carbon fractions in sewage.

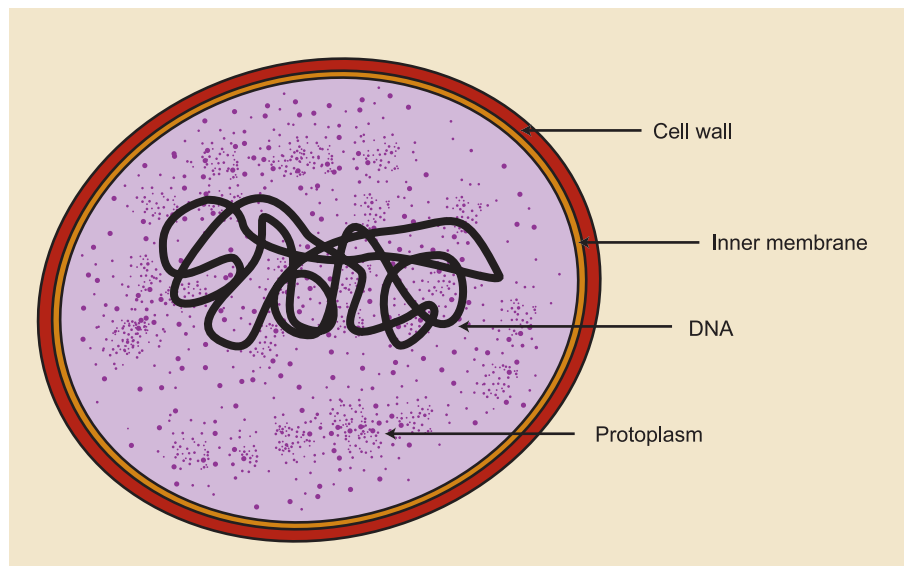
The composition of activated sludge

Activated sludge bacteria

The activated sludge of the aeration basin of a wastewater treatment works is a complex ecosystem of competing organisms. The dominant organisms are the bacteria, of which there may be 300 species present. Bacteria are amongst the smallest and most abundant living organisms. Each comprises a single cell varying in size from about 0.5 – 2 μm . On the outside, the cell is bounded by a membrane that regulates the inflow of ions and molecules from the surrounding water. This, in turn is surrounded by a rigid cell wall, made of a sugar polymer. The interior of the cell contains the cytoplasm and the thousands of different chemicals whose reactions are regulated by enzymes. The bacterial cell does not have a nucleus. Most bacteria are spherical, but some may be rod shaped or have a spiral form. Filamentous bacteria comprise long chains of small bacterial cells, sometimes surrounded by a tubular sheath, and can reach lengths of 100 μm .

Small molecular weight compounds diffuse into the bacteria (ingestion) through the cell wall. At the same time, some larger complex molecules that have been synthesised within the bacteria, pass outwards. This process is referred to as secretion.

Figure 2 Cross section through a bacterium. The protoplasm inside contains a DNA molecule, but no nucleus. It is surrounded by an inner membrane and a cell wall.



The secretions include slimes and gels, that may bond the bacteria together, and also enzymes. The enzymes break down large organic molecules into smaller monomers that are small enough to be ingested.

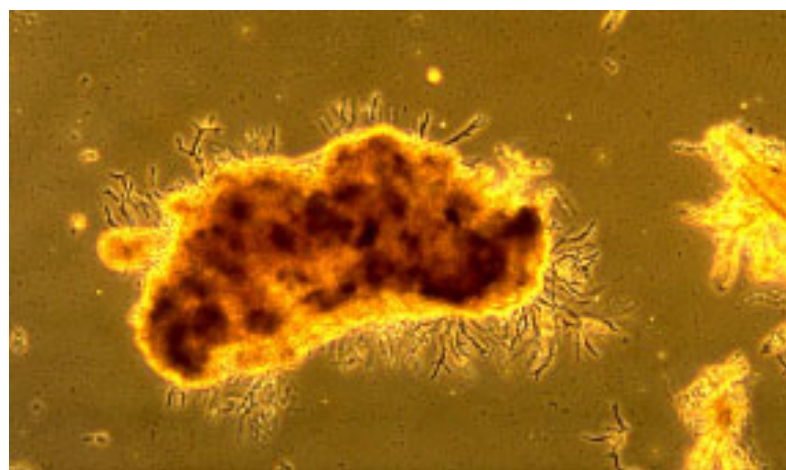
The bacteria use the ingested molecules for the synthesis of new molecules, in the process of growth. When they have reached normal size, the bacterium divides into two, and the process is repeated. If nutrient molecules are not limiting, this results in exponential growth in the numbers of bacteria.

The bacteria in a wastewater treatment plant comprise both heterotrophs and autotrophs. The heterotrophic or carbonaceous bacteria are the predominant group of organisms. They are characterised by feeding mainly on organic carbon molecules rather than inorganic ones. By contrast, the autotrophs take in inorganic chemicals, and use these in the synthesis of organic compounds. The nitrifying bacteria that remove ammonia from the wastewater are the most important of this group. There are relatively few species of autotrophs, and since they have low growth rates, they tend to be out-competed by the faster-growing heterotrophs.

Bacterial flocs

In a well-maintained aeration tank, the bacteria are concentrated in the flocculent material of the activated sludge, although some always occur free in the wastewater. The flocs are formed from aggregates of non-living organic polymers that are probably secreted by bacteria. They have an open porous structure, and are sufficiently robust to withstand the shear forces created by water movement, during aeration of the tanks. They vary in size from less than 10 μm up to 1mm (1000 μm).

Figure 3 Micrograph of activated sludge flocs. The dark brown colour indicates a high population of bacteria in the floc. The small floc in the centre shows filamentous bacteria extending out from the surface, but since they do not link up with neighbouring flocs, this sludge will settle well. (Photograph courtesy of Ray Kenny).

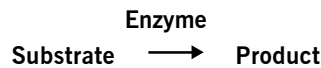


The bacteria are adsorbed on to the internal and external surfaces of the floc, and a medium sized floc may harbour several million bacteria. Immediately after the wastewater enters the aeration tank, the fine particulates, colloidal particles and large molecules, become entangled with, and adsorbed to, the floc material. This has the advantage that the enzymes that are secreted by the bacteria into the water, will tend to be confined in the vicinity of the substrate, thereby facilitating their digestion. However, for the bacteria living on the inside of the floc, oxygen availability may be a problem. This is because oxygen has to diffuse along a concentration gradient from the wastewater through the floc material to the inside. The bacteria of disaggregated flocs may continue to grow when the oxygen concentration of the mixed liquor is only 0.6 mg O_2/l , whereas to ensure this concentration on the inside of a large floc, a mixed liquor oxygen concentration of 1.2 – 2.0 mg O_2/l may be required. Quite often, when the aeration tank is operated at below 2.0 mg O_2/l , the centre of the flocs may become oxygen depleted, and colonised by facultative anaerobic bacteria. The outer surface of the activated sludge flocs are frequently colonised by microorganisms of a higher trophic level, including protozoa and rotifers. These feed on bacteria and particulate material in the wastewater.

As in all ecosystems, the constituent organisms are in a dynamic steady state. Thus the dominant bacterial species may change, sometimes on a daily basis, in response to changes in the composition of the wastewater. Those species of bacteria that have the ability to secrete the enzymes to break down a novel food source will grow more rapidly, thereby increasing in relative number. This process is known as *adaptation* or *acclimation*. In some cases exposure to low levels of potentially toxic chemicals, such as phenol, may result over a period of days in the induction of enzymes that will digest them. These species of bacteria can then exploit the toxicant as a food source.

Metabolism of bacteria

Treatment of sewage in the aeration tank involves the removal of organic carbon from the mixed liquor by Ingestion by the bacteria. Once inside, the carbon compounds are metabolised. Metabolism comprises the thousands of simultaneous chemical reactions that are going on at any one time inside the bacterium. In each of these reactions, a substrate, in the presence of an enzyme (which acts as a catalyst), is converted into a product.



The product then becomes the substrate for the next step in the chain, and is almost immediately converted, in the presence of another specific enzyme, into a different product - and so on. For some of these reactions to take place, chemical energy needs to be provided (endergonic reactions). In other reactions (exergonic reactions), energy is given off, usually in the form of heat. The major divisions of metabolism that concern us here are:

Catabolism or Energy Metabolism This comprises a series of reactions in which carbon compounds, are broken down to yield cellular energy. This is biological oxidation and involves oxygen uptake by the bacterium. This is also the basis of the process referred to as *Respiration*.

Anabolism This is a series of biosynthetic reactions in which small molecules are joined together to form large molecular weight macromolecules. This requires an input of energy from Catabolism, and is the basis of the process of *Growth*.

The 3 major processes in a bacterium

Although there are many thousands of chemical reactions involved in the metabolism of a bacterium we can identify the three major *processes* that are relevant to the biological treatment of sewage. These are:

- **Ingestion**
- **Respiration**
- **Growth and division**

These processes are very highly integrated and the relationship between them in a single bacterial cell can be shown thus:

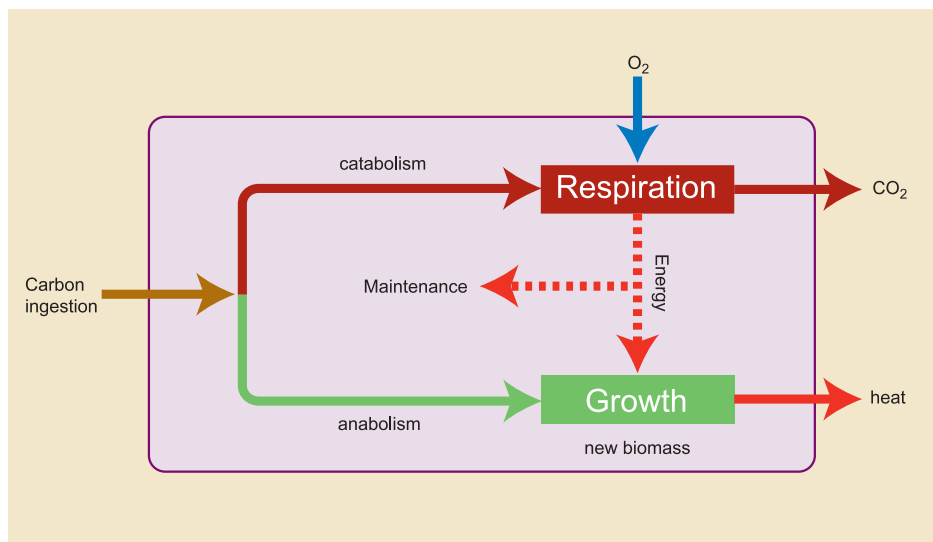


Figure 4 Representation of a single bacterium showing the relationship between the 3 processes.

Figure 4 shows the pathway of the ingested organic carbon. Some goes along the pathway of catabolism or Respiration and ends up as carbon dioxide. This carbon is lost to the system. The remaining organic carbon follows the anabolism or Growth pathway and ends up in new biomass. This carbon is therefore retained in the system. The purpose of respiration is to provide the energy that is required for growth and for the maintenance of the bacterium*.

These three processes – Ingestion, Respiration and Growth - are very highly coupled or meshed. No one process can go faster than the other. One implication of this is that, for instance, if you measure the respiration rate, you are indirectly also measuring the rate of growth and the rate of carbon ingestion.

Growth is the driver and rate-limiting step. Every bacterium has a genetically programmed maximum rate of growth that will be achieved under ideal conditions. As it grows, it withdraws carbon compounds from the internal pool in its

*For those who are familiar with BOD₅ measurements, it will be obvious from Fig 4 that the test is measuring only the carbon that is used to provide the energy for growth.

cytoplasm. Carbon flows in (or is Ingested) from the mixed liquor in order to keep this pool topped up. At the same time, energy is used for biosynthesis and growth, and hence the catabolism pathways of Respiration also withdraw carbon from the internal pool, and this also results in carbon being drawn in by Ingestion.

It will be noted that the 3 processes correspond to the major processes that we shall see when we examine the operation of the treatment works aeration basin. They can be summarised as:

Bacterial process	Treatment plant process
Ingestion	Biodegradation
Respiration	Aeration requirement
Growth and division	Biomass production

Ingestion

This involves the passage of organic carbon compounds, other molecules and ions from the mixed liquor into the bacterium. To do this, they have to pass through the cell wall and the inner membrane. The cell wall does not present much of a barrier, and control over entry is exercised at the inner membrane. Ions such as sodium diffuse in because the concentration in the mixed liquor is higher than inside the bacterium. They then have to be 'pumped' back out again to maintain the internal steady state. Small organic molecules similarly pass in along a concentration gradient, or may be assisted in entry by various mechanisms located in the inner membrane. Most large molecules are excluded. In order to use these for their nutrition and growth, the bacteria secrete enzymes into the water to digest them into small monomers, which can then pass into the cell. Different species of bacteria are specific for what enzymes they secrete, and this determines which chemicals they can exploit as a food source. The ability to secrete a particular enzyme may be latent. In other words, the bacterium requires the presence of the particular chemical compound in the water to switch on the genes for the synthesis of the enzyme required for its digestion. This is the basis of the process of Acclimation or Adaptation in activated sludge, mentioned previously.

Growth of bacteria

Bacteria show prodigious feats of growth. Some bacteria may double their biomass in as little as 20 minutes, provided they have the right conditions of temperature, pH and an abundance of organic carbon, other nutrients, trace elements etc. Note that an individual bacterium has limited capacity for growth, only growing from the size of a daughter cell produced at the time of division to the normal cell size. Growth rate is therefore measured as the increase in number of cells with time.

The conditions required for growth vary between species of bacteria. However, there are some general principles. The growth rate observed is a result of both genetic and environmental factors. The shape of the growth curves, and the maximum rate of growth under optimal conditions is genetically determined. The effects of environmental factors are described below.

Substrate concentration The main substrate for growth is the BOD, or degradable organic carbon in the mixed liquor. With increase in the concentration of substrate, the growth rate increases exponentially and then levels off. So with further increase in concentration of substrate in the medium, there is no further increase in growth. The bacteria are at their maximum growth rate.

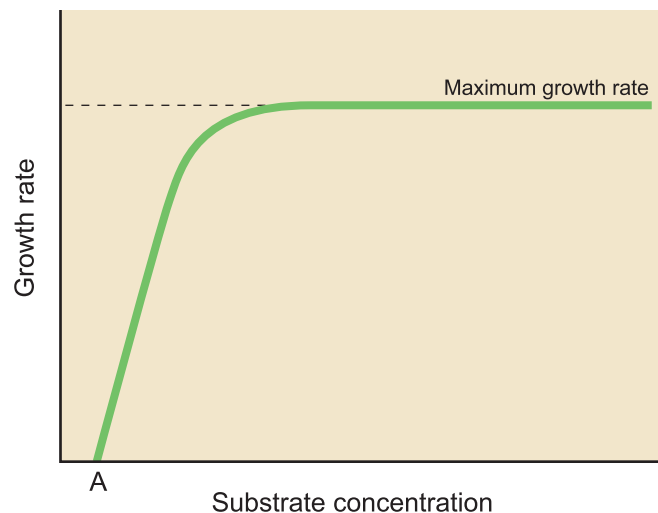


Figure 5 The growth rate increases exponentially with increase in substrate concentration, to a maximum.

Note that the curve does not pass through the origin. This is because at very low concentrations, substrate is being used for respiration simply to keep the bacterium alive. At concentrations below A in Figure 5, the bacteria remain alive, but are not growing.

The slope of the growth v substrate concentration curve can be important. A steeper slope indicates a greater affinity for, or ability to use, substrate. In the example below, species Y has a greater affinity for substrate than species X at low concentrations. At these concentrations, it will grow faster and out-compete X. At higher concentrations, X has a higher maximum growth rate and will then out-compete Y - see Figure 6 overleaf.

Some filamentous bacteria found in aeration tanks are examples of high substrate affinity forms.

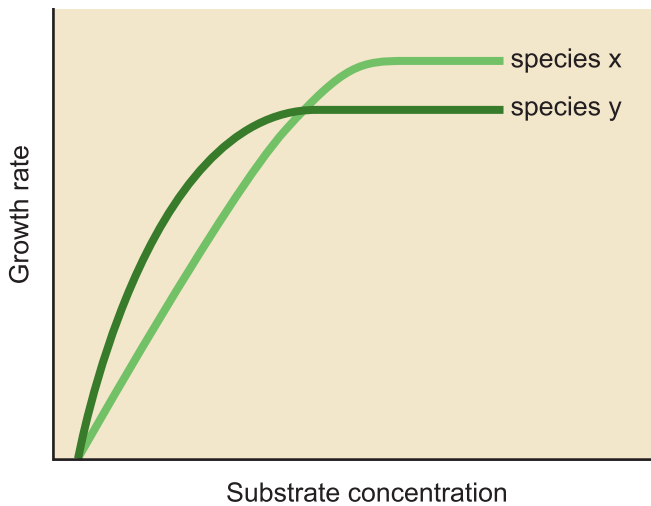


Figure 6 Species X has a higher maximum growth rate than species Y. However, Y has a higher affinity for the substrate than X at low concentrations.

Availability of other nutrients Whilst the major substrate requirement is for carbon, growth is also dependent on the intake of nitrogen and phosphorus. The optimum ratio of C:N:P in the mixed liquor is generally thought to be 100 : 5 : 1. The ratio of these nutrients in settled domestic sewage is variously reported as 100 : 17 : 5 or as 100 : 19 : 6. This indicates that nitrogen and phosphorus will not be limiting for growth. Trace components, which include S, Na, Ca, Mg, K, and Fe are also required, and are available in abundance in domestic sewage. By contrast, the wastewater from brewing, pulp and paper, and food-processing industries can be deficient in nitrogen and phosphorus. Nutrients therefore need to be added to the mixed liquor to obtain maximum bacterial growth and to optimise carbonaceous treatment. From an operational point of view, lack or an insufficiency of a critical nutrient may result in incomplete treatment, because the bacteria are unable to grow optimally.

Oxygen Growth can be inhibited if oxygen concentration falls to very low levels in the aeration tank. This is because oxygen becomes limiting for respiration. This is dealt with more fully in the section below, on Respiration.

Temperature Bacteria have a genetically determined viable temperature range. For most carbonaceous bacteria of the activated sludge, this is from about 0 to 30°C. However thermophilic bacteria survive and grow between about 30°C and 60°C. In general, growth rate follows the rule of Arrhenius, that chemical reactions double in rate for a 10°C increase in temperature. Thus as the temperature increases, the rate of growth, and hence requirement for oxygen for respiration, increases.

Toxicity Toxic chemicals in the wastewater can enter the bacteria and inhibit one or more enzymes of the pathways involved in either anabolism or catabolism. If the catabolic reactions of respiration are affected, the rate of respiration and energy production is reduced and the rate of growth is therefore reduced. On the other hand, if the anabolic pathways of biosynthesis are inhibited, the rate of growth is reduced, and this is accompanied by a fall in the rate of respiration, as the requirement for energy is reduced. This is shown in the figure below.

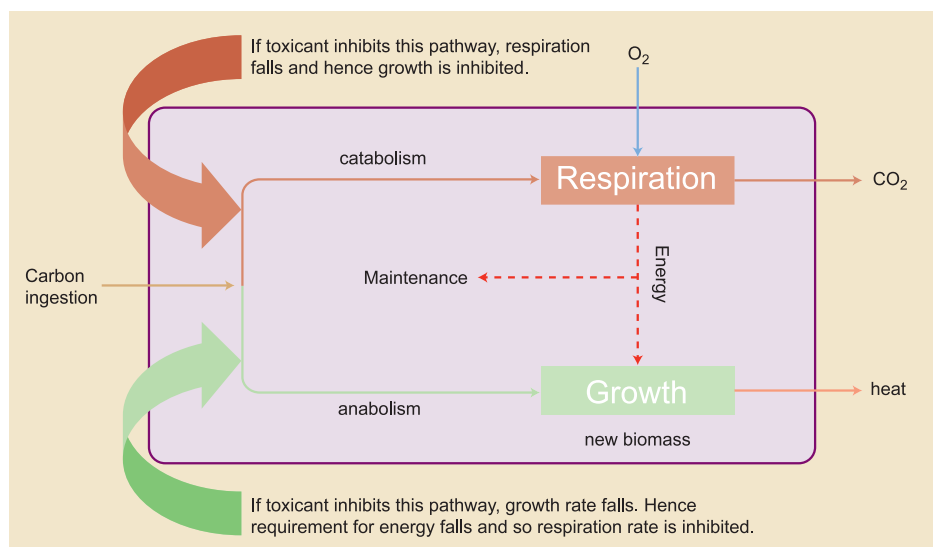


Figure 7 Toxic chemicals can inhibit either the Respiration metabolic pathway or the Growth metabolic pathway. In both cases the influx of organic carbon into the bacterium, which corresponds to the rate of biodegradation in the aeration tank, is inhibited as a result.

The tight coupling between Ingestion, Respiration and Growth was mentioned previously. It follows that irrespective of where the toxic chemical exerts its inhibitory effect, Growth, Respiration and Ingestion will be equally inhibited. In the aeration tank therefore, toxicity will have the effect of reducing the rate at which organic carbon is degraded. This can be easily monitored by observing changes in the rate of respiration of the activated sludge.

Respiration

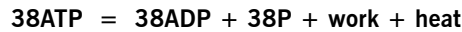
This is a chain of metabolic reactions by which a substrate molecule is oxidised, and the energy made available to do work inside the cell. The energy contained in a substrate such as glucose is rapidly liberated as heat when it is oxidised by burning it in air. When glucose is metabolised in respiration, the same amount of energy is ultimately liberated, but only after some of it has been used to carry out cellular work. During respiration, the energy is initially captured by the molecule adenosine diphosphate (ADP). This adds on another phosphate group to form adenosine triphosphate (ATP). The energy that is captured or transferred is stored in what is sometimes called a 'high energy phosphate bond'.

So when glucose is metabolised the overall reaction is:



This is not a perfectly efficient energy capture mechanism, and some of the energy is lost as heat.

The ATP then moves to another site within the cell and releases the energy to do work, as described below. At the same time the phosphate group is released, regenerating ADP again. So overall, we have:



The ATP is used as quickly as it is produced. The rate-limiting step is in fact the requirement for energy. The faster the cell is using energy, the faster the reactions in respiration proceed.

From the equation above, it will be clear that the rate of respiration could be measured by the rate of oxygen uptake, by the rate of CO₂ production or by the rate of heat liberation. Carbon dioxide is difficult to measure in aqueous media. Heat production can be measured in a calorimeter, but the simplest measure of respiration rate is by measuring the oxygen uptake rate with an activated sludge respirometer.

Why do bacteria need energy? All living organisms need an input of energy simply to maintain the steady state. For instance, each bacterium is involved in pumping out ions that diffuse through the cell wall, and in various processes of self-repair. All of those processes can be grouped together as maintenance, and all require energy. If the bacterium is mobile (and most are not) energy is used in propulsion. However the main use of energy in bacteria is for biosynthesis for growth. Growth involves the joining together of small molecular weight compounds to form macromolecules, which may then be further modified and assembled to form structures such as membranes, cell walls etc. Thus simple hexose sugars such as glucose, are joined together by glycosidic bonds, amino acids are joined together by peptide bonds to form proteins, and so on. Energy transferred by the ATP molecules is used in doing this work. In the course of this, some of the energy is lost as heat. So as the bacteria grow, they release heat, and this causes the temperature of the aeration tank to be above ambient air temperature.

Endogenous respiration In a normal growing bacterium, there are a certain number of molecules laid aside as storage products. These are mainly in the form of glycogen and poly-β-hydroxybutyrate (PHB). When all of the biodegradable carbon in the mixed liquor has been used up, as may happen at the end of a plug flow reactor, growth ceases, and the bacterium is then starving. In order to remain alive it still requires energy for maintenance processes. It therefore starts to metabolise its storage products to provide this energy.

Although growth has stopped, a low rate of respiration continues in order to provide the energy for maintenance. This is referred to as the Endogenous respiration rate. When the storage products have become exhausted, the bacterium then begins to metabolise cellular proteins and other structural molecules in order to provide the carbon for endogenous respiration. However, this is a like chopping up and burning the furniture to keep the house warm. Eventually the cell dies and splits open, thereby releasing the residual internal molecules, which then become available as potential food source for other bacteria.

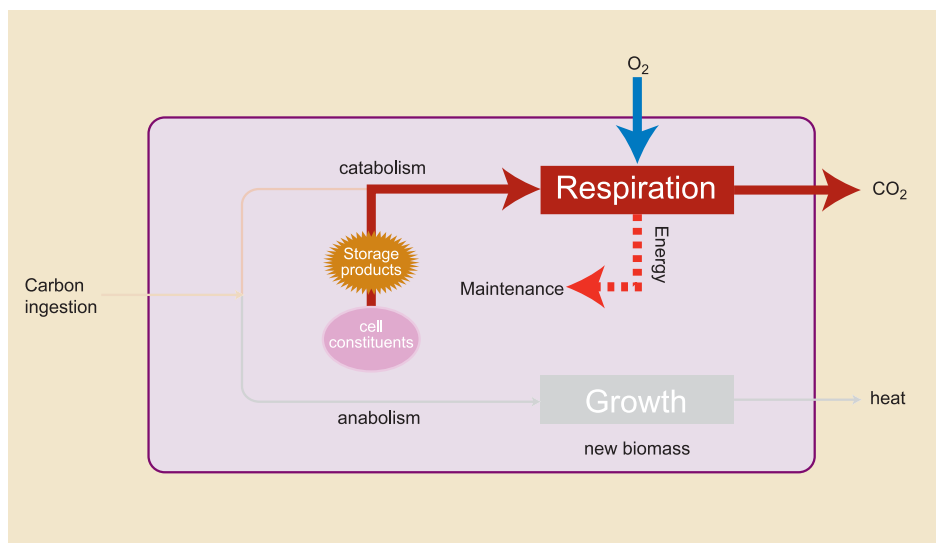


Figure 8 When the bacterium is starving, it has to rely on storage products for the carbon to sustain its endogenous respiration in order to keep itself alive. When these have been exhausted, cell constituents are metabolised.

When a bacterium in the no-growth Endogenous phase is presented with feed again, the first process is to rebuild the cell constituents used up. This is followed rapidly by the resumption of growth and the rebuilding of the storage products - see Figure 9 overleaf.

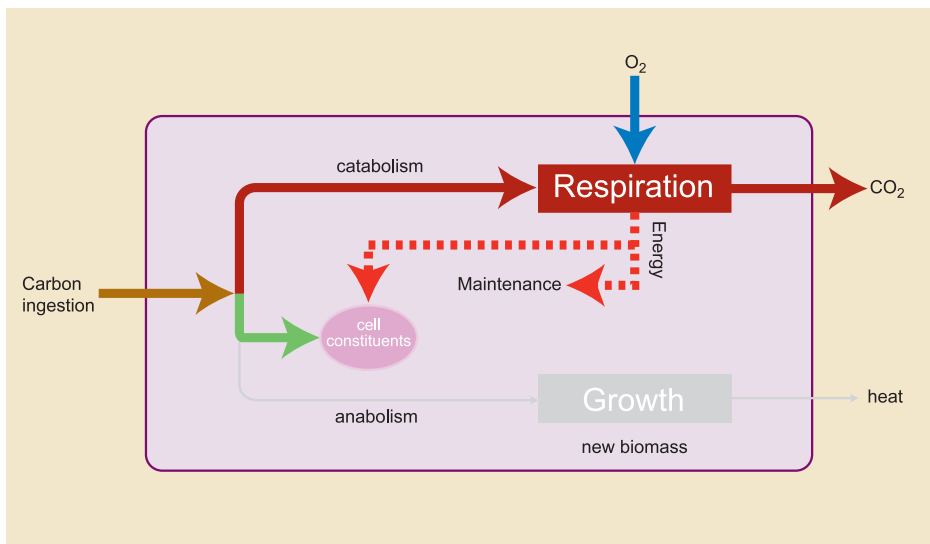


Figure 9 When substrate is provided to a bacterium in Endogenous respiration phase, the carbon is used initially to rebuild that used up during starvation. It also respire some of the carbon to provide the energy for the biosynthesis reactions involved.

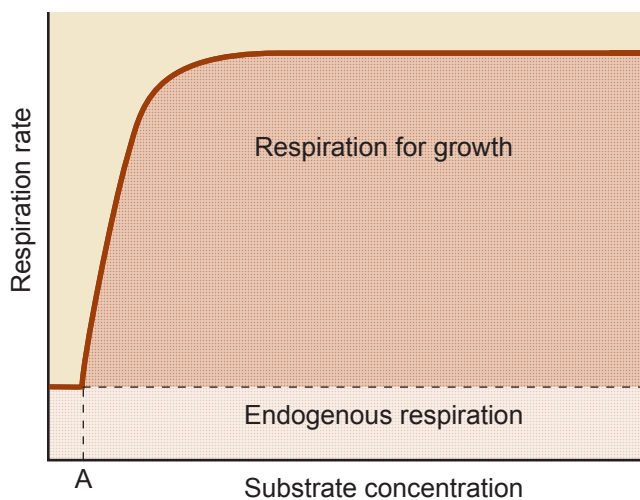


Figure 10 Relationship between respiration rate and substrate concentration in a bacterium. At concentrations higher than A, the bacteria are growing, but the endogenous rate is still a part of the total respiration rate.

It is important to remember that in a growing bacterium in the aeration tanks, part of its respiration is to provide the energy used in biosynthesis and growth, but there is still a small component that is being used in cell maintenance, as shown in Figure 4.

Because of the tight linking between Ingestion, Growth and Respiration, we find that the rate of respiration is affected by the same factors as those affecting growth rate, viz. substrate concentration, availability of nutrients, oxygen concentration, temperature and toxicity.

Effect of substrate concentration When the substrate concentration is at zero, as happens at the end of a plug-flow aeration tank, the bacteria are respiring endogenously, and there is no growth. As the substrate concentration increases, the point is reached at which there is now sufficient carbon intake for growth to take place. As the concentration increases further, the growth rate increases, and the respiration rate also increases. The relationship is essentially similar to the growth rate curve shown in Figure 5.

The biosynthetic machinery is switched on very rapidly when bacteria in the endogenous state are presented with food. This can be vividly demonstrated in the lab. If a concentrated feed is added to a flask of endogenous mixed liquor, within seconds it becomes transformed into a foaming and frothing cauldron, as the carbon dioxide bubbles produced in respiration are released.

Oxygen If oxygen levels in the mixed liquor are too low, respiration will be inhibited and hence energy will not be available for growth. When saturated with air at 20°C, wastewater will hold approx 9.2 mg O₂/l. Oxygen passes from the water into the bacteria along a concentration gradient (or more accurately a PO₂ gradient). The higher the oxygen concentration in the water, the larger is the gradient to the inside of the bacterial cell, where the oxygen concentration is close to zero. As mentioned previously, oxygen is not limiting above concentrations of about 1.5 – 2.0 mg O₂/l for bacteria in flocs and about 0.6 mgO₂/l for dispersed bacteria. Below these critical concentrations, the respiration rate falls rapidly due to the unavailability of oxygen, as shown in Figure 11 opposite. Filamentous bacteria have a greater tolerance of low oxygen levels than floc bacteria. At oxygen concentrations below the critical concentration, filamentous bulking (see later) can occur, as their relative biomass increases.

Figure 11 Respiration rate remains constant as the oxygen concentration in the mixed liquor falls. Below the critical concentration, respiration rate falls rapidly.

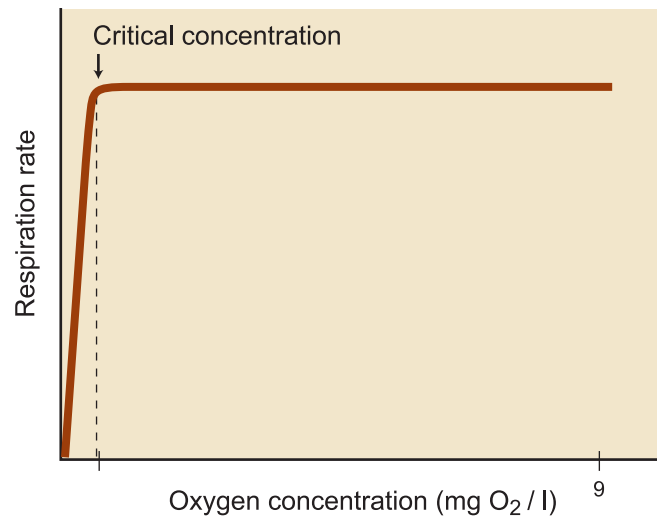
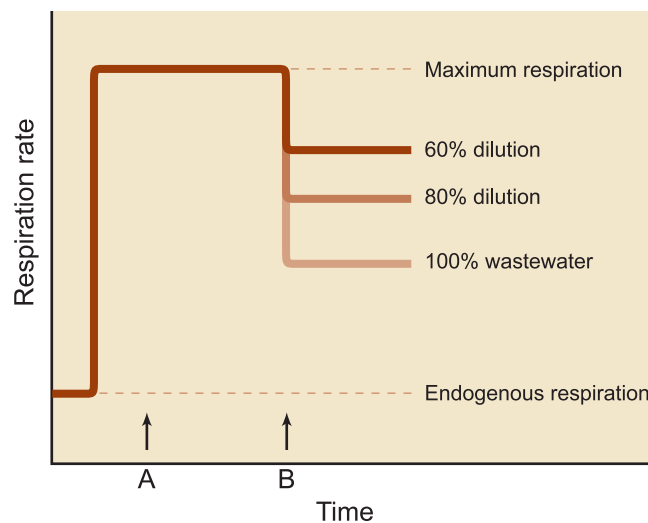


Figure 12 Time course of a laboratory experiment in which samples of activated sludge in endogenous phase are presented (A) with feed in excess, and then (B) with 3 different concentrations of toxic wastewater. The higher the concentration of toxic waste, the greater is the inhibition or the lower is the respiration rate.



Temperature The respiration rate approximately doubles for every 10°C increase in temperature, as noted for growth, above. However, the solubility of oxygen in water decreases with increase in temperature. One consequence of this is increase in the critical oxygen concentration value. Optimum aeration therefore becomes more and more difficult as the temperature in the tanks rises. It is for this reason that most thermophilic plants, operating at 40-60°C, have to use pure oxygen for aeration.

Toxicity As shown in Figure 7, toxic chemicals can inhibit either the catabolic pathways of respiration or the anabolic pathways of synthesis and growth. Irrespective of which pathway is actually inhibited, all three processes of Ingestion, Growth and Respiration will be similarly inhibited. It is for this reason that toxicity tests conventionally measure the inhibition of respiration, since oxygen uptake rate is easily measured using respirometry. The sequence of events taking place when endogenous activated sludge is presented with feed and then with a slug of toxic waste is shown in Figure 12. Immediately after feeding, the respiration rate rises rapidly to its maximal value. When a toxic wastewater is introduced, the respiration rate falls to a new lower level. The difference between this new rate and the maximal rate is a measure of the inhibition. Inhibition is normally expressed as a percentage of the uninhibited maximum rate of respiration. The percentage inhibition increases with increase in concentration of the toxic chemical in the mixed liquor, as shown in the figure below.

Conventionally, toxicity is expressed as the EC₅₀, EC₂₀ or EC₁₀ i.e. the concentration causing an inhibition of 50%, 20% or 10% of the respiration rate.

We have seen that the rates of both growth and respiration are affected by substrate concentration, availability of other essential nutrients, temperature, and oxygen concentration.

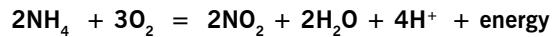
Because of the tight coupling of these processes with Ingestion, the uptake rate – which corresponds to the rate of biodegradation in the aeration tanks – responds in the same way.

Up to this point, we have dealt exclusively with the heterotrophic or carbonaceous bacteria that predominate in activated sludge. However, in many treatment works, nitrifying bacteria play a significant role, and so we shall briefly review this group in order to understand the fundamental differences that they display.

Nitrifying bacteria

If the treatment works receives a significant amount of nitrogenous matter in its influents, it will need to be removed by the nitrifiers, in the course of treatment. Nitrifying bacteria are autotrophs, requiring only inorganic chemicals as the starting point for their energy metabolism and growth. Thus ammonia is taken up and oxidised to provide the energy required for growth. Carbon dioxide is used as the carbon source, and this is metabolised into organic carbon compounds inside the bacteria - a process which also requires energy. There are relatively few species of nitrifiers, and their contribution to the total bacterial biomass is small.

The process of ammonia oxidation is referred to as nitrification, and is carried out by two different groups of nitrifiers. The first group oxidise ammonia to form nitrite. The most abundant genus is *Nitrosomonas* but there are other nitrifiers as well. The overall reaction is:



The oxidation of nitrite to nitrate is carried out by *Nitrobacter* and by other minor species. The overall reaction here is:



Note that this process should not be referred to as respiration, although oxygen is consumed and the purpose-energy capture is the same. Respiration is the process found in carbonaceous bacteria and in plants and animals. (In mixed activated sludge, the oxygen uptake may comprise both heterotrophic respiration and oxidation by nitrifiers. For convenience only the oxygen uptake rate of activated sludge is referred to as its respiration rate).

Chemical oxidation by nitrifiers is not as efficient as the process of respiration in heterotrophs. Relatively large amounts of oxygen are required per unit of energy produced. They therefore have a greater relative oxygen requirement and are slow growing.

The major metabolic processes can be represented as if occurring in a single bacterial cell in Figure 13.

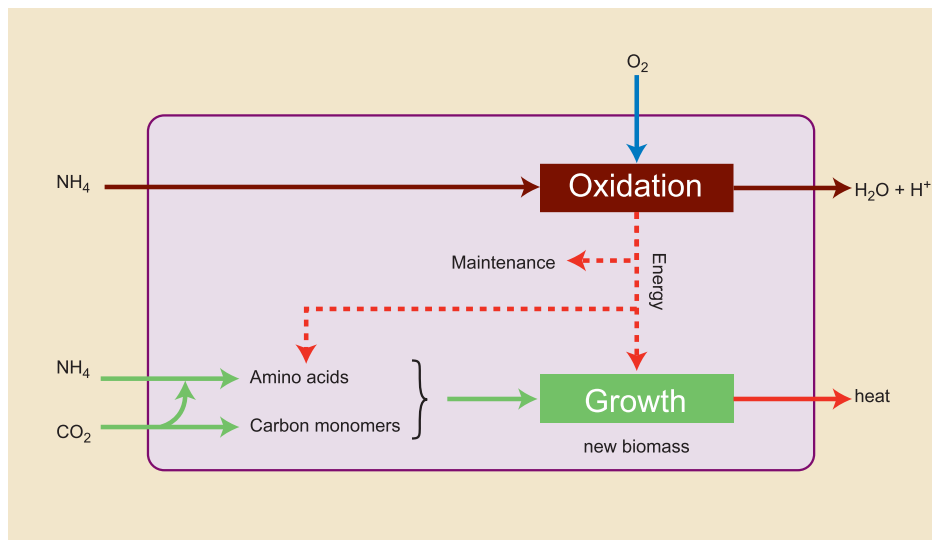


Figure 13 For convenience, the major processes of the two groups of nitrifiers are here combined into a single cell. Note the basic similarity to the carbonaceous bacteria processes in Figure 4. Here oxidation is the oxygen-consuming process instead of Respiration.

Nitrifiers are also characterised by having a low range of temperature tolerance, from c $8^\circ\text{C} - 30^\circ\text{C}$ and they exhibit a very low metabolic rate below $15-20^\circ\text{C}$. They have a higher critical oxygen concentration of c $2.0 - 2.5 \text{ mg O}_2/\text{l}$ at 20°C , and their growth is easily disrupted by changes in environmental variables. They are also very much more susceptible to inhibition by toxic chemicals than carbonaceous bacteria, the nitrite oxidising species more so than the ammonia oxidising species.

Biological treatment plant operation

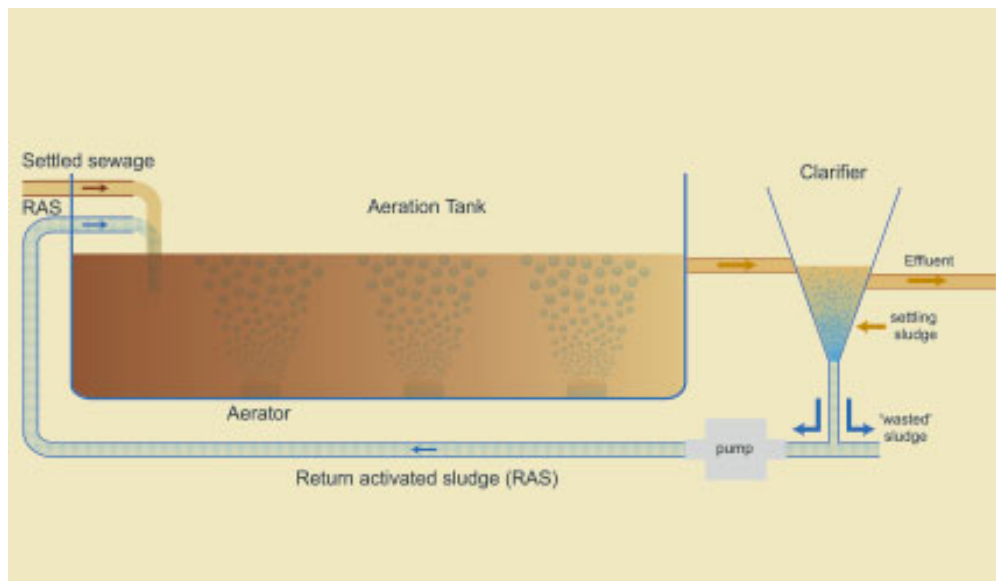
Basic treatment plant layout

A typical treatment plant comprises three phases of treatment – primary, secondary and tertiary. Primary treatment involves settlement of solids in a clarifier tank. The wastewater then passes to the secondary treatment or aeration tanks. This is the major biological phase of treatment by the activated sludge bacteria. A tertiary phase may be used to further improve the quality of the secondary effluent, by removing nitrogen, phosphates, suspended solids or pathogens, as required.

There are many designs of aeration tank, including plug-flow, completely mixed, percolating filter, sequencing batch reactor (SBR), and so on. The simplest of these is the plug-flow system, and we will use this to illustrate the basic principles of biological treatment.

A plug-flow system might consist of a long rectangular tank, 3-5m in depth, with aerators to supply oxygen for respiration, and to keep the activated sludge in suspension.

Figure 14 Layout of a plug-flow plant. In its passage through the aeration tank, there is an increase in the activated sludge biomass, as a result of bacterial growth. At the end of the tank, the mixed liquor passes to the clarifier, to allow the sludge to settle. Some sludge is recycled back to the beginning of the tank. The rest, corresponding to the net new biomass, is 'wasted' i.e. dewatered and dried.



At the inlet it receives the inflow of settled sewage from the primary clarifier. It also receives a flow of Return or Recycled Activated Sludge (RAS). This combination of wastewater and activated sludge is referred to as the mixed liquor.

As the influent enters the aeration tank, it mixes with the activated sludge. Very small particles and large organic molecules adsorb on to the polymers of the floc material. At the same time, the activated sludge bacteria, which are at the endogenous respiration stage, come into contact with a high concentration of the small molecular weight organic carbon molecules that they require in order to grow. Growth starts immediately, and the respiration rate rises rapidly in order to supply the energy required for this.

As the mixed liquor flows along the tank, the concentration of soft BOD will begin to fall, and the bacteria then feed on the small molecular weight organic carbon compounds produced by the action of enzymes released by the sludge bacteria. The concentration of substrate made available by this is lower than at the initial stage of treatment. Hence the growth rate is lower than at the first part of the tank, and the respiration rate is correspondingly lower. The actual course of treatment along the length of a plug flow reactor can be easily monitored by measuring the respiration rate, (or the specific oxygen uptake rate (SOUR)) of samples of the activated sludge.

Since the respiration rate declines, the need for aeration also falls with distance from the inlet point. Ideally by the time the mixed liquor has reached the end of the tank, 90-95% of the influent BOD should have been removed. If it reaches this state before the point of discharge, there is under-utilisation of capacity. Conversely, if it has not reached this at the outlet, the effluent will be discharged to the environment with significant BOD still untreated, and may cause eutrophication.

After treatment, the mixed liquor from the aeration tank passes to a conical-shaped receiver, called the clarifier. The activated sludge is allowed to settle to the bottom of the tank whilst the clear liquid above flows away as the treated effluent, and is ultimately discharged to the receiving waters. Sufficient of the settled activated sludge to maintain the operational concentration of bacteria in the aeration tank, is pumped back to the inlet as the Return Activated Sludge. This normally represents about 25-50% of flow through the aeration tank. The remainder of the settled sludge, referred to as the 'wasted' sludge, is pumped away to be dewatered, dried, and disposed of.

It will be obvious that the flow of effluent plus the flow of wasted sludge equals the rate of sewage inflow to the aeration tank. Under steady-state conditions, the activated sludge that is wasted corresponds to the net production of sludge resulting from the growth of biomass during passage through the aeration tank.

Treatment plant operation

An ideal biological treatment plant would have the following features:

- Fast throughput of sewage
- High rate of BOD removal
- Good settlement of sludge in the clarifier
- Low rate of sludge production
- Minimal aeration costs
- High quality effluent - low in BOD suspended solids etc.

In operational practice it is difficult to achieve all of these, and process control involves various compromises to achieve optimisation. Fast throughput with rapid BOD removal can be achieved by using a relatively high BOD concentration (a high f/m ratio - see later). However, high BOD concentrations result in high growth rates and a high rate of biomass or sludge production (Figures 5 and 15). Operationally, it may cause aeration problems, and will result in poor sludge settling characteristics.

A high sludge production incurs high costs in dewatering, drying and removal. It is possible to reduce the relative amount of sludge produced by operating the plant at low BOD levels (low f/m – explained later). At the resultant low biomass growth rates (Figure 15), a high proportion of the carbon in the BOD is used by the bacteria in maintenance, or in simply keeping alive, i.e. for endogenous respiration. Consequently, most of the BOD ends up as CO₂, rather than in sludge. This is illustrated in Figure 16.

Thus in a low rate system, very little sludge will be produced, and it is characterised by having good setting characteristics. The down side of this mode of operation, of course, is that the overall treatment time is increased and so is the total amount of aeration required.

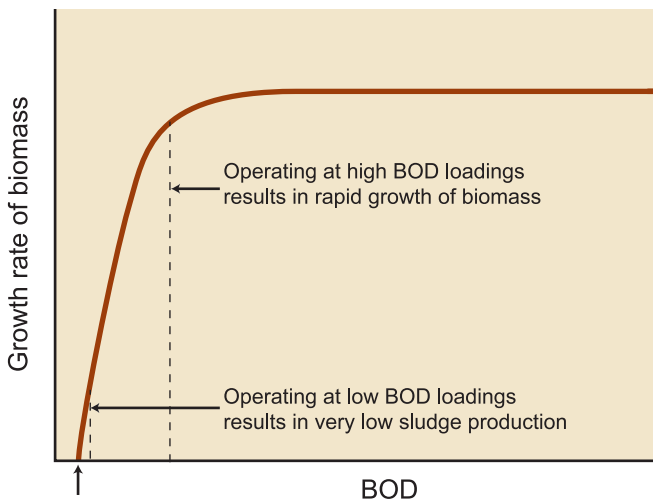


Figure 15 The relationship between biomass growth rate and BOD loading.

At this loading the bacteria are in endogenous state and there is no net sludge production

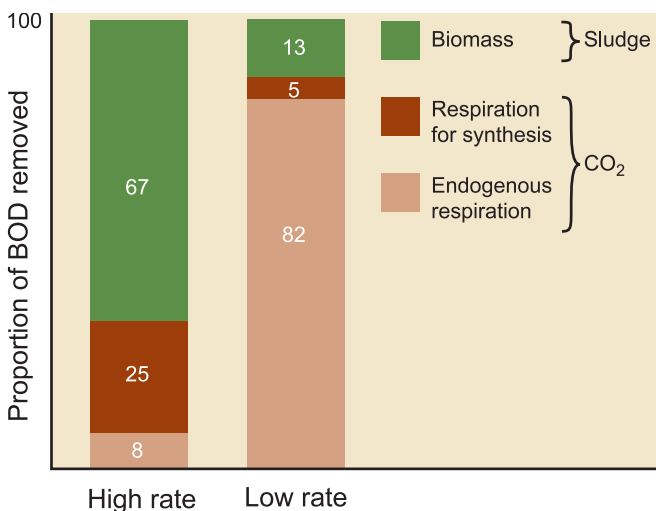


Figure 16 Proportions of BOD removed that appears in sludge production and as CO₂ from respiration in a high rate and low rate (oxidation ditch) system. In the low rate plant, not only is the rate of growth lower, but the relative proportion of the BOD transformed into sludge is also very much lower.

The manipulation of this relationship between growth rate or sludge production rate and BOD in process control gives rise to 3 main types of biological treatment plant.

- **High rate** For pre-treatment or partial treatment of high BOD Sewage, as in some pharmaceutical and dairy wastes etc.
- **Conventional** Medium rate, characteristic of most municipal treatment works.
- **Low rate** Low BOD loading, and characteristic of small, extended aeration works and oxidation ditches and lagoons, occupying a large land area.

The process characteristics of these differing types of plant are summarised later (Table 1), but first we shall deal with the main process variables involved.

Process variables used in control of the biological processes

Some small municipal treatment works may receive domestic sewage characterised by only small variations in composition and flow. Here, process control can be automated so that very little on-site direct management is required. By contrast large treatment works, particularly those treating industrial wastewater from many sources, require vigilant control in order to manage the biological activity of the aeration tank. The following section describes the commonly used process variables that may be used in control of the treatment: measurements of sludge biomass, treatment duration and retention times in the system, and the ratio of the concentration of influent BOD to the activated sludge biomass.

In order to apply basic equations to these variables, the simple plug flow system can be represented diagrammatically - see Figure 17.

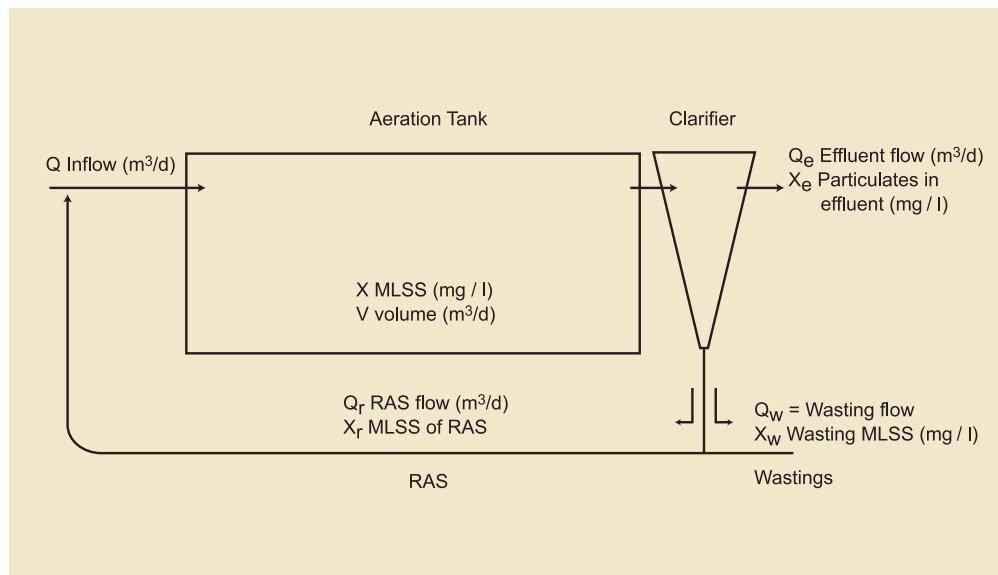


Figure 17 Notations for variables used in process equations.

Mixed Liquor Suspended Solids (MLSS)

In a well-operated plant, most of the bacterial biomass is associated with the activated sludge floc. By filtering and drying a sample of the suspended solids, and then weighing the dried residue, a measure of the biomass may be obtained. It is referred to as the Mixed Liquor Suspended Solids, or MLSS, and is expressed in mg/l. However, under some circumstances a significant proportion of the MLSS may be inorganic material. For this reason, some process engineers prefer to derive a weight for the organic matter in the sludge. This is done by combusting the dried residue in a furnace at 500°C, reweighing, and obtaining the volatilised organic matter, by subtraction. This is referred to as the Mixed Liquor *Volatile* Suspended Solids or MLVSS. However even this weight is an imprecise measure of the active microbial biomass, since a significant part of the floc comprises inert organic matter.

Despite its shortcomings, MLSS is universally used in process control as a measure of biomass. MLSS values range from about 800 - 1,500 mg/l for extended-aeration and other low-rate systems, to about 8,000 mg/l or more, for high-rate systems. It may be intuitive to think that higher efficiency of treatment would be achieved by increasing the MLSS, since the more organisms that are present in the mixed liquor, the faster the BOD should be ingested. However, high MLSS concentrations create problems in aeration and also in settlement of sludge in the clarifier.

Hydraulic retention time or volumetric loading

This is the average time spent by the influent sewage in the aeration tank. It is calculated as the tank volume (m³) divided by the flow rate. Since flow rate Q is normally expressed in m³/d and hydraulic retention time is normally expressed in hours, the formula used is:

$$\text{HRT} = \frac{V}{Q} \times 24 \text{ hours}$$

Clearly the higher the inflow rate Q, the sooner the sewage influent will reach the outlet and therefore the lower will be the residence time or hydraulic retention.

The hydraulic retention time must be sufficiently long for removal of the requisite proportion of BOD from the mixed liquor. In a conventional activated sludge system the HRT will be between 5 and 14 hours. The process engineer has little control over HRT, and following heavy rainfall the increased influent flow Q may reduce the actual retention time to as little as 1 hour.

Sludge residence time or sludge age

Sludge age is the mean residence time of the microorganisms in the system. It is calculated as the total amount of MLSS in the system divided by the MLSS that is lost in wasting and in the effluent, each day i.e.

$$\frac{\text{Tank volume m}^3 \times \text{MLSS mg/l}}{(\text{Wastings flow m}^3/\text{d} \times \text{Wastings MLSS mg/l}) + (\text{Effluent flow m}^3/\text{d} \times \text{Effluent particulates mg/l})}$$

or using the usual notation:

$$\text{SRT or } t_s = \frac{V \cdot X}{Q_w \cdot X_w + Q_e \cdot X_e} \text{ days}$$

The MLSS in the RAS is not included in the calculation. It will be noticed that under steady-state conditions, the denominator equals the net sludge production each day. If it is large, as a result of rapid growth of the activated sludge, the sludge age will be low. Conversely if very little growth of sludge is produced (for example by using a very low ratio of food to biomass f/m (see later), the 'average age' of the sludge in the system increases, since it is recycled in the RAS many times.

Values of sludge age may vary from < 0.5days, in a very high-rate system, to 75 days in low growth-rate systems, such as extended aeration systems. In a conventional plant, SRT would normally be between 3 - 4 days. Sludge settlement is correlated with SRT. Low SRT values are associated with non- or poorly-flocculating sludge, and poor settling characteristics.

Sludge loading or f/m ratio

The rate of biomass growth, and rate of respiration (and hence rate of BOD removal by bacterial ingestion) increases with increase in BOD loading as shown in Figs 5 and 10. However, the rate of BOD removal in the aeration tank is also related to sludge biomass. The higher the biomass, the higher the rate of BOD removal. In order to measure the amount of feed available to a unit of biomass, the BOD is divided by the MLSS. The value obtained is the so-called sludge loading, more commonly referred to as the f/m ratio or the food/microorganism ratio. As the ratio of food (BOD) to microorganism increases, so will the rate of BOD removal, growth rate, and respiration rate.

The f/m ratio is a useful value for the treatment plant manager since there are predictable consequences of running the plant at different f/m ratio values. It is calculated as the daily flow of BOD divided by the total MLSS in the aeration tank (derived from the product of the MLSS and the volume of the aeration tank). Thus:

$$f/m = \frac{\text{BOD g/l} \times \text{flow m}^3/\text{d}}{\text{MLSS g/l} \times \text{tank volume m}^3} = \frac{\text{g O}_2 \cdot \text{g MLSS/d}}{\text{g MLSS/d}}$$

Or, using the previous notation:

$$f/m = \frac{\text{BOD} \times Q}{X \times V}$$

We are again using BOD as a measure of the organic carbon available to the activated sludge bacteria and the use of oxygen units, can be somewhat confusing.

The values for f/m range from about 0.5 to 1.0. For conventional plants an f/m of between 0.2 and 0.5 is usually aimed for. At higher values, the rate of treatment increases, but at the cost of poor settlability of the sludge. f/m values below 0.2 are associated with slow BOD removal rates, but with very good sludge settlement (see table below)

Another measure of sludge loading is the instantaneous f/m, or BOD_{ST}/B , where B is the MLSS. This is a measure of the loading at any point in the tank. It can be easily measured on a grab sample, and used as a measure of the progress of treatment at various points along the length of the tank. It is not yet in general use. Probably because of the lack of a rapid and effective way to measure BOD_{ST} . However with the availability of rapid respirometers such as Strathtox, this management tool may become more widespread.

Comparison of process variables - different treatment plant types

As shown before, plug-flow treatment can be divided into 3 main types, each characterised by different process variables strategies. These are summarised in Table 1 below.

Table 1

Treatment Rate	HRT (h)	Sludge Age (d)	Sludge Loading (f/m) kg BOD/kg MLSS.d ⁻¹	Sludge Production kg sludge/kg BOD removed
Conventional	5 - 14	3 - 4	0.2 - 0.5	0.5 - 0.8
High Rate	1 - 2	0.2 - 0.5	>1.0	0.8 - 1.0
Low Rate	24 - 72	>5 - 6	<0.1	<0.4

Settlement problems

As noted above, high-rate treatment is often associated with poor settlement, and the opposite is true for low-rate treatment. Settlement is affected also by nutritional imbalance, changes to the microbial components of the activated sludge, and the presence of toxic chemicals in the mixed liquor. The settlability of the sludge in the clarifier is measured by the sludge volume index (SVI) - the volume of sludge occupied by 1g of MLSS in a standard measuring cylinder, after 30 minutes of quiescent settling. An alternative measure is the sludge specific volume index (SSVI) in which the volume occupied is measured in a specially designed cylinder that incorporates a weak horizontal stirring, to mimic the non-quiescent conditions experienced in a clarifier.

In conventional well-settling activated sludge, the flocs often have a core structure formed by the filaments of filamentous bacteria. However these filaments may grow to project out of the flocs (Figure 3). If this growth continues, they may form a bridge between adjacent flocs (Figure 18) and prevent their independent motion. Conversely, flocs without a filamentous skeleton may break up into smaller flocs due to the shear exerted by the turbulence of aeration. In some cases these may round up to form spherical clumps of bacteria with little apparent extracellular polymeric substance. Finally in some circumstances flocs may fail to form, and the bacteria comprise separate individual cells or only very small aggregates. The different forms that the flocs can take are related to various settlement problems.

Deflocculation This is caused by failure of the bacteria to form flocs, or by the break-up of flocs as a result of severe aeration turbulence. Settlement is minimal, and as a result there is little clarification. This gives rise to a turbid effluent that would fail its particulate-matter consents. Another consequence is that the continued loss of bacteria in this effluent may make it impossible to maintain MLSS levels in the aeration tank via the return activated sludge. This will in time result in an increase in the f/m ratio.

Deflocculation can be caused by inadequate aeration, resulting in low dissolved oxygen concentration, and by high sludge loadings. These two factors may occur simultaneously, if good aeration management is not practised. It can also be caused by low pH and by the presence of certain toxic chemicals in the influent.

Pin-point floc Here the flocs are very small and compact. Since they have little extracellular polymeric substance (EPS) to bond them together, they readily break into smaller units as they grow, due to the shear forces exerted by aeration. This sludge settles poorly and there is again loss of biomass in the effluent, with the inevitable consequences for maintaining MLSS in the aeration tank. It is often associated with long treatment times, e.g. in extended aeration systems where the sludge age is above 5-6 days and the f/m ratio is very low. However it can also appear in high-rate plants treating chemical or pharmaceutical waste, where it is thought to be a consequence of toxic chemicals inhibiting the growth of the filamentous bacteria that would otherwise assist floc formation.

Foaming and mousse formation Foaming is sometimes associated with the presence of non-degradable detergents in the wastewater. This causes a light frothy foam. However a more intractable type of foaming, usually referred to as mousse formation, has a different cause. Here filamentous fungal hyphae of the genus *Nocardia* bind the foam into a dense blanket. This can trap the activated sludge flocs. Thus foaming can result in a loss of MLSS from the return activated sludge.

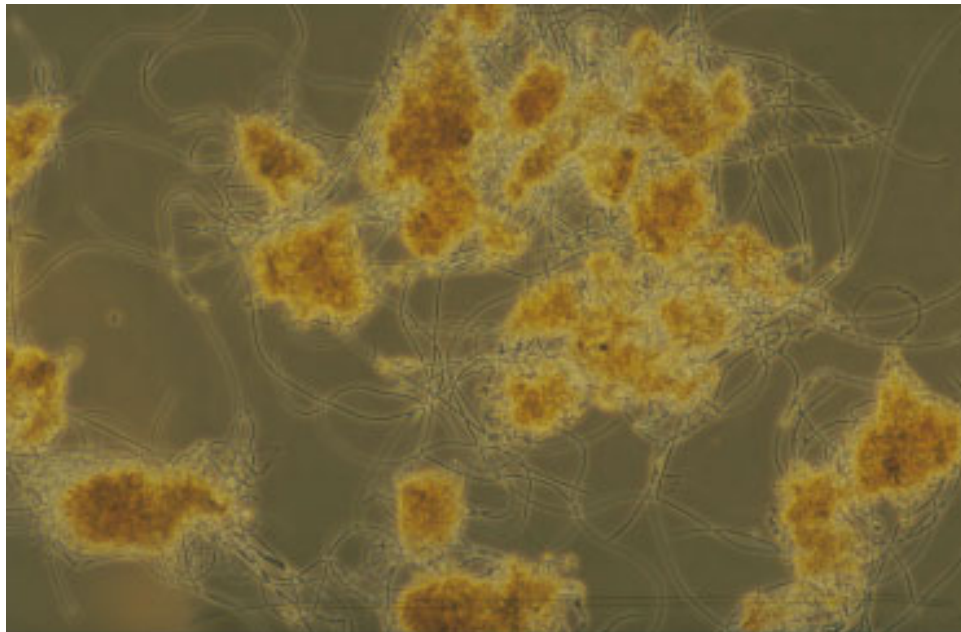


Figure 18 Micrograph of activated sludge showing filamentous bulking. Filamentous bacteria have grown out from the flocs, forming bridges with adjacent ones. (Photograph courtesy of Ray Kenny)

The usual solution to the problem is to reduce the sludge age by increasing the waste sludge flow, thereby washing the *Nocardia* out of the system. Scum traps are usually unable to cope with the volume of foam produced, and anti-foaming chemical treatment and surface chlorination is sometimes used.

Filamentous bulking This occurs when the filamentous bacteria extend out of the flocs, becoming entangled with filaments from adjacent flocs. The network of filaments may then act as a filter, entrapping small particles, so that a very clear final effluent is produced. However, the bulked flocs settle poorly and the settled sludge is less compact or more diffuse. As a result, the level of the sludge blanket at the base of the clarifier extends up towards the surface, causing

loss of flocs in the effluent. The poor compaction of the sludge in the clarifier also results in a low MLSS return in the return activated sludge.

Filamentous bulking is often associated with changes in process variables, including sludge loading; nutrient concentration and oxygen concentration. In general, filamentous bacteria have a lower BOD uptake rate and lower maximum specific growth rate than floc bacteria. However, they also have a higher substrate affinity (see Figure 6), which means that they can out-compete floc forming bacteria under low substrate or f/m ratio conditions. Ideally sludge loading should result in f/m ratios of between 0.2 and 0.45 Kg BOD / Kg MLSS/d. This can be managed by changing the influent flow, the BOD of the influent or the MLSS concentration. The latter is the only one that is normally available to the process engineer, and is achieved by changing the wastage of sludge.

The ideal BOD : N : P ratio as noted previously is 100 : 5 : 1. If N or P are deficient, filamentous bacteria will out-grow floc-forming bacteria. High-carbohydrate wastes such as those produced by the brewing and some food processing industries, are particularly conducive to sludge bulking, and this may be due to nutrient imbalance. In order to remedy this, it is possible to use respirometry to assess the level of nutrient addition required to maximise the sludge respiration rate, and then adding the required nutrients at the inflow of the aeration tank.

Low oxygen concentration will also favour the growth of some filamentous bacteria, since they have a greater affinity for oxygen. This form of bulking can be overcome by accurate determination of the critical oxygen concentration for the activated sludge (Figure 10), using a rapid closed chamber respirometer, together with good aeration management.

In a study carried out in the US, 80% of bulking incidents caused by the Type O21N microorganism, were associated with the treatment of industrial wastes, suggesting that this bacterium exhibits greater tolerance of certain toxic chemicals.

Control of bulking may be achieved by process variable changes, where this is the identified cause. However, in many cases the process engineer will choose to use chlorination of the RAS flow or the aeration tank, to selectively kill filamentous bacteria. Promotion of sludge settling may also be attempted by the addition of lime, iron salts or synthetic organic polymers.

Conclusion

Wastewater treatment is mainly concerned with the biodegradation of organic carbon or BOD. This is carried out by the heterotrophic or carbonaceous bacteria in the aeration tank of the wtw. These bacteria take up, and hence remove, organic carbon molecules from the mixed liquor, and use them for either Respiration or for the Growth of new biomass.

The 3 processes of Ingestion, Respiration and Growth are tightly coupled. By measuring Respiration you are indirectly measuring biodegradation and biomass growth.

As a consequence of this, respiration can be used as a management tool for any of the following applications in wastewater treatment:

- **Carbonaceous capacity: how much BOD can be removed/unit time by the sludge bacteria?**
- **Nitrification capacity: how much ammonia can the plant deal with?**
- **Nutrient requirement: how much nutrient needs to be added, in order to maximise respiration and hence biodegradation rate?**
- **Bioaugmentation: will the addition of cultured bacteria increase the rate and efficiency of biodegradation?**
- **Short-term BOD: how much readily biodegradable BOD is in the influent sewage?**
- **Toxicity management: By how much will an industrial discharge inhibit the respiration, and hence rate of biodegradation?**
- **Activated sludge health: Is the respiration rate and hence plant efficiency being compromised by toxic chemicals in the mixed liquor?**
- **Active biomass: what proportion of the MLSS is live and viable biomass?**
- **Critical oxygen concentration: what is the critical oxygen concentration that needs to be maintained before respiration and biodegradation rates are inhibited, and filamentous bulking increased?**

Many of these applications have not hitherto been routinely used in process management. With the advent of new generation respirometers such as Strathtox, optimisation of process control can now bring substantial cost savings in wastewater treatment.

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