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## Synopsis

The following will be discussed in this chapter:

- Typical components of textile effluent waste streams: the soluble components are dyes, oxidants, dissolved salts, surfactants, starches, etc. Insoluble enzyme substrates will mainly be fibres such as cotton, flax, viscose, lyocell, wool, silk, polyester, acrylics, polyamides and others. Details of the types of components, which are considered to be hazardous waste, will also be considered.
- Possible synergistic effects of other effluent components on colour removal with activated sludge, together with inhibition of the colour removal process, for example by heavy metals and dissolved salts. The physical state of the dye is also considered to be important in dye degradation mechanisms, such as the degree of aggregation of the dye. This can largely depend upon pH and other factors and components within the effluent during treatment.
- The influence of pH, temperature and concentration of other components.
- Mechanistic studies of aerobic and anaerobic enzyme activity. Enzyme activity under extremes of temperature, pH and other such conditions, which promote such activity.
- Aerobic and anaerobic dye degradation mechanisms that are likely to be in operation during treatment. Both are important and pertinent in an effluent treatment plant and the degree to which each predominates will depend on the rate and degree of oxygenation. Variation in degree of agitation within a plant, for example, will promote the formation of localised deadspots in which reduced oxygenation might favour a reductive dye degradation mechanism.
- Likely future trends encompassing minimisation of waste components. In the case of dyes, improved reactive dye bath exhaustion rates will be of importance.

- Alternative technology involving coagulation, flocculation and precipitation techniques.
- The current demand for stable dye systems so that resistance to dye degradation inherent within the dye structure. However, with increasing environmental awareness, there may be a tendency to move away from increased dye fastness especially on substrates, which have a limited service life.

A book edited by Cooper (1995a) has significant contributions from other authors, many from different parts of the industrial sector.

## 6.1 Hazardous waste

Hazardous waste includes material described by the two groups shown below. Such wastes can be solids, sludges, liquids or gases.

- **Manifestation** – irritants, strong sensitisers, infectious, carcinogenic, mutagenic, teratogenic.
- **External influence** – flammable, explosive, radioactive, toxic or poisonous, corrosive.

Hazardous wastes include major types of heavy metals used in textiles and dyestuffs. Other lesser textile wastes – especially from dyeing and finishing – are acids and alkalis, bleaches, adhesives and polymers, crosslinking agents, carbonising agents, conditioner for wool, catalysts, detergents, dye carriers, chemical finishes including flame retardants and solvents. Of these, dye carriers, chemical finishes, and solvents are chemicals most likely to be potentially hazardous.

### 6.1.1 Heavy metals

These are potential toxicants dependent on their concentration and chemical form in the environment. Note that although arsenic is not a metal, it is included in this data because of its toxic nature. Table 6.1 gives metals frequently found in textile waste streams:

Processes and process materials in which these metals are commonly used are as follows:

- premetallised dyes with 3 to 4% metal content;
- double salt preparation of zinc in some basic dyes with approximately 3% metal content;
- dichromates used to oxidise and fix some dyes;
- chromium compounds as used in topchroming;
- catalysts for wash and wear applications;

*Table 6.1* Metals frequently found in coloured textiles

Metal	[M] ppm	Dye type with highest [M]
Arsenic	<1–1.4	fibre reactive
Cadmium	<1	all types
Cobalt	3–83	acid
Copper	33–110	vat
Lead	6–52	fibre reactive
Mercury	0.5–1	vat
Zinc	3–32	basic

[M] refers to the concentration of the metal in the waste stream in ppm.

- improvement agents for wash and lightfastness in certain fabrics;
- flame-retardants.

### 6.1.2 Dyestuffs

Dyes tend to be non-biodegradable by conventional aerobic treatment systems. Some contain heavy metals such as Cr, Cu and Zn. Commercial dyestuffs comprise approximately 50% dye by weight, the remainder usually being non-hazardous filler, for example sugar and surfactant. Dye molecules contain a chromophore and an auxochrome, and the very position of substitution of the auxochrome can markedly affect the toxicity of the dyestuff. Many dyes contain heterocyclic compounds, which may exhibit chelating action. Therefore, toxicity could result, either by the removal of metals essential to the environment or by synergistic action to increase the toxic effects of metals normally present. Generally, from a dye class standpoint, basic dyes appear to be the most toxic because of their cationic nature. Direct and vat dyes tend to be non-hazardous.

From bioassay studies, basic (cationic) dyes and some acid and disperse dyes are toxic to fish and algae. The remaining dye classes are refractory, but may degrade anaerobically, typically in landfill sites, resulting in possible carcinogenic metabolites. Thus all dye-containing waste streams may be considered to be potentially hazardous.

## 6.2 Types of textile effluent

The scouring of cotton generates quite high concentrations of organic matter from natural impurities and from sizing materials in the fabric. This effluent tends to be concentrated, and therefore needs to be diluted prior to treatment, which also prevents clogging of the treatment plant.

Scouring synthetic materials yields effluent with appreciable amounts of size and antistatic agents in cloth. Raw wool gives high concentrations of emulsified wool grease resulting in highly polluted liquor.

In the bleaching process, the contents of bleach liquor effluent are mainly residues of oxidising agents, for example sodium hypochlorite ( $\text{NaClO}$ ), sodium chlorite ( $\text{NaClO}_2$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and alkalis or acids, which must be neutralised. Peroxides are not too troublesome but other oxidising agents must be reduced or kept out of the treatment plant altogether.

In mercerisation where caustic solutions are concentrated enough for processing, these can be further concentrated later by evaporation if necessary, when it may be economically viable to recover them. The effluent is a solution and suspension which is usually highly caustic and could be put to good use at minimal cost. For example, it could be mixed with acidic industrial effluent which is frequently high in heavy metals in order to precipitate out the metal hydroxides.

In dyeing, different types of dyeing processes will give rise to various kinds of liquor which may be discharged, and most or all will contain surface active agents. In sulphur dyeing, the liquor contains sodium sulphide, which can result in staining. This has to be removed or at least diluted. In chrome dyeing, liquors and some after-treatment solutions contain metals that can interfere in biochemical processes. These must be removed or made innocuous.

Printing wastes contain thickeners and dye pigment. There are five possible sources of these:

- Pastes surplus to requirements: these can be minimised by precise calculations in making up pastes.
- Residues on machines, screens and rollers: washings can be treated separately by sedimentation.
- Loose colour on fabric.
- Finishing residues from various starch fillings usually from surplus materials in mixing and machine boxes.
- Weaving size residues from washing out becks and sow boxes. Water jet looms utilise appreciable quantities of wastewater.

Corrosion in soft water at low pH seriously affects tanks and pipe work. Natural water and free  $\text{CO}_2$  with no alkali or dissolved  $\text{O}_2$  attacks iron pipes resulting in  $\text{FeCO}_3$ . Raising the pH with alkali prevents both types. Foaming was a serious problem in the 1950s. Detergents and soaps are now biodegradable. Unpleasant odours usually arise from organic wastes and solvents. The remedy for this is usually the use of flocculation, filtration and sedimentation techniques. We can also use adsorption onto active carbon or chlorination.

### 6.3 Methods of water treatment for incoming water

Clarification removes suspended solids, thereby making the water less turbid, and surface waters are the main type requiring this treatment. The exact level of treatment depends on the source; for example whether from river, lake, canal and so on.

The demand for water in printing, dyeing and finishing is usually quite variable, owing to the number of different operations that can be taking place at any one time. A system is therefore required which allows storage of both raw and treated water.

When demands are made on the treated water, it is replenished from the raw supply automatically, after having been treated first. Water can be drawn off at several depths, allowing take-off at or near the surface regardless of the level and ensuring that maximum settling has occurred, this being the first stage in clarification. Therefore a greater storage capacity means that a longer time for settling is necessary. Very small suspended particles will adsorb ions from the water and thereby acquire a charge. A charge layer will then exist around each particle, preventing coalescence. This charged layer therefore stabilises the particles and produces a finely divided suspension which, for many applications in wet processing is undesirable. Fine suspended solids may be removed by one of the following processes:

- 1 Coagulation, which is brought about by neutralisation of surface charge.
- 2 Flocculation, which is micelle formation by addition of a suitable flocculent.
- 3 Sedimentation, which is the settling of flocculated solids.
- 4 Filtration, which filters out flocculated solids.

Other substances, when present in significant concentrations, may be toxic. Volumes of wastewater and pollution loads are costly to treat; the lower the volume, the lower the cost of treatment. The volume is an important factor in determining the size of treatment plant. River authorities (now the Environment Agency in the UK) have high standards and because these require a high standard of treatment, it pays to reduce the volume.

### 6.4 Treatment of wastewaters from the textile industry

When they reach the manufacturer, cotton fibres contain natural waxes and fats which must be removed before bleaching or colouring matter is introduced to dyed yarn or fabrics. Scouring or kiering removes most of these.

Table 6.2 Typical scouring waste liquor from cotton linters

Properties and constituents	Concentration (mg/dm <sup>3</sup> )
Permanganate value (4 hour)	8850
BOD (5 day)	10650
Ammoniacal nitrogen	52
Total nitrogen	492
Phosphate	35
Potassium	148
Alkalinity (CaCO <sub>3</sub> )	16750

Woven cotton fabric or yarn in the raw state is boiled in solutions of alkali, often under pressure.

Impurities possessed by woven cotton goods include natural impurities like those mentioned above. Size and starch, or various substitutes, are used to facilitate weaving. These can be removed by hydrolysis with acids or enzymes, followed by rinsing, or may be removed during the scouring process.

Scouring liquors tend to be alkaline and contain large amounts of organic matter (see Table 6.2), which are both expensive to treat. The standards of the river authorities, for example the Environment Agency in the UK, are very high.

After scouring, the cotton is bleached using:



Usually, potassium or calcium chlorite, salts or peroxide are used in bleaching. These remove and destroy natural colouring matter. Wastewaters from this process contain partly spent bleaching solution and impurities removed from the fibres. After bleaching, cotton is washed and then immersed in a solution of sodium bicarbonate or weak H<sub>2</sub>SO<sub>4</sub>, which destroys the bleach. Finally, a thorough wash with a soap solution is necessary.

Mercerisation increases lustre and dye affinity. The process involves treating the cotton fabric with sodium hydroxide, NaOH, and then washing with hot water whilst it is under tension. Any residual sodium hydroxide is neutralised with acid and the cotton material is thoroughly washed.

Dyeing of fabric results in large volumes of liquor being discharged. These liquors vary in character, depending on the nature and class of dye used; for example they may be acidic or alkaline and will contain high concentrations of salt and synthetic surface-active agents. Post-dyeing treat-

ments involve the use of a variety of finishes such as starches, gums and waxes, resins and other materials, depending on the required finish, although these tend to be low in concentration because of the large volumes of water that are added during the finishing processes.

Wastewaters from the processing of wool include scour, bleach and dye liquors, plus associated washing waters. The two main differences from cotton wastes are the degree of alkalinity and the nature of the organic matter. Also, waste liquor from wool treatment may be acidic. Scouring wastewaters contain a high proportion of wool grease (lanolin), in an emulsified form, soluble organic matter and sand. Lanolin contains cholesterol and other complex organic substances, which are readily absorbed by the skin and are used in ointments and cosmetics.

Back washing liquor and yarn scouring liquor contain emulsified oils, grease and fatty matter derived from soaps. Emulsions must be centrifuged and then cracked with acid or other chemicals. Cracking is the process of breaking down into smaller molecules which are more amenable to further treatment processes. This aids wastewater treatment by making the soluble components more able to be broken down by biological treatment.

Greasy solids are normally removed by flocculation and filtration prior to mixing with other wastewater. Mixing and balancing waste liquors evens out large variations in composition, temperature and flow. Balancing involves neutralising acid or alkali so that the liquid is in a suitable condition to go forward for secondary treatment.

Waste liquor from wool treatment is similar to that from cotton dyeing but may be acidic. It contains large amounts of wetting agents and softeners plus some salts and potentially toxic materials. These can be inhibitory to biochemical processes, i.e. they retard or tend to prevent naturally occurring biological processes. Waste liquor from the treatment of synthetic fibres contains a variety of organic substances at several hundreds of milligrams per litre many only slowly degraded by special microorganisms.

## 6.5 Effluent treatment

The methods of treatment available are the same whether the effluent is treated on site or at the local sewage treatment works (STW). The various methods of treatment can be categorised under the following headings:

- membrane technologies.
- chemical treatment
- biological treatment

The way in which these treatments work is discussed in the following sections.

### 6.5.1 Membrane technologies

A variety of filters are used to remove the contaminants from textile effluent and these include the following techniques:

- Reverse osmosis is a high-cost treatment where the effluent, under pressure, passes across a semipermeable membrane. A purified permeate of relatively clean effluent and a concentrate of the contaminants are formed which have to be disposed of in some other way. The permeate will still contain some residue of contaminants as approximately 20% of the effluent remains untreated. The membrane needs frequent cleaning which results in additional cost and a more labour intensive process (Cooper, 1995a).
- Nanofiltration involves the use of a membrane as a filter. It retains any materials with a relative molecular mass greater than approximately 200. The permeate may still contain high levels of dissolved inorganic salts and therefore cannot be used for recycling. The concentrate contains all the organic impurities and some of the inorganic ones and will subsequently need further treatment. The need for frequent cleaning, together with high capital costs and high running costs make the units very expensive to run, even more so than reverse osmosis (Cooper, 1995a).
- Ultrafiltration or microfiltration aims to reduce suspended solids and organic materials with a particle size of 0.02 mm or greater. These solids form sludge, which is subsequently disposed of. They have little effect on the reduction of colour and any colour that is removed is adsorbed onto the other materials during removal (Cooper, 1995a). Both microfiltration and ultrafiltration are suitable for reducing chemical oxygen demand (COD) and suspended solids but their use would have to be as part of a multitechnique treatment plant.

### 6.5.2 Chemical treatment

Chemical treatment is usually simply coagulation and flocculation. Under optimum conditions only approximately 50–60% biological oxygen demand (BOD) (organic matter) can be removed by addition of coagulants such as  $\text{Al}_2(\text{SO}_4)_3$  or  $\text{Fe}_2(\text{SO}_4)_3$  and lime. Therefore this process alone will not achieve the standards required by river authorities.

The method is well established as an effective and economic way of treating effluent. A separation process, i.e. sedimentation or flotation, is always used in conjunction with this process. Chemicals are used to form a precipitate, which during formation or settlement adsorbs the colour and other unwanted materials in the effluent. The precipitate is removed as a sludge, which will probably be dewatered to reduce the volume. In the past, inorganic coagulants such as lime, magnesium and iron salts were used to



remove modern dyes but now organic polymers have been developed for more efficient treatment of the colour. These coagulants are not without problems and in some cases colour consent conditions are not met. Some polymers can cause toxicity problems in the discharging effluent. Many of the polymers used are cationic and cause toxicity problems in addition to adverse effects on nitrification in the discharging effluent, which could in turn affect any activated sludge process that the effluent subsequently passes through.

Chemical oxidation may be employed to remove colour from effluent. Strong oxidising agents such as hydrogen peroxide, chlorine, ozone etc. are used to degrade the organic molecules and the dye. This is however a costly treatment (Socha, 1992). Partial oxidation has been tried as an economically viable study but problems lie in the nature of the products formed, which could be more harmful to the environment than the constituents of the original effluent (Cooper, 1995a).

### 6.5.3 Biological treatment

Most materials of animal and vegetable origin can be broken down into simpler compounds such as water and carbon dioxide by microorganisms such as bacteria, fungi, protozoa and so on. This can be done in the presence of oxygen by aerobic degradation or in the absence of oxygen by anaerobic degradation. Both happen as the bacteria and other microorganisms convert the effluent into more acceptable products (Socha, 1992). It is important that the type and concentration of substances, and conditions such as pH, temperature and so on that will kill off the microorganism population are carefully controlled.

Aerobic treatment can be carried out in stabilisation ponds, aerated lagoons, activated sludge or percolating filters. Aerobic treatment uses oxygen dissolved in the wastewater together with microorganisms in the activated sludge to convert the wastes to more microorganisms and CO<sub>2</sub>.

Organic matter is partially oxidised and some of the energy produced is used for making new cells with formation of flocs. The flocs are allowed to settle and then removed as sludge (Laing, 1991). A proportion of the sludge removed is recycled back to the aeration tank to maintain the colony of microorganisms and the remainder of the sludge can either be disposed of or further reduced by anaerobic treatment. Disposal of the sludge can be through agricultural use as a fertiliser, in landfill or by drying and incineration (although, disposal to agriculture is prohibited by law in many countries because of the presence of heavy metals).

Anaerobic treatment occurs in sealed tanks and converts the waste into methane and carbon dioxide. Where nitrogenous and sulphide-containing pollutants are present, ammoniacal substances and hydrogen sulphide are

**Table 6.3** Conditions necessary to ensure no inhibition of biological treatment (Laing, 1991)

Variable	Condition
Temperature	35°C maximum (can be higher for anaerobic treatment)
Ratio BOD: nitrogen	Approx. 17:1
Ratio BOD: phosphorus	Approx. 100:1
pH	6.5–9 (preferably 7 maximum)
Metals (Zn, Cu, Cr)	Less than 10 mg/dm <sup>3</sup>

produced. At some municipal sewage treatment works, the sludge formed by the aerobic treatment process passes into tanks for anaerobic treatment. Considerable heat is produced from anaerobic treatment and after extraction by heat exchangers can be used to heat administration buildings within the sewage treatment works (STW). The methane produced is collected, compressed and then used in generators to produce electricity. The electricity produced can power site processes and the surplus is sold to the national grid. The production of this power not only reduces the running costs of the STW but also provides a welcome income, thus reducing costs further.

For biological treatment to succeed there must be sufficient nitrogen and phosphorous present in the effluent. It may be necessary to add these nutrients in an industrial treatment plant. At a STW where domestic effluent is mixed with industrial effluent, these nutrients should appear in high enough concentrations to keep the microorganism population healthy. Table 6.3 shows other conditions that are necessary to ensure the success of biological treatment.

## 6.6 The use of activated sludge for the removal of colour

The extent of the problem posed by colour depends very much on the class of dye that is used. Some dyes are very much more easily removed from effluent than others. Insoluble vat and disperse dyes can be removed in quite high proportions by primary settlement. Basic and direct dyes respond well to treatment by the activated sludge process but reactive dyes and some acid dyes seem to cause more of a problem. It is generally considered that the activated sludge process removes only low levels of these dyes. Some dyes will respond better to anaerobic conditions than aerobic conditions (Paul *et al.*, 1997). Many dyes are not biodegraded but adsorbed under

aerobic conditions. Studies have found that many azo dyes can be degraded under anaerobic conditions (Cooper, 1995a). Twenty-two water soluble dyes were assessed for degradability under anaerobic conditions. Results showed that all except one of the anthraquinone acid dyes, CI Acid Blue 80, showed signs of significant colour removal.

Conclusions were drawn and suggestions made that these dyes were likely to be broken down in the environment by anaerobic conditions. Concerns were raised that the aromatic amines formed during this process could prove more toxic to the aquatic environment than the intact dye molecule. ETAD (The Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers), which was formed in 1974 to represent the interests of these industries on matters relating to health and environment, investigated the anaerobic degradation of simple aromatic amines and found they were unlikely to be persistent (Cooper, 1995a). Trials were carried out in simulated landfill conditions using sludge contaminated by azo dyes and no evidence of dye in the leachate was found nor any amines that would occur as metabolic byproducts (Laing, 1991).

Today's consumers demand textiles that are fast, not only to washing but also to light and perspiration. Much research has produced dyes with structures that are much more stable to such conditions than in the past. This stability also makes microbial attack more difficult, limiting their biodegradability.

It has long been known that the ease of elimination of acid dyes from effluent was directly related to the solubility of the dyes (Hitz *et al.*, 1978). The more sulphonic acid groups that are present in the structure of the dye, the more soluble and therefore less responsive to treatment is the dye by the activated sludge process. The removal of acid dyes by bioelimination, which is adsorption onto the biomass rather than biodegradation, was found to be related, not to the number of sulphonic acid groups (and thereby the solubility), but to the size of the dye molecule. It is thought that the greater the molecular size, the greater the degree of adsorption (Cooper, 1995b).

Early colour removal researchers found in their study that medium to high rates of colour removal were obtained for the disperse dyes (Hitz *et al.*, 1978). The removal of water-soluble basic and direct dyes is good although this is probably by adsorption onto the biomass rather than biodegradation. The reactive dyes showed little evidence of colour removal, which bore no relationship to the number of sulphonic acid groups or the ease of hydrolysis. No vat dyes were used in this study because of the more complicated procedures needed to prepare the insoluble vat dyes. It would be expected that much of the insoluble disperse and vat dyes could be removed by primary sedimentation prior to any treatment. It was found in this study (Hitz *et al.*, 1978) that reactive dyes respond poorly to the activated sludge process.

Adsorption onto the biomass is poor, with a maximum of 30% colour removal, and biodegradation is virtually non-existent.

In a more recent study (Binkley *et al.*, 1998), different results were obtained. It was found that using an activated sludge obtained from a conditioned textile effluent treatment plant, a removal rate well in excess of the 30% previously mentioned was obtained. In this study removal rates well in excess of 80% were found using the conditioned activated sludge.

A broad range of microorganisms is needed in the activated sludge process to treat the diverse range of pollutants found in industrial and domestic effluent that passes through the STW prior to discharge to the watercourse. At the STW the constituents of the incoming wastewater can change considerably on an hourly or daily basis.

The industrial on-site effluent treatment plant has a more consistent quality to its effluent. The range of pollutants is much more specific to particular industrial processes and it is therefore known on a daily basis what the effluent will contain. The diversity of the microorganism population is probably much less than that in a municipal STW and therefore more specialised. As a result, the sludge will be conditioned to the chemicals normally found in the effluent. Consequently, any shock loading with foreign pollutants can severely hamper the smooth running of the plant. This is also the case for municipal STW plants.

The precise composition of diverse microorganisms used in the treatment of wastewater is difficult to define. A complete ecosystem is developed which feeds on components of the incoming effluent and each other. The composition of this ecosystem will depend on the environment created by such conditions as pH, temperature and the availability and variety of the food (Horan, 1991).

The largest component of the population in this mix of microorganisms is the bacteria. Bacteria are very small, the majority having diameters of 0.2–1.5  $\mu\text{m}$  and are very difficult to see using an optical microscope although a microscope with a good resolving power will allow identification of some of the shapes of the larger bacteria. Staining techniques, using a dye, can make the bacteria easier to see and identify, and the whole microorganism or just a selected part of it can be coloured.

Four different shapes of bacteria can be identified: sphere, straight rod, curved rod and spiral.

The sphere can be found singly (coccus), in pairs (diplococci) or in a chain (streptococci). The straight rods are the most commonly found bacteria and include the frequently seen genera such as *Pseudomonas*, *Zooglea*, *Escherichia* and *Salmonella*. The curved rods (vibrio) are a single curve whereas the spirals (spirilla) (normally only found in water) can vary from one complete turn to many turns and can measure from 0.5–60  $\mu\text{m}$  in length (Horan, 1991).

Protozoa are also an important component of this microbial mix. They are single cell organisms which can be found in several forms. Some are well known as pathogens, i.e. they are agents of disease and death, but most are harmless. Some are parasitic but most live quite freely in all types of habitat. Protozoa prove to be very valuable in activated sludge treatment plants. They are more common in aerobic than in anaerobic treatment (Curds, 1992).

The larger protozoa are clearly visible under the microscope making identification a lot easier than the bacteria. Most common protozoa vary in size from 5–250  $\mu\text{m}$  although some very rare ones have been found as large as 6 mm in length (Pechenik, 1991). Three types of protozoa can be found in wastewater treatment processes. These are:

- *Mastigophora* (flagellated protozoa), which possess one or more flagella that are used to assist in feeding and motility, giving them the ability to move spontaneously under their own power. They can be subdivided into two classes, *phytomastigophora* – algae fall into this class – and *zoomastigophora*, most commonly found in activated sludge plants.
- *Sarcodina* (amoeba), which have pseudopodial structures that are involved in feeding and the locomotion of the organism. Amoeba can be naked, lacking any skeletal structure or testate with shells composed of proteinaceous, silicaeous or carbonaceous material.
- *Ciliophora* (the ciliates), which are the largest in terms of species. A characteristic of these is the arrangement of cilia over the surface of the cell, used for purposes of locomotion. The ciliates can be subdivided into four groups:
  - i *holotrichia* – free swimming, cilia all over the body
  - ii *spirotrichia* – flattened body with cilia on the underside
  - iii *peritrichia* – inverted bell-shaped bodies, mounted on a stalk, with cilia arranged around the top of the body. The stalk anchors the organism to a sludge floc.
  - iv *suctoria* – cilia in early life; they develop a stalk and feeding tentacles later (Horan, 1991).

Some protozoa feed on bacteria and other organic matter while others prey on smaller protozoa and as such are considered predators. Also present in this microbial mix are rotifers and nematodes.

- Rotifers are simple multicellular invertebrate animals, 50–250  $\mu\text{m}$  in length. They have a ring of cilia surrounding the mouth, which sweep bacteria and other organic matter in. Some rotifers swim but others move by a creeping motion. The body anchors itself to a floc and can be seen stretching out from the floc surface. They are quite widespread and are desirable for the two roles they play in an activated sludge process. Rotifers remove freely suspended bacteria, i.e. non-flocculated bacteria,

and they make a contribution to the floc-forming process by producing faecal pellets surrounded by mucus (Bitton, 1994).

- Nematodes are unsegmented cylindrical worms, 0.5–3 mm in length. They feed on bacteria and other microorganisms. They are often found in the activated sludge process but it is thought that they have little to do with the process of decreasing COD (Henz *et al.*, 1995).

Organic and inorganic particles together with bacterial cells form the constituents of an activated sludge floc. The size of flocs can range from less than 1 µm to 1000 µm (Bitton, 1994). The flocculation process brings together primary particles to form a floc. The primary particles are very small, too small to settle out and if unable to flocculate will remain as suspended solids in the settlement tanks. As the floc size increases, the number of active aerobic bacteria will decrease and anaerobic bacteria such as methanogens take over in the inner regions of the floc (Bitton, 1994). It has been suggested that the structure of an activated sludge floc can be explained as filamentous microorganisms forming the backbone of the floc to which zoogaea and floc-forming microorganisms attach themselves. Some flocs do not have this filamentous backbone; therefore a different theory has been proposed suggesting that some of the activated sludge microorganisms produce polymers that are responsible for floc formation (Bitton, 1994). Although these are just two of the theories put forward, the exact composition and structure of the sludge flocs is uncertain (Henz *et al.*, 1995). Dense and fairly regular flocs with evidence of protozoa amongst them are a sign that the biomass is in a healthy state. Mistreatment of the activated sludge will result in the protozoa disappearing and disaggregation of the flocs into their primary particles. It is only when these primary particles are brought together that the flocs are formed and are able to settle out of the process for disposal. Some form of agitation is necessary to ensure that the primary particles meet up but the flow rate should not be high enough to inhibit settlement. In plants with a high flow rate, flocculation is poor, a high degree of suspended solids is found and turbidity of the effluent is evident. It therefore takes the skill of the operative to maintain the correct level of agitation.

## **6.7 Decolourisation – by enzymes, fungi, and by biosorption and enrichment cultures**

### **6.7.1 Biotechnological methods**

For microbial dye decolourisation not connected to the activated sludge process, bioreactors can be designed containing defined bacterial or fungal cultures, isolated enzymes or enzyme mixtures immobilised on a solid

carrier material. Such immobilised biocatalysts are much more susceptible to damage by harsh chemical environments than are the mixed cultures present in activated sludge systems. Thus, they are preferentially used to treat partial process streams within the plant where the composition and physical properties are rather more readily controlled than the overall plant effluent.

For instance, exhausted dyeing bath solutions have been decolourised enzymatically using a laccase formulation and subsequently reused for the preparation of new dyeing baths (Abadulla *et al.*, 2000). Similarly, hydrogen peroxide has been removed enzymatically from bleaching solutions using a catalase in an industrial pilot experiment (Paar *et al.*, 2001). Owing to the use of high-specificity enzymes, only the target molecules are attacked while valuable additives or fibres are kept intact and can be reused.

### 6.7.2 Enzymes

Because enzymes are protein molecules they do not metabolise dyes but they do catalyse a specific type of transformation with them. Mineralisation of dyes can never be expected with enzymes. However, enzymatic modification of dyes may often be sufficient at a certain stages in the process to destroy chromophores and reduce toxicity. Additionally, enzymatic modification can render the dyes more susceptible to subsequent biodegradation in a municipal wastewater treatment plant where complete elimination takes place.

Although dye molecules display a high structural variety, they are degraded by only a few different enzymes. These biocatalysts have one common mechanistic feature: They are all redox-active molecules and thus exhibit the desired broad substrate specificities.

In the early 1980s, there were reports about a specific enzyme responsible for azo dye cleavage (Zimmermann *et al.*, 1982, Zimmermann *et al.*, 1984). This enzyme was called an azoreductase. The authors had isolated a bacterial strain capable of degrading the azo dye Orange II. Subsequently they succeeded in the isolation and characterisation of a single protein that catalysed the azo bond reduction with remarkable specificity towards the dye used for the long-term adaptation of the organism with remarkable specificity towards Orange II. The reduction of the azo compound took place in the presence of oxygen, which is especially notable since normally anaerobic conditions are required.

Since then, a number of reports have been published where azo dye decolourisation has been linked to some azoreductase activity of the described organisms. However, the difficulties in finding organisms that can grow solely on azo dyes as carbon and energy sources showed that such

azoreductase activity often is a secondary metabolic effect caused by the action of unspecific reductive enzymes such as cofactor-dependent oxidoreductases or cytochrome P450 reductases.

Moreover, azo dye reduction does not necessarily depend on the action of enzymes at all. They may simply be reduced by the reductive environment generated within an organism. Thus, cleavage of azo bonds under anaerobic conditions is often due to unspecific reduction processes mediated by redox-active compounds such as quinone-type substances, biochemical cofactors such as NADH or reduced inorganic compounds such as  $\text{Fe}^{2+}$  or  $\text{H}_2\text{S}$  which are formed by certain strictly anaerobic bacteria as metabolic end products. As a consequence, types of dyes, other than azodyes, that are susceptible to reduction can be transformed as well. In contrast, the azoreductase described by Zimmermann *et al.* (1984) displays very restricted substrate specificity and is limited to a very narrow range of structurally related azo dyes.

Generally, in the presence of oxygen, intracellular mono- and di-oxygenases that are ubiquitously present in living organisms may transform dyes in the course of secondary metabolism. They cause the breakdown of aromatic rings via incorporation of oxygen atoms (biohydroxylation) and subsequent cleavage of the ring system resulting in carboxylic acids, which are further used in metabolism (Fig. 6.1).

The enzymes mentioned so far are dependent on cofactors such as NAD(P)(H) or FAD(H). They are never used in isolated form but only within whole cell systems where the cofactors are regenerated continuously. For technical applications of single enzymes or enzyme cocktails less complicated systems are required.

In terms of enzyme remediation of textile dyes, laccases seem to be the most promising enzymes. Laccases are polyphenoloxidases which accept a wide range of aromatic alcohols and amines as substrates. Their major advantage lies in that there are no expensive cofactors required. Only oxygen (i.e. air) needs to be present as a cosubstrate. Laccases have been shown to decolourise a wide range of industrial dyes and in the presence of small amounts of low molecular weight redox mediators their versatility can be extended even more (Reyes *et al.*, 1999). Not only azo dyes are attacked by laccases. Various other types of dyes are also attacked. For example, indigoid dyes are decolourised very efficiently by laccases from various fungi. As shown in Fig. 6.2, indigo (1), the most important dye in the manufacturing of blue jeans, was demonstrated to be cleaved under laccase catalysed electron transfer to give isatin (2) and upon further decarboxylation anthranilic acid (4) as the final stable oxidation product. It was suggested that the degradation might proceed via dehydroindigo as a reaction intermediate (Campos *et al.*, 2001). This process is used industrially to achieve the stonewashed effect of indigo-dyed denim fabric by means of milde enzymatic decolourisation.







### 6.7.3 Fungal decolourisation

Both types of oxidative enzymes described previously, laccases and peroxidases, are predominantly generated by white-rot fungi (one of their natural functions being to degrade lignin), a complex aromatic matrix in wood. The fact that laccases and peroxidases are excreted by the fungi makes the latter organisms themselves interesting for application in bioremediation. As living whole-cell systems they are suitable for use as end-of-the-pipe solutions as an alternative to activated sludge processes. Various white-rot fungi like *Phanerochaete chrysosporium*, *Trametes versicolor* or *Pleurotus ostreatus* have been employed, immobilised on various supports. Fluidised-bed reactors, for instance, which utilise fungi, contain freely-mobile pellets covered with layers of immobilised biomass, while packed-bed reactors contain organisms that are fixed onto a suitable support material (Zhang and Yu, 2000). Packed-column reactors and rotating drum reactors using fungal biofilms have also been constructed (Kapdan *et al.*, 2000). In all cases, the fungal mycelia are grown on the solid support directly in the reactor. By addition of various types of nutrient, the efficiency of the systems can be manipulated. For an informative compendium of the literature describing fungi used to decolourise dyes, see the review of Fu and Viraraghavan (2001).

Although both immobilized fungi and isolated enzymes have a good potential for the treatment of process waters in the textile industry, enzyme reactors display one major advantage over whole-cell systems; with the latter, enzymes are produced *in situ*. Thus, the concentration of the active compound, which is always an enzyme, is limited by the growth of the living organism. In contrast, with isolated enzymes appropriate amounts can simply be dispensed. To support fungal growth, nutritional compounds must be added while in parallel other substances are generated in addition to the desired biocatalyst. This may lead to unwanted side-effects and, at least, will incorporate new loadings of chemicals into the effluent. Furthermore, enzyme expression and thus its secretion by a fungus is dependent on its metabolic state and therefore presents far from constant release into the medium over time.

### 6.7.4 Decolourisation by biosorption

A prominent method for removing colour from effluents is to adsorb coloured particles physically onto various materials like charcoals, clays, soils, diatomaceous earth, activated sludge, compost, living plant communities, synthetic polymers or inorganic salt coagulants (Slokar and Marechal, 1998). If biomass is used for such an adsorption, the process is called biosorption.

Colour removal via biosorption is usually achieved by adsorption on fungal mycelia. Fungal cells for biosorption applications may either be used as growing cells or in the form of dead biomass; thus decolourisation can take place with or without concomitant biodegradation. Not surprisingly, decolourisation with active biomass is usually much more effective owing to parallel digestion (Aretxaga *et al.*, 2001). In the former case, the problem of elimination of waste dyestuff is not actually solved since the recalcitrant molecules are still present and have to be treated in a subsequent step.

Azo dyes have been shown to bind effectively onto the mycelium of *Aspergillus niger* resulting in extensive colour removal at greater than 95% (Sumathi and Manju, 2000). A stationary culture of this fungus was also used to decolourise a complex wastewater from a textile company by an airlift bioreactor over a relatively wide range of pH values. Between pH 3 and 7 there was 100% decolourisation; with pH 12 still about 60%. Thus, the process displays a high tolerance towards harsh changes in the pH. Furthermore, it does not seem to be limited to a certain type of dye. Acid, basic, direct, reactive and disperse dyes are reported to be removed from solution within a couple of hours (Assadi and Jahangiri, 2001).

Pellets consisting of activated carbon and mycelium of *Trametes versicolor* were used for textile dye decolourisation by Zhang and Yu (2000). Combining biodegradation with adsorption, high decolourisation rates could be achieved as was also reported for a system using bacteria and carbon black as a carrier material (Walker and Weatherly, 1999).

Biosorption on agricultural residues was suggested by Nigam and co-workers as a first step prior in microbial treatment to concentrate dyes (Robinson *et al.*, 2001). Here, dyestuff is first adsorbed on a waste product that later serves as a growth substrate for solid-state fermentation of a suitable fungus. This seems to be a very promising alternative, since in a first step, very rapid decolourisation of the effluent takes place by physical means compared to the rather slow decolourisation by growing organisms which may take up to a couple of days until satisfactory decolourisation is achieved. This slow step of fermentation of dyes is carried out separately, when time plays a less significant role. Moreover, the growth conditions and thus the decolourisation activities are optimal for white-rot fungi during solid-state fermentation. Comparable decolourisation rates could never be achieved with the fungi growing in the effluent.

### 6.7.5 Enrichment cultures

Enrichment cultures are populations of microorganisms or single organisms which have developed a certain property via natural adaptation. In general, enrichment of microorganisms with special effectiveness in dye digestion occurs at any site where dyes are present in large amounts. Dye

decolourising microorganisms can hence be obtained simply by isolation of existing dye-degrading cultures from environmental samples such as textile effluents.

Applying methods of directed evolution, adaptation of promising strains to the conditions present in textile effluents is possible as well. Under chemostat (an apparatus for growing bacterial cultures at a constant rate by controlling the supply of nutrient medium) conditions, enrichment of bacteria capable of growing on dye molecules as the only carbon source takes very long periods of time; from several months up to years. Alternatively, employing genetic methods, DNA encoding for enzymes involved in dye metabolism can be transferred and new organisms with desired properties can be tailor-made.

Several enrichment cultures have been described which have been optimised for special requirements. For instance, thermophilic bacteria, selected by adaptation from a textile effluent, have been shown to decolourise textile dyes at temperatures up to 60°C. One isolate of this mixed culture was able to decolourise commercial azo, diazo, reactive and disperse dyes (Banat *et al.*, 1997).

Complete mineralisation of dyes under various conditions by using enrichment cultures has also been accomplished. A methanogenic consortium was found to detoxify aromatic amines formed during the prior azo reduction step resulting in the complete mineralisation of an azo dye under strict anaerobic conditions. This mixed population was grown on the amines as the sole nitrogen sources (Razo-Flores *et al.*, 1997) and the azo dye azodisalicylate was continuously degraded in a bioreactor for more than 100 days.

Complete mineralisation of an azo compound by an isolated aerobic bacterial strain has also been successful. Via continuous adaptation of *Hydrogenophaga palleronii* S1, a strain was developed growing on 4-carboxy-4'-sulphoazobenzene as the sole source of carbon and energy (Blümel *et al.*, 1998).

## 6.8 References

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