Table 1.27. Comparative strengths of wastewaters from food-processing industries.

	BOD (mg l^{-1})	COD (mg l^{-1})	PV (mg l^{-1})	Suspended solids (mg l^{-1})	pH	Population equivalent per m^3 of waste
Brewery	850	17000	—	90	4–6	14.2
Cannery						
citrus	2000	—	—	7000	Acid	33.3
pea	570	—	—	130	Acid	9.5
Dairy	600–1000	—	150–250	200–400	Acid	10.0–16.7
Distillery	7000	10000	—	Low	—	116.7
Farm	1000–2000	—	500–1000	1500–3000	7.5–8.5	16.7–33.3
Silage	50000	—	12500	Low	Acid	833.3
Potato processing	2000	35000	—	2500	11–13	33.3
Poultry	500–800	600–1050	—	450–800	6.5–9.0	8.3–13.3
Slaughterhouse	1500–2500	—	200–400	800	7	25.0
Sugar beet	450–2000	600–3000	—	800–1500	7–8	7.5–33.3

the BOD (2,000–15,000 mg l^{-1}), total nitrogen (800–900 mg l^{-1}) and phosphate (20–140 mg l^{-1}), almost entirely present in the dissolved or colloidal fractions with the suspended solids content rarely in excess of 200 mg l^{-1}. Slaughterhouse and meat packing wastewaters are strong and unpleasant, being comprised of faeces and urine, blood washings from carcasses, floors and utensils, and the undigested food from the paunches of slaughtered animals. These wastewaters are high in dissolved and suspended organic matter, in particular proteins and fats, high in organic nitrogen and grease, as well as pathogens.

The strengths and volumes of wastewaters from the main food-processing industries are summarised in Table 1.27.

1.3. Micro-organisms and Pollution Control

Micro-organisms have a number of vital functions in pollution control. It is the microbial component of aquatic ecosystems that provides the self-purification capacity of natural waters in which micro-organisms respond to organic pollution by increased growth and metabolism (Sec. 1.4.1). It is essentially the same processes which occur in natural waters that are utilised in biological treatment systems to treat wastewater. Apart from containing food and growth nutrients, wastewater also contains the micro-organisms themselves, and by providing a controlled environment for optimum microbial activity in a treatment unit or reactor, nearly all the organic matter present can be degraded (Chap. 3). Micro-organisms utilise the organic matter for the production of energy by cellular respiration and for the synthesis of protein and other cellular components in the manufacture of new cells. This overall reaction of wastewater treatment can be summarised as:

$$\text{Organic matter} + \text{O}_2 + \text{NH}_4^{2+} + \text{P} \longrightarrow \text{New cells} + \text{CO}_2 + \text{H}_2\text{O}$$

Similar mixed cultures of micro-organisms are used in the assessment of wastewater and effluent strength by the biochemical oxygen demand test (BOD_5), in which the oxygen demand exerted by an inoculum of micro-organisms growing in the liquid sample is measured over five days to give an estimate of the oxidisible fraction in the wastewater (Sec. 1.4.2). Many diseases are caused by waterborne micro-organisms, a number of which are pathogenic to man. The danger of these diseases being transmitted via wastewater is a constant threat to public health (Chap. 9). Therefore the use of micro-organisms, such as *Escherichia coli*, as indicator organisms to assess the microbial quality of water for drinking, recreation and industrial purposes, as well as in the assessment of wastewater treatment efficiency is an essential tool in pollution control (Sec. 9.2).

1.3.1. *Nutritional classification*

In wastewater treatment, it is the bacteria that are primarily responsible for the oxidation of organic matter. However, fungi, algae, protozoa (collectively known as the Protista) and higher organisms all have important secondary roles in the transformation of soluble and colloidal organic matter into biomass, which can be subsequently removed from the liquid by settlement prior to discharge to a natural watercourse. In order to function properly, the micro-organisms involved in wastewater treatment require a source of energy and carbon for the synthesis of new cells as well as other nutrients and trace elements. The micro-organisms are classified as either heterotrophic or autotrophic according to their source of nutrients. Heterotrophs require organic matter both for energy and as a carbon source for the synthesis of new micro-organisms, whereas autotrophs do not utilise organic matter but oxidise inorganic compounds for energy and use carbon dioxide as a carbon source.

Heterotrophic bacteria, which are also referred to as saprophytes in older literature, utilise organic matter as a source of energy and carbon for the synthesis of new cells, respiration and mobility. A small amount of energy is also lost as heat during energy transfer reactions. The heterotrophs are subdivided into three groups according to their dependence on free dissolved oxygen.

Aerobes require free dissolved oxygen in order to decompose organic material:

$$\text{Organics} + O_2 \xrightarrow{\text{aerobic micro-organisms}} \text{Aerobic micro-organisms} + CO_2 + H_2O + \text{energy}$$

Like all microbial reactions it is autocatalytic, that is the micro-organisms that are required to carry out the reaction are also produced. Aerobic bacteria predominate in natural watercourses and are largely responsible for the self-purification process (Sec. 1.4.1). They are also dominant in the major biological wastewater treatment processes such as activated sludge and percolating filtration. Aerobic processes are biochemically efficient and rapid in comparison with other types of reactions, producing by-products that are usually chemically simple and highly oxidised such as carbon dioxide and water.

Anaerobes oxidise organic matter in the complete absence of dissolved oxygen by utilising the oxygen bound in other compounds such as nitrate:

$$\text{Organics} + NO_3 \xrightarrow{\text{anaerobic micro-organisms}} \text{Anaerobic micro-organisms} + CO_2 + N_2 + \text{energy}$$

or sulphate:

$$\text{Organics} + SO_4^{2-} \xrightarrow{\text{anaerobic micro-organisms}} \text{Anaerobic micro-organisms} + CO_2 + H_2S + \text{energy}$$

Anaerobic bacterial activity is found in freshwater and estuarine muds rich in organic matter and in the treatment works in the digestion of sludge. Anaerobic processes are normally biochemically inefficient and generally slow, giving rise to chemically complex by-products which are frequently foul-smelling (Chap. 7). The end products of proteins, carbohydrates, and fats which have undergone microbial breakdown under anaerobic and aerobic conditions are summarised in Table 1.28. Facultative bacteria use free dissolved oxygen when available but in the absence of oxygen are able to

Table 1.28. End-products of the aerobic and anaerobic microbial breakdown of the major organic substrates found in sewage (Berthouex and Rudd 1977).

		Enzymes of		Representative end-products	
Substrates	+	micro-organisms	→	(Anaerobic conditions)	(Aerobic conditions)
Proteins and other organic nitrogen compounds		Enzymes of micro-organisms		Amino acids	Amino acids
				Ammonia Hydrogen sulphide Methane Carbon dioxide	Ammonia→nitrites→nitrates Hydrogen sulphide→sulphuric acid $\left.\begin{array}{l}\text{Alcohols}\\\text{Organic acids}\end{array}\right\}\rightarrow CO_2+H_2O$
				Hydrogen Alcohols Organic acids Phenols Indol	
Carbohydrates		Enzymes of micro-organisms		Carbon dioxide Hydrogen Alcohols Fatty acids Neutral compounds	$\left.\begin{array}{l}\text{Alcohols}\\\text{Fatty acids}\end{array}\right\}\rightarrow CO_2+H_2O$
Fats and related substances		Enzymes of micro-organisms		Fatty acids + glycerol Carbon dioxide Hydrogen Alcohols Lower fatty acids	Fatty acids + glycerol $\left.\begin{array}{l}\text{Alcohol}\\\text{Lower fatty acids}\end{array}\right\}\rightarrow CO_2+H_2O$

gain energy anaerobically and so are known as facultative aerobes. An example of a facultative bacterium is *E. coli*, a common and important coliform, this and other such bacteria are common in both aerobic and anaerobic environments and treatment systems. Often, the term obligate is used as a prefix to these categories of heterotrophic bacteria to indicate that they can only grow in the presence (obligate aerobe) or absence of oxygen (obligate anaerobe).

Using these basic reactions as guides, it is possible to write balanced equations for the utilisation of the organic substrate and the synthesis of new micro-organisms. For example, using glucose as the organic substrate and the formulae $C_5H_7O_2N$ to represent the composition of the organisms, equations for respiration and the production of energy for cell maintenance and synthesis can be written:

$$\text{Respiration: } C_6H_{12}O_6 + O_2 \longrightarrow 6CO_2 + H_2O + \text{energy}$$

while the equation for synthesis of new micro-organisms is:

$$\text{Synthesis: } C_6H_{12}O_6 + NH_4^+ \longrightarrow C_5H_7O_2N + 3H_2O + H^+$$

These two 'half-reactions' can be combined to give the basic organic transformation reaction brought about by aerobic micro-organisms in biological wastewater treatment plants and which is discussed in more detail in Sec. 3.1.

$$C_6H_{12}O_6 + 0.5NH_4^+ \longrightarrow C_5H_7O_2N + 3.5CO_2 + 5H_2O + O.5H^+$$

Bacteria are comprised of 80% water and 20% dry matter. Of the dry matter, 90% is organic and the remainder is inorganic. Hoover and Porges (1952) used the equation $C_5H_7O_2N$ to describe the organic fraction of bacteria in wastewater with 53% of the weight of the organism assumed to be organic carbon. More comprehensive equations have been formulated to describe the chemical composition of bacteria, for example the one used by Mara (1974) which takes into account the phosphorus content of bacterial cells, $C_{60}H_{87}O_{25}N_{12}P$. The remaining 10% of the cells are comprised of phosphorus (50%), sulphur (15%), sodium (11%), calcium (9%), magnesium (8%), potassium (6%), and iron (1%). As all these inorganic elements are required for microbial growth, any deficiency will result in growth limitation or inhibition.

The amount of energy biologically available per unit of organic matter broken down by heterotrophs depends on the oxygen source used. The greatest yield of energy comes from the use of dissolved oxygen in oxidation, while least energy is from strict anaerobic metabolism. With a mixed culture of micro-organisms, as is found in wastewater treatment, the micro-organisms seek the greatest energy yield in order to achieve maximum synthesis. This is illustrated by the microbial activity which occurs when an organically enriched water is put into a closed container. At first, aerobic and facultative bacteria will decompose organic matter, gradually depleting the dissolved oxygen. After all the dissolved oxygen is exhausted, the facultative bacteria continue to use oxygen bound as nitrate and sulphate. At this state, other facultative and anaerobic bacteria begin to break down the organic matter to organic acids and alcohols which produce least energy:

$$\text{Organics} \longrightarrow \text{Organic acids} + CO_2 + H_2O + \text{energy}$$

If methane forming bacteria are present, then the anaerobic digestion process is completed by converting the organic acid to methane and carbon dioxide:

$$\text{Organic acids} \longrightarrow CH_4 + CO_2 + \text{energy}$$

Autotrophic bacteria cannot utilise organic matter, instead they oxidise inorganic compounds for energy and use carbon dioxide or carbonate as a carbon source. There are a number of autotrophs in the aquatic ecosystem, however only the nitrifying, sulphur and iron bacteria are particularly important in wastewater oxidation. The nitrifying bacteria oxidise ammonia nitrogen in a two step reaction, initially to nitrite, which is unstable, and finally to nitrate.

$$NH_4^+ + \text{oxygen} \xrightarrow{\textit{Nitrosomonas}} NO_2^- + \text{energy}$$

$$NO_2^- + \text{oxygen} \xrightarrow{\textit{Nitrobacter}} NO_3 + \text{energy}$$

The reaction occurs in secondary treatment units although it is very sensitive to environmental conditions, occurring most efficiently at low organic loadings and warm temperatures (Sec. 3.5.2).

In sewers, hydrogen sulphide is given off by sulphate reducing bacteria if the wastewater becomes anaerobic. The slightly acidic gas is absorbed into condensation water which collects on the top or crown of the sewer or on the side walls. Here, sulphur bacteria, which are able to tolerate pH levels of 1.0 oxidise the hydrogen sulphide to strong sulphuric acid using atmospheric oxygen:

$$H_2S + \text{oxygen} \xrightarrow{\textit{Thiobacillus}} H_2SO_4 + \text{energy}$$

The sulphuric acid reacts with the lime in the concrete to form calcium sulphate, which lacks structural strength. Gradually the concrete pipe can be weakened so much that it eventually collapses. Crown corrosion is particularly a problem in sewers which are constructed on flat gradients, in warm climates, in sewers receiving heated effluents, with wastewaters with a high sulphur content or in sewers which are inadequately vented. Corrosion-resistant pipe material such as vitrified clay or PVC plastic, prevents corrosion in medium size sewers, but in larger diameter sewers where concrete is the only possible material, corrosion is reduced by ventilation which expels the hydrogen sulphide and reduces condensation. In exceptional circumstances, the wastewater is chlorinated to prevent sulphate-reducing bacteria forming hydrogen sulphide or the sewer is lined with a synthetic corrosion-resistant coating.

Not all species of iron bacteria are strictly autotrophic, however, those that are can oxidise inorganic ferrous iron to the ferric form as a source of energy:

$$\mathrm{Fe}^{2+}\,(\text{ferrous}) + \text{oxygen} \xrightarrow{\textit{Leptothrix}} \mathrm{Fe}^{3+}\,(\text{ferric}) + \text{energy}$$

The bacteria are filamentous and deposit oxidised iron ($Fe(OH)_3$) in their sheath. They occur mainly in iron rich mine wastewaters but can also occur in biological wastewater treatment units. For example, they are common in percolating filters which treat domestic effluents receiving infiltration water from coal mining areas and so are rich in iron (Gray 1980). If the domestic water supply contains dissolved iron, the bacteria can become established in water pipes, forming yellow or reddish-brown slimes and tainting the water as the mature bacteria die.

Autotrophs derive energy from either sunlight (photosynthetic) or from inorganic oxidation-reduction reactions (chemosynthetic). Chemoautotrophs do not require external sources of energy but utilise the energy from chemical oxidation, while phototrophs require sunlight as an external energy source:

$$\mathrm{CO_2} + \mathrm{H_2O} \xrightarrow{\text{Photosynthetic micro-organisms}} \text{Phototrophs} + \mathrm{O_2}$$

Free dissolved oxygen is essential for the aerobic processes of heterotrophic and autotrophic bacteria. When aerobic organisms utilise organic nutrients, they consume dissolved oxygen at the same time. Each molecule of glucose, which is the basic building block of all carbohydrates, requires six molecules of oxygen for complete conversion to carbon dioxide and water by aerobic bacteria:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{bacteria}} 6CO_2 + 6H_2O$$

There is also a considerable oxygen demand during the nitrification of nitrogenous compounds by autotrophic nitrifying bacteria:

$$2NH_4 + +3O_2 \xrightarrow{\text{bacteria}} 2NO_2 + 4H^+ + H_2O$$

$$2NO_2 + O_2 + 2H^+ \xrightarrow{\text{bacteria}} 2NO_3^- + 2H^+$$

If the dissolved oxygen is not replaced, then aerobic growth will eventually stop when the oxygen is exhausted, allowing only the slow anaerobic processes to continue. Microbial activity is not only oxygen-limited in the case of aerobic micro-organisms, it is also restricted by the availability of adequate supplies of carbon, nutrients such as nitrogen and phosphorus, trace elements and growth factors. It is the actual composition of micro-organisms that controls the nutrient requirements of organisms, and as proteins are composed mainly of carbon, nitrogen and smaller amounts of phosphorus, it is these three elements which are essential for microbial growth. The requirements of carbon, nitrogen and phosphorus by microbial cultures in wastewater treatment processes is expressed as a ratio (C:N:P) and if the waste is deficient in any one of these basic components, complete utilisation of the wastewater cannot be achieved.

Many inorganic ions, mainly metals, are required to ensure that bacterial enzymatic reactions can occur. Therefore, trace amounts of calcium, magnesium, sodium, potassium, iron, manganese, cobalt, copper, molybdenum and many other elements are required. These are found in adequate amounts in sewage, as are growth factors such as vitamins. However, if any of these materials are deficient or absent, then microbial activity will be restricted or may even stop (Jefferson *et al.* 2001).

The mixed microbial cultures found in biological wastewater treatment units degrade and subsequently remove colloidal and dissolved organic substances from solution by enzymatic reactions. The enzymes are highly specific, catalysing only a particular reaction and are sensitive to environmental factors such as temperature, pH, and metallic ions. The major types of enzyme-catalysed reactions in wastewater biochemistry are:

Oxidation	the addition of oxygen or the removal of hydrogen;
Reduction	the addition of hydrogen or the removal of oxygen;
Hydrolysis	the addition of water to large molecules which results in their breakdown into smaller molecules;

Deamination	the removal of an NH_2 group from an amino acid or amine; and
Decarboxylation	the removal of carbon dioxide.

Microbial energetics, metabolism, population and community dynamics are fully explored in Chap. 3.

1.4. Microbial Oxygen Demand

It is important to know how much oxygen will be required by micro-organisms as they degrade organic matter present in wastewater for two reasons: (a) to ensure that sufficient oxygen is supplied during wastewater treatment so that oxidation is complete and (b) to ensure receiving waters do not become deoxygenated due to the oxygen demand of these micro-organisms, which results in the death of the natural fauna and flora. The amount of organic matter that a stream can assimilate is limited by the availability of dissolved oxygen. This is largely determined by the rate oxygen is utilised by microbial oxidation and the rate at which it can be replaced by reaeration and other processes.

1.4.1. *Self purification*

The term self-purification is defined as the restoration, by natural processes, of a river's natural clean state following the introduction of a discharge of polluting matter. In natural river systems, organic matter is assimilated by a number of processes which include sedimentation which is enhanced by mechanical and biological flocculation, chemical oxidation, and the death of enteric and pathogenic micro-organisms by exposure to sunlight. Of course, the assimilative capacity of rivers, i.e. the extent to which the river can receive waste without significant deterioration of some quality criteria, usually the dissolved oxygen concentration, varies according to each river because of available dilution, existing quality and the rate of the self-purification capability (Benoit 1971). The most important process in self-purification is biochemical oxidation, i.e. the aerobic breakdown of organic material by micro-organisms. Biodegradable organic matter is gradually eliminated in rivers due mainly to bacterial action, by methods very similar to those occurring in wastewater treatment. Complex organic molecules are broken down to simple inorganic molecules in a process requiring oxygen. In this process of self purification, it is the attached micro-organisms, collectively known as periphyton, that are normally responsible for the greatest