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Biotechnology and Effluent Treatment

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Introduction

Effluent treatment is the largest and one of the most important controlled applications of micro-organisms in the manufacturing industries. *Table 1* compares the quantity of sewage treated on an annual basis with other biological and non-biological products in the UK. The Department of the Environment reports that Water Authority spending on effluent treatment and pollution control in the UK in 1978 was £1100 million (Water Data Unit, 1979). It is European Community policy (Ellington and Burke, 1981) that spending on pollution should continue to rise in accordance with increased expectations of environmental quality and inflation. A US Government technology report (Congressional Office of Technology Assessment, 1981) estimated that municipal, agricultural and industrial spending on pollution control will be $400 000 million between 1976 and 1986. The size and impact of this market for the combined use of microbiology, biochemistry and engineering will be second only to fine chemicals in the foreseeable future (Bull, Holt and Lilly, 1982).

Table 1. Quantities of sewage effluent treated compared with other common industrial products (UK figures) (modified from Dunnhill, 1981).

Product	Weight (tonnes/year)	Price (£/t)
Water as sewage	6000×10^6	0·10
Milk	16×10^6	25
Steel	12×10^6	300
Beer	$6{\cdot}6 \times 10^6$	280
Sugar	1×10^6	350
Cheese	$0{\cdot}2 \times 10^6$	1300
Bakers yeast	$0{\cdot}1 \times 10^6$	460
Citric acid	$0{\cdot}015 \times 10^6$	700
Penicillin	$0{\cdot}003 \times 10^6$	45 000

Abbreviations: ADF, alternating double filtration; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; RBC, Rotating Biological Contacter; TOC, total organic carbon; UASB, Upflow Anaerobic Sludge Blanket.

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The traditional aims of effluent treatment have been to reduce the concentration of organic matter in waste water which otherwise exerts an oxygen demand expressed as BOD (biological oxygen demand), and to decrease the number of potential pathogens in the waste. This allows the effluent to be discharged into the environment without adverse effect.

The recent discoveries of how to alter the characteristics of micro-organisms by genetic manipulation, combined with new advances in reactor design, offer new opportunities for waste treatment technology, and broader aims for environmental biotechnology can now be identified such as (1) the recycling of materials for economic self-defence, (2) conservation of resources and (3) management of environmental problems. Not all of these strategic goals can be stimulated by normal market forces but the economic deficit can be rectified by legislation on pollution, government subsidy for conservation of vital resources and as a consequence of disruption in existing supplies. In Italy, for example, effluent treatment plant to recover biogas from wastes attracts a 70% grant because the country is short of indigenous energy (Alfani, 1983). This chapter reviews existing effluent treatment, emphasizing processes available for by-product recovery, and discusses the future prospects for environmental biotechnology for further recovery of useful materials and the breakdown of recalcitrants (used here in the sense of a persistent organic compound).

The characteristics of waste

Waste water is treated to prevent any adverse effects on the receiving water and to allow reuse. The polluting load is measured by a range of analyses to assess its physical, chemical and biological effects on the environment. The biggest problem with domestic, agricultural and a wide range of industrial wastes (i.e. food, drinks, and fermentation wastes) is caused by their organic content. The metabolism of this organic matter by the natural organisms contained in the receiving water causes rapid oxygen depletion and elimination of the normal aquatic flora and fauna. The organic content of waste water is determined by three tests: of chemical oxygen demand (COD), total organic carbon (TOC), and biological oxygen demand (BOD). COD and TOC are measures of total organic carbon determined by chemical oxidation and pyrolysis (with infra-red absorption of the carbon dioxide formed) respectively. BOD is the biologically oxidizable fraction measured by incubation over 5 days. These are standardized tests and the methods and procedures are described in several texts (Department of the Environment, 1972; American Public Health Association, 1975, Department of the Environment 1979). The amount of suspended solids, determined by filtration and turbidity tests, is a parameter which is used to assess the effects of pollution from wastes on light penetration in streams (which controls algal growth) and the amount of precipitation on to the river bed (which affects bottom fauna and fish eggs).

The ammonia (as nitrogen) content of wastes is also normally measured. In domestic wastes nitrogen is derived from urea and the deamination of protein. Residual ammonia is directly toxic to aquatic life and its oxidized products can cause nutrient enrichment and excessive algal growth or eutrophication.

An additional complication arises if the waste water is recycled for water supply: nitrate combines with haemoglobin and can be toxic to young growing children. Nitrogen is, however, an essential additional nutrient for any biological treatment process. *Table 2* compares the character and strength of a typical domestic agricultural and industrial waste water.

Table 2. A comparison of some different types of waste water.

Characteristics	Type of waste water		
	Domestic	Agricultural	Industrial
pH	7·8	8·5	4·5
BOD (mg/ℓ)	370	15 000	8000
COD (mg/ℓ)	670	25 000	17 370
TOC (mg/ℓ)	219	N.K.	N.K.
Total solids (mg/ℓ)	1309	22 000	3200
Suspended solids (mg/ℓ)	146	14 500	570
Ammonia (as N) (mg/ℓ)	46	2000	0·5

N.K., not known.

A detailed characterization of sewage has been carried out by Painter and co-workers (Painter 1958, 1971; Painter and Viney, 1959; Painter, Viney and Bywaters, 1961), who have identified 75% of the organic nutrient present. They found that 30–40% of the total organic material present was in solution; 10–15% was present as colloidal solids (0·1–1 μm); 20–25% as supra-colloidal solids (1–100 μm); and 30–35% as settleable solids ($> 100\,\mu$m). The single largest organic constituents in both solution and suspension were the fatty acids, representing about 30% of the total organic carbon. The next largest constituents were the sugars (about 15% of the total organic carbon). It is probable that the sugars are progressively converted into the fatty acids and that the amount of fatty acid varies according to the age of the sewage. The total fat and grease content of sewage is 40–100 mg/ℓ and all the fatty acids up to C20 have been identified in sewage, including those with odd numbers of carbon. The most common are the simple acids acetic, butyric and propionic derived

Table 3. Concentration of inorganic materials (mg/ℓ) in domestic sewage.

Element	Soft water	Hard water
Cl	20·1	68
Si	3·9	N.K.
Fe	0·8	0·8
Al	0·13	N.K.
Ca	9·8	109
Mg	10·3	6·5
K	5·9	20·0
Na	23	100
Mn	0·47	0·05
Cu	1·56	0·2
Zn	0·36	0·65
Pb	0·48	0·08
S	10·3	22·0
PO_4 as P	6·6	22·0

N.K., not known.

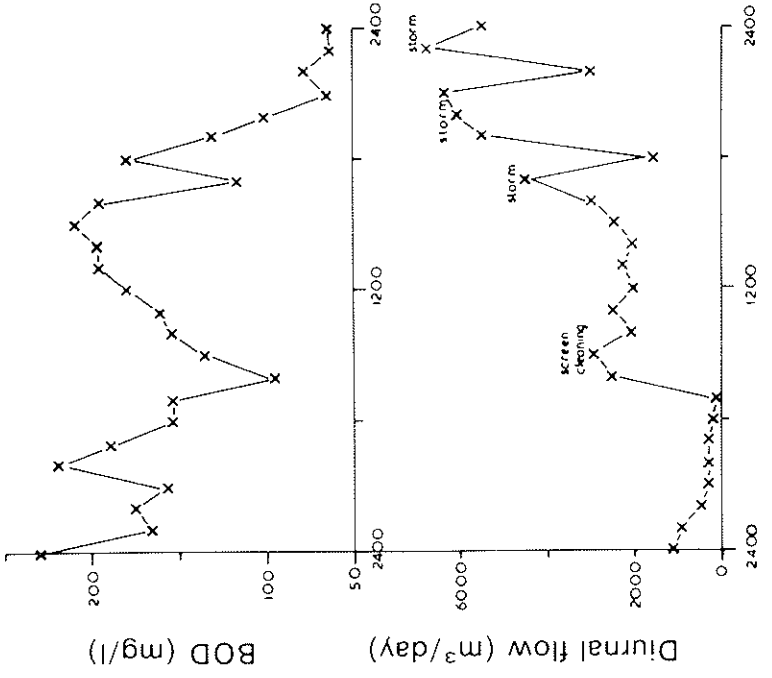

Figure 1. Diurnal flow and BOD for domestic sewage (typical day in early summer). Flow monitored at sewage works on standard meter calibrated in m^3/day.

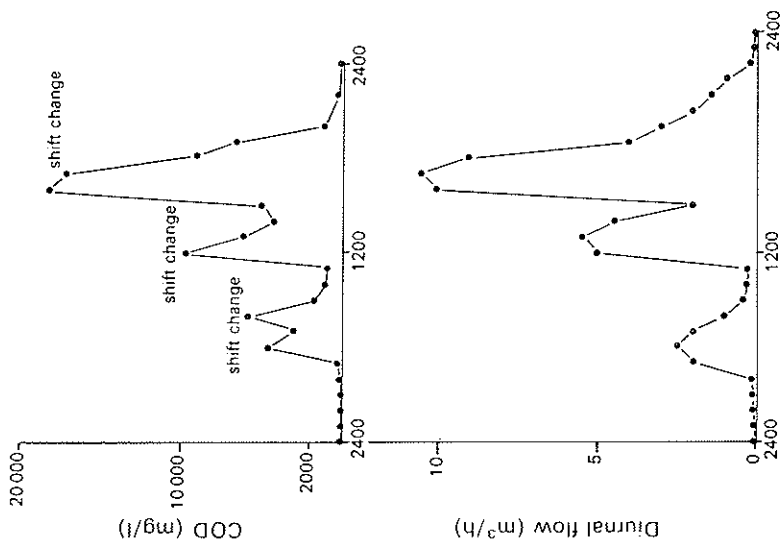

Figure 2. Diurnal flow and COD for an industrial waste (typical midweek production day).

from the simple sugars. Glucose, sucrose and lactose are the predominant sugars, followed by galactose, fructose, xylose and arabinose; most of the sugar is glucose. Carbohydrate in suspension is mainly cellulose and starch.

The inorganic substances present in waste waters are influenced by the characteristics of the water supply, as well as by the nature of any industrial processing. The differences between soft- and hard-water domestic sewage are shown in *Table 3*.

There are also major changes in the flow and strength of waste waters according to the diurnal patterns of behaviour and production. *Figures 1 and 2* show the diurnal changes in flow and strength of a domestic and an industrial waste (Wheatley and Williams, 1976; Wheatley and Cassell, 1983). Changes of up to 300% of average are common with domestic works when affected by storm run-off. The flows never stop completely, because of the infiltration of ground water.

Present treatment technology

Waste waters are normally complex in composition, and treatment plants need to acquire a diverse range of micro-organisms with the metabolic capacity to degrade various types of wastes and to allow the effluent to be discharged to the environment without adverse effect. Effluent treatment depends on mixed-culture fermentation but also includes higher grazing organisms so that a complete ecosystem is formed with various trophic levels. There are two common types of fermenter—plug-flow fixed-film systems (referred to as biological or percolating filters) and completely mixed flocculant processes or activated sludge (*Figure 3*).

Most effluents are heterogeneous and contain both dissolved and suspended matter. The primary step in biological treatment is, therefore, adsorption of the substrate on to the biological surface. This is then followed by a sequence of of steps with the breakdown of the adsorped solids by extracellular enzymes, the absorption of dissolved materials into cells, growth, endogenous respiration, release of excretory products and the ingestion of the primary population by secondary grazers (*Figure 4 and 5*). The artifical ecosystem can be in complete balance in such a way that all the available substrate and subsequent primary growth are consumed or leave the system. Thus the only products of waste treatment are simple inorganic salts and gases.

The earliest type of treatment was the percolating filter, which developed directly from experiments carried out on the percolation of sewage through soil. The first biological filters were built at Salford in 1890 and despite nearly 100 years of use, the current basic design is virtually unchanged. They are circular or rectangular tanks made from brick or concrete containing a graded bio-support medium—usually clinker, slag, stone or gravel, depending on local availability. The components of the medium are normally 30–100 mm in size and the packed depth 1·8 m. Biological filters are classified by the organic and hydraulic load which they receive (*Table 4*). They are easy to maintain, incur low running costs, produce only small quantities of surplus biomass and have a plant life of 30–50 years. The process has proved to be very reliable

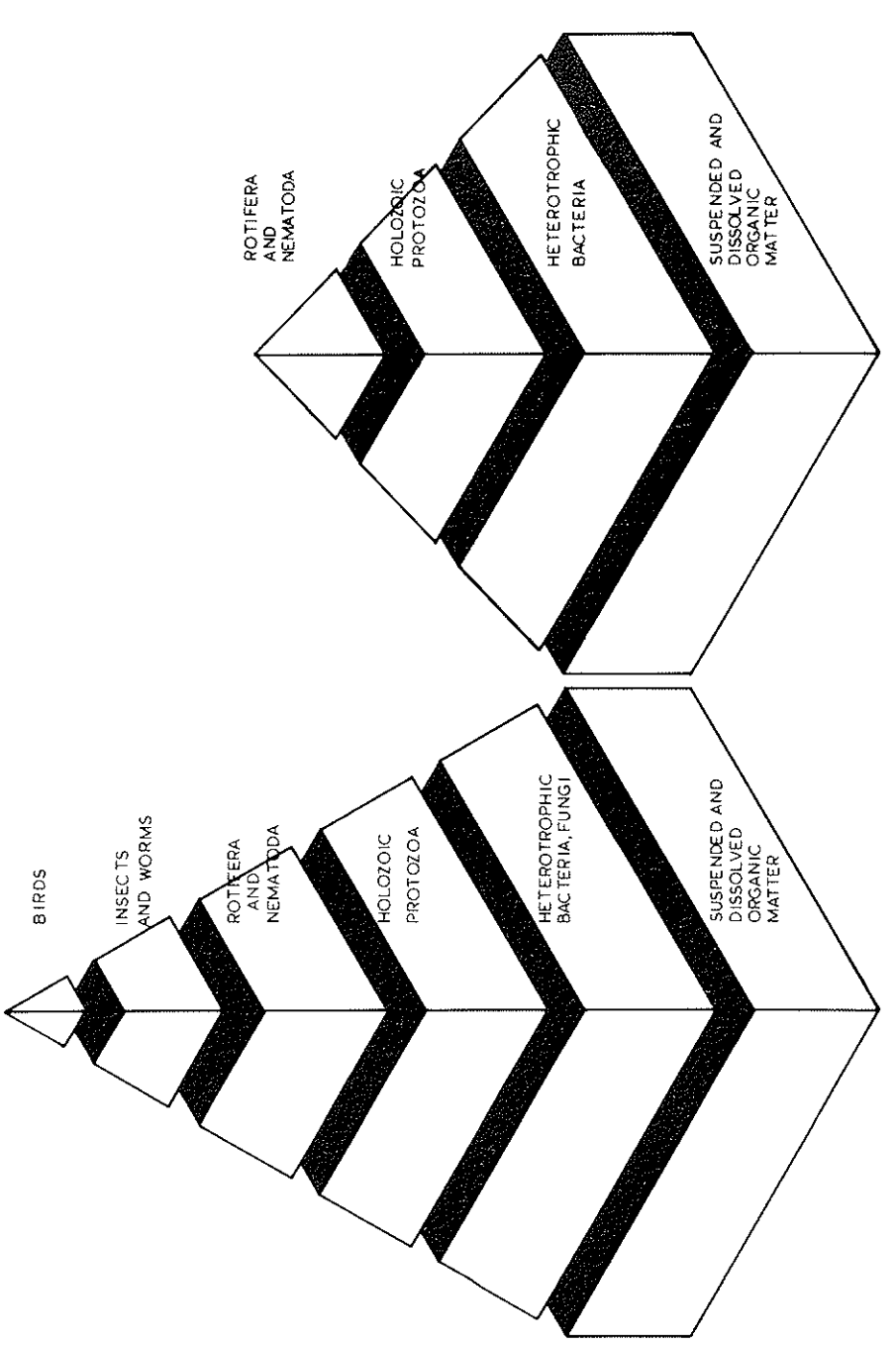

Figure 3. Diagram of the main food links in biological waste treatment.

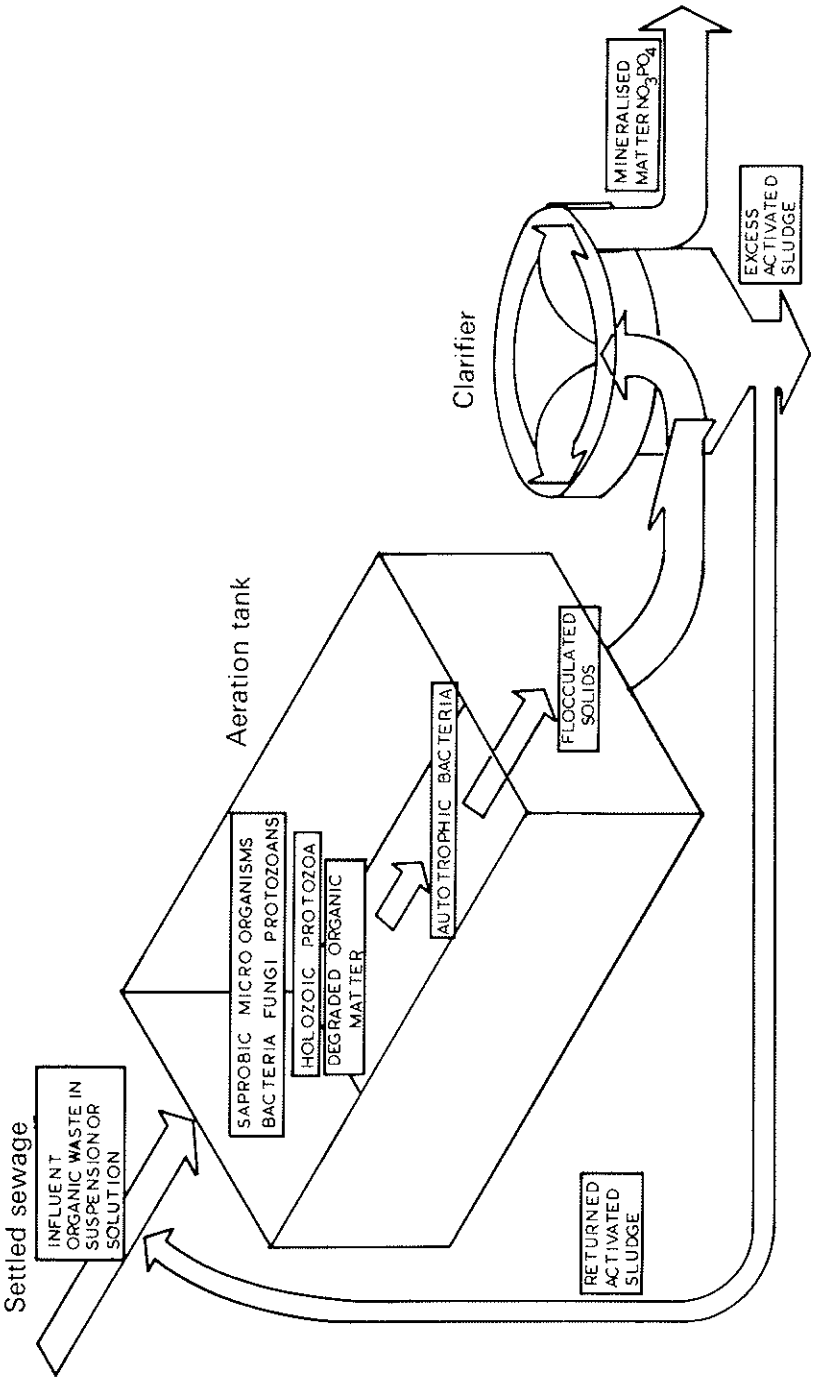

Figure 4. Mass transfer processes in activated sludge.

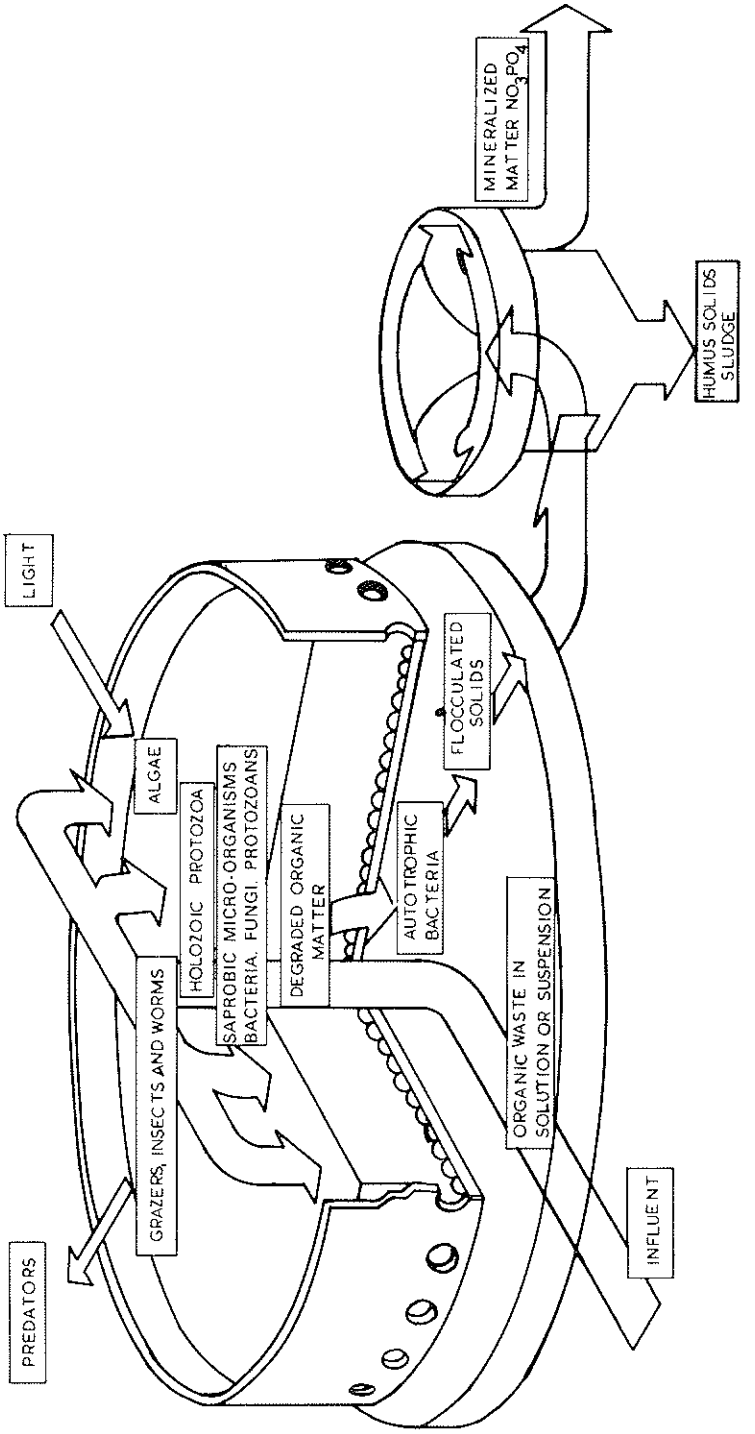

Figure 5. Mass transfer processes in biofiltration.

Table 4. Biological filter loadings.

Type of filter	BOD load (kg/m^3 of media)	Efficiency of BOD removal (%)	Sludge conversion (%)
Septic tank systems	0·05	95	5
Low-rate percolating	0·05–0·1	95	15–20
Mid-range double filtration, ADF, recirculation	0·1–0·5	85–90	20–50
High rate filtration	2·0–6·0	50–70	50–90

and it is estimated that 70% of European and American effluent treatment plants use this type of system.

The most common alternative is the activated sludge process which is a completely mixed process, aerated and stirred by bottom diffusion or surface agitation; it was first used at Manchester in 1914. The incoming screened degritted settled waste is aerated with active sludge or bios recycled from a settling tank. The residence time in the aeration tank is 4–10 hours, after which the final effluent is separated from the active sludge by settlement (1 hour) and most of the sludge is returned to mix with the incoming waste (*Figure 5*). The process is more intense than that of biological filters and is able to treat approximately 10 times the effluent per volume of reactor and is therefore much cheaper to build. It is, however, more difficult to operate and maintain, incurs high running costs through mixing and aeration, and produces large quantities of surplus biomass or sludge.

The extra operational difficulties result both from increased mechanical plant and from a reduced ecological trophic diversity. Growths of fungi or filamentous bacteria, both of which are natural organisms in polluted waters and normal components of biological filter film, seriously reduce the density of the activated sludge bios and interfere with the settlement and return of the active sludge to the aeration tank. A second effect of the reduced ecological diversity seems to be a reduction in the resilience of activated sludge (compared with that of biological filters) to shock or variable organic loads. Despite these operational difficulties, and because of the large areas of land required for equivalent biological filtration, activated sludge has been the preferred type of treatment for loads from population equivalents of more than 50 000. Normal BOD loads are 0·2–0·5 kg/kg dry biomass for a 90% removal of BOD (*Table 5*). The majority of effluent in the UK is treated by activated sludge; *Table 6* shows the size of the largest treatment works.

Table 5. Activated sludge loadings.

Type of plant	BOD load per kg MLSS* (kg)
Extended aeration	0·02–0·06
Conventional load	0·2–0·5
High rate	1·5–2·0

* Mixed liquor suspended solids.

Table 6. Sewage works in England, Wales, Northern Ireland and Scotland in 1978 with a theoretical dry-weather flow greater than 100 000 m^3/day.

Sewage treatment works	Water Authority or River Purification Board	Dry-weather flow (000's m^3/day)	Population (000's)
Beckton	Thames	912	2250
Crossness	Thames	505	1550
Minworth	Severn Trent	384	1050
Davyhulme	North West	314	714
Mogden, Isleworth	Thames	439	1330
Deephams	Thames	171	685
Stoke Bardolph	Severn Trent	146	465
Blackburn Meadows	Yorkshire	128	466
Derby (Raynesway)	Severn Trent	108	210
Knotstrop (High Level)	Yorkshire	150	481
Finham	Severn Trent	112	339
Maple Lodge	Thames	113	434
Avonmouth	Wessex	160	500
Rye Meads	Thames	78	322
Dalmuir	Clyde	219	440
Dalmarnock	Clyde	136	300
Belfast (Duncrue Street)	Northern Ireland	110	530

Source: Water Data Unit, 1979.

TREATMENT PLANT DESIGN

The basis of aerobic treatment design is to provide an environment in which the waste, the organisms responsible for purification, and the air, are brought into contact. Treatment efficiency is, in principle, therefore proportional to the amount of biomass and contact time between waste and biomass. Normally the limiting factor is aeration efficiency: excessive biomass rapidly occludes the voidage in biological filters and exerts too great an oxygen demand in activated sludge. Early work (Dunbar and Calvert, 1908; Royal Commission on Sewage Disposal, 1908) rapidly established empirically the amounts of biomass, the concentrations of waste and the aeration or ventilation required to ensure a treated effluent suitable for river discharge. Improvements followed as the understanding of the process grew. O'Shaughnessy (1931) conducted some early experiments on growth rate and noted that BOD removal was first order: dilution of the waste reduced the amount of biomass generated. Several practical methods of controlling biological treatment evolved from this early appreciation of the kinetics. A common problem at that time was excessive biological growth in filters, thus restricting both ventilation and effluent flow. This led to a deterioration in performance and ultimately, if the filter blocked, complete failure. It affected biological filters mainly in the winter when film autolysis and grazing activity was low. Wishart and Wilkinson (1941) described some experiments in which they successfully controlled biomass by washing the filter with biologically treated effluent. They then suggested a scheme of alternating double filtration (ADF) where the waste normally applied to two filters in parallel was instead applied to two filters in series. Biomass accumulates in the 1st filter as it receives and consumes virtually all of the applied substrate. The order of the filters is then reversed before the bios in the first filter prevents the free

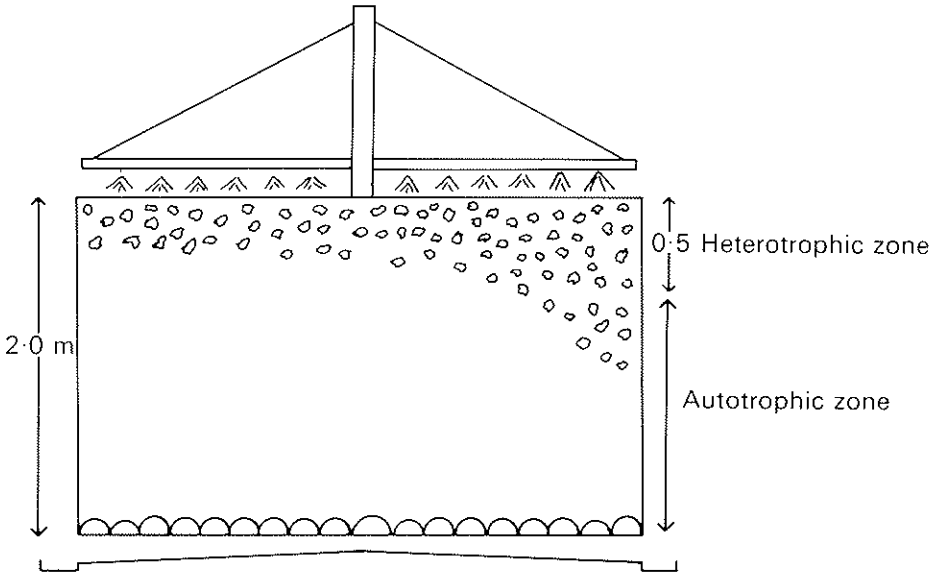

Figure 6. Ecological zones in biological filtration.

flow of waste and air through it. The original primary filter then receives treated effluent which has passed through the new primary filter. This results in the rapid autolysis and consumption of the starved biomass. ADF enables filters to be operated at more than twice the normal load with few operational problems. Additional experiments by Mills (1945a) established that the best frequency of alternation was weekly, and Tomlinson (1946) reported on the effects on ecology. ADF is now extensively used, particularly with industrial wastes. Mills (1945b) noted another change regarding the distribution of biomass through the depth of the filter. Normally the biomass was in two distinct zones because the system was plug flow and the surface of the filter received and removed the greatest amount of applied substrate. Thus most of the active organic-consuming organisms are in this upper 0·5 m (*Figure 6*), known as the heterotrophic layer. Below this is the autotrophic zone, which is about 1·5 m deep. The bios material in the base of the filter occurs in flocculated deposits rather than as prostrate slime adhering closely to the support media, as in the case of surface growth (Wheatley, 1981) (*Figure 7*). The material is the debris from the primary population or the surface micro-organisms. There is very little BOD removal in these lower layers but considerable autotrophic nitrification. Higher rates of instantaneous irrigation such as those from ADF, recirculation and intermittent dosing are thus used to increase the efficiency of biological filtration by dissipating the load deeper into the filter. Tomlinson (1946) showed that 62% of the biomass occurred in the top 0·5 m of a conventional filter, whereas there was only 44% in the top of an ADF filter. This improves total BOD removed by making more efficient use of the filter media but normally reduces the nitrifying activity. Dilution of the incoming waste by recirculation is also used with activated sludge and biological filters: the strength of the waste

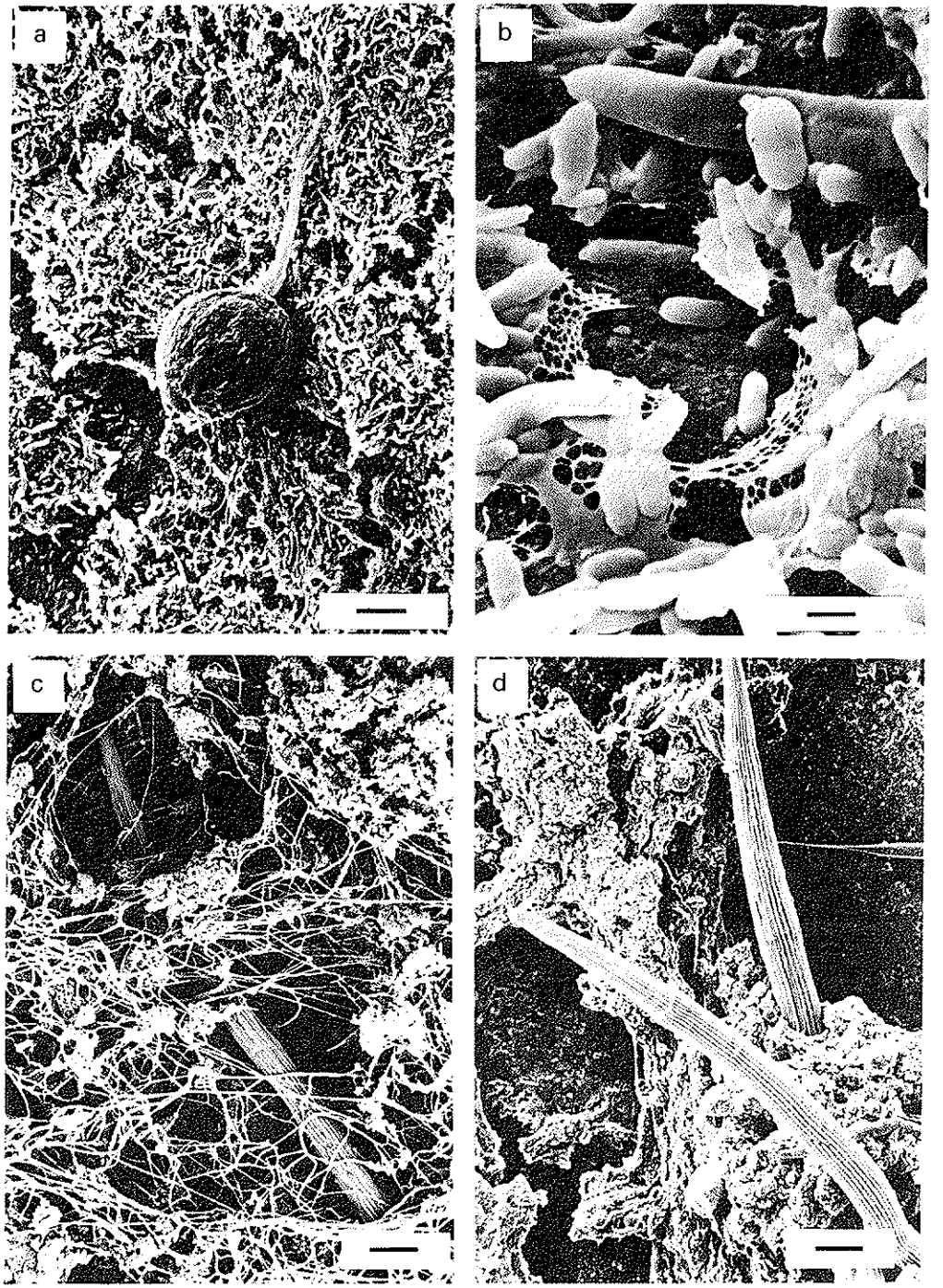

Figure 7. Electron micrographs of biological growth adhering to support media. (a) Bacterial growth and stalked protozoan *Opercularia* from a filter treating domestic sewage (surface growth) (bar = 1 μm); (b) Higher magnification of bacterial growth showing bacterial cells and extracellular polymer (bar = 0·73 μm); (c) Fungal growth from a biological filter treating dairy waste (also shown is a nematode worm) (bar = 22·5 μm; (d) Humus solids and debris from the base of a biological filter (two nematode worms are also visible) (bar = 22·5 μm).

is diluted, important trace nutrients are returned and the effluent is reaerated (Mills, 1945b; Lumb and Eastwood, 1958; Lumb, 1960). Other work (Lumb and Barnes, 1948; Tomlinson and Hall, 1955; Hawkes and Shephard, 1972) established that slowing down the distributor on biological filtration also improved performance by encouraging an even spread of biomass through the filter depth. One of the more recent techniques was direct double filtration with the first filter of a series of two containing a larger medium (150–300 mm) to accommodate more biological growth but at a lower BOD removal efficiency (about 70%). The second filter contains conventional media and completes the removal of organic waste to 95%. Overall loading is higher. Different combinations of processes are now also used, such as high-rate activated sludge followed by filters, or high-rate filters followed by activated sludge (Tebbutt, 1971). These are often simple extensions to existing plant. Similar modifications have been made to the activated sludge process to even out energy and aeration demand through the tank. A common method of operation is tapered aeration, which is designed to match oxygen demand to aeration capacity. Based on a plug flow through the aeration basin, the oxygen demand at the outlet is much less than that at the inlet, so that aeration can be progressively reduced along the length of the tank. This is normally accomplished by a reduction in the number of diffuser domes in the base of the tank. A similar modification, more applicable to surface aeration, is step aeration which introduces the waste at intervals throughout the length of the tank. Aeration of the return sludge without addition of further waste is also used to encourage the organisms to utilize any stored nutrient. The activated sludge is then able to assimilate a greater amount of substrate or waste when re-introduced into the main treatment tank. This process is known as contact stabilization.

ECOLOGY

The ecology of waste treatment by open mixed culture is complex and there are problems in differentiating between the organisms that are actively growing and those organisms that can be isolated from the fermenters. There is a fundamental difference between the two processes: activated sludge is a truly aquatic environment, whereas the biological filter contains only a thin film of water over the surface of the bios. The bacteria are the basis of both processes and the Zoogloea (Gram-negative, non-sporing, non-motile capsulated rods) are normally referred to as being the major group responsible for treatment (Wattie, 1943; Hawkes, 1963) (*Figures 8* and *9*). There are still some doubts as to whether Zoogloea are a defined group or a strain of *Pseudomonas* which develop a slime layer because of the particular conditions under which they are grown (McKinney, 1956). It is assumed (Pike and Carrington, 1972; Pike, 1975) that only a small proportion of the sludge floc and slime is actively growing but most of the bios could still carry out biochemical reactions by extracellular enzymes and adsorption of organic matter on to the floc. A number of other bacteria have also been shown to be active in waste-water treatment processes (*Chromobacter*, *Achromobacter*, *Flavobacter*, *Arthrobacter*) (Pike, 1975). Work has also been carried out on the filamentous bacteria because of their association

Figure 8. Common bacteria and algae in effluent treatment processes. (a) *Sphaerotilus* (actual filament diameter 2–3 μm); (b) *Leptothrix* (filament diameter 1–2 μm); (c) *Phormidium* (filament diameter 5 μm); (d) *Ulothrix* (filament diameter 15–17 μm); (e) *Zoogloea* (cells 0·5–1·0 μm in diameter and 1–2 μm long; finger-like projections normally approx. 50 μm in diameter).

with poorly operating activated sludge plants. The four common filamentous bacteria that have been identified and characterized are *Beggiatoa*, *Sphaerotilus*, *Leptothrix* and *Nocardia* (Eikelboom, 1975; van Veen, Mulder and Deinema, 1978; Dhaliwal, 1979; Beccari, Mappeili and Tandoi, 1980). A wide variety of enteric organisms, including some potential pathogens, can be detected in fresh sewage, but they have no role in the treatment process and their numbers fall rapidly (Pike and Carrington, 1979) (*Table 7*). The organisms responsible for treatment are derived from aerial inoculation and via infiltration water.

Six filamentous fungi have also been shown to grow actively in waste-water treatment systems: these are *Fusarium*, *Geotrichum*, *Subbaromyces*, *Saprolegnia*,

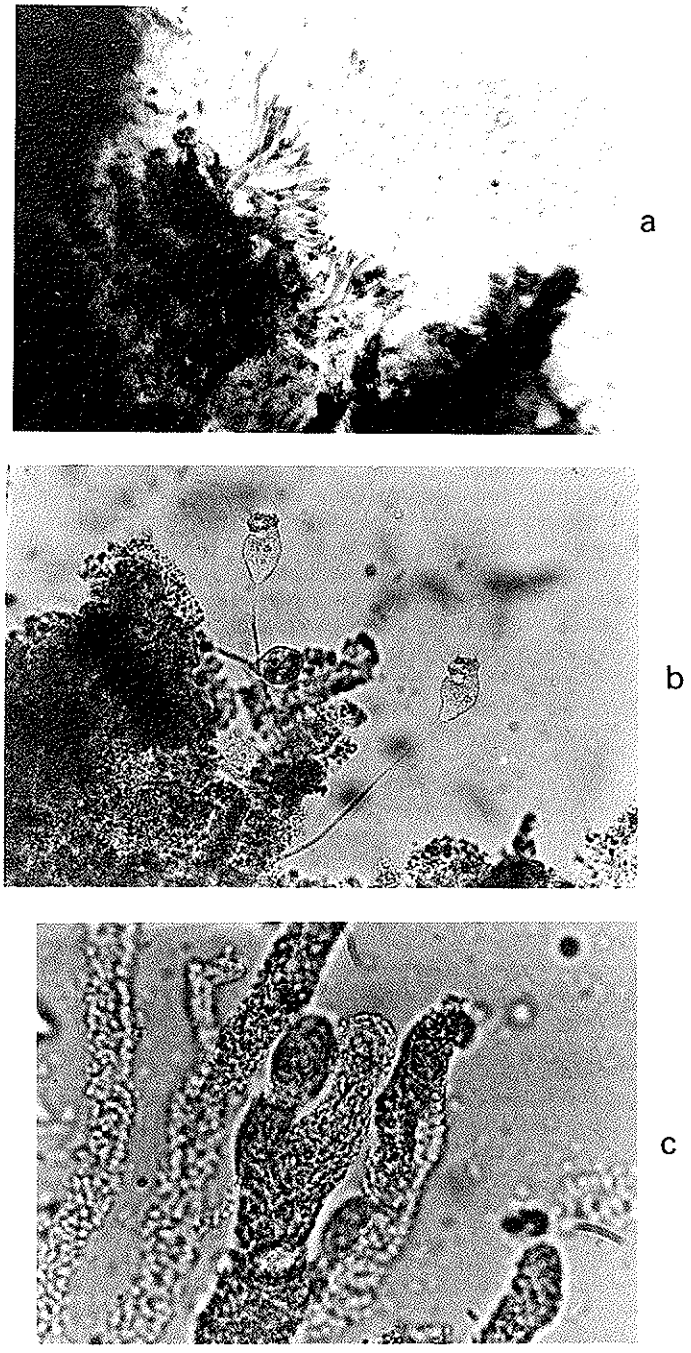

Figure 9. *Zoogloea* and *Sphaerotilus* bacteria growing in activated sludge. (a) Showing distinct finger-like projections (50 μm in diameter) of zoogloea; (b) Cells of zoogloea and peritrichous protozoa (*Vorticella*, cell approx. 50–90 μm in length); (c) Higher magnification of finger-like projections of zoogloea.

Table 7. Summary of pathogen removal by various sewage treatment processes†.

Organisms	Parameters*	Primary sedimentation	Trickling filter with primary and secondary sedimentation, sludge digestion and sludge drying	Activated sludge with primary and secondary sedimentation, digestion and sludge drying	Oxidation ditch with sedimentation and sludge drying	Septic tanks	Land application or slow sand filtration as tertiary treatment	Anaerobic digestion (30–40°C)
Enteric viruses	A	10^3–10^5	10^3–10^5	10^3–10^5	10^3–10^5	0–10^9	10–10^4	May survive for over 3 months
	B	10^3–10^5	10^2–10^4	10–10^4	10–10^4	10–10^3	0–10^2	
	C	0–30	90–95	90–99	90–99	50	99–100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated		
Salmonellae	A	10^3–10^4	10^3–10^4	10^3–10^4	10^3–10^4	0–10^9	10–10^3	May survive for several weeks
	B	10^2–10^3	10^2–10^3	10–10^3	10–10^3	0–10^8	0/1	
	C	50–90	90–95	90–99	90–99	50–90	100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated		
Shigellae	A	10^3–10^4	10^3–10^4	10^3–10^4	10^3–10^4	0–10^9	10–10^3	Unlikely to survive for more than a few days
	B	10^2–10^3	10^2–10^3	10–10^3	10–10^3	0–10^8	0/1	
	C	50–90	90–95	90–99	90–99	50–90	100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated		
E. coli	A	10^6–10^8	10^6–10^8	10^6–10^8	10^6–10^8	10^7–10^9	10^4–10^7	May survive for several weeks
	B	10^5–10^7	10^5–10^7	10^4–10^7	10^4–10^7	10^6–10^8	0–10^3	
	C	50–90	90–95	90–99	90–99	50–90	99·99–100	
	D	Contaminated	Contaminated	Contaminated	Contamined	Contaminated		
Cholera vibrio	A	10–10^3	10–10^3	10–10^3	10–10^3	0–10^9	0·1–10^2	May survive for 1 or 2 weeks
	B	1–10^2	1–10^2	0·1–10^2	0·1–10^2	0·10^8	0/	
	C	50–90	90–95	90–99	90–99	50–90	100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated		

Leptospires	A	Very few	Very few	Very few	Very few	Very few	Very few	Survive for not more than 2 days
	B	Very few	Very few	Very few	Very few	0/1?	0	
	C	0	0	0?	0	100	100	
	D	Safe	Safe	Safe	Safe	Safe	—	
Entamoeba histolytica cysts	A	$10–10^4$	$10–10^4$	$10–10^4$	$10–10^4$	$0–10^5$	$10–10^3$	May survive for 3 weeks
	B	$5–10^4$	$5–10^3$	$5–10^3$	$5–10^3$	$0–10^5$	0	
	C	10–50	50?	50?	50?	0?	100	
	D	Contaminated	Safe	Safe	Safe	Contaminated	—	
Hookworm ova	A	$10–10^3$	$10–10^3$	$10–10^3$	$10–10^3$	$0–10^4$	$10–10^2$	Ova will survive
	B	$10–10^2$	$10–10^2$	$10–10^2$	$10–10^2$	$0–10^3$	0	
	C	50	50–90	50–90	50–90	50–90	100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated	—	
Ascaris ova	A	$10–10^3$	$10–10^3$	$10–10^3$	$10–10^3$	$0–10^4$	$0–10^2$	Ova will survive for many months
	B	1–10	$0–10^2$	$0–10^2$	$0–10^2$	$0–10^3$	0	
	C	30–80	70–100	70–100	70–100	50–90	100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated	—	
Schistosome ova	A	1–100	1–100	1–100	1–100	1–100	1–10	Ova may survive up to 1 month
	B	1–10	1–10	1–10	1–10	1–10	0	
	C	80	50–99	50–99	50–99	50–90	100	
	D	Contaminated	Safe	Safe	Safe	Contaminated	—	
Taenia ova	A	1–100	1–100	1–100	1–100	$0–10^3$	0·1–50	Ova will survive for a few months
	B	0·1–50	0·1–50	0·1–50	0·5–50	0–50	0	
	C	50–90	50–95	50–95	50?	50–90	100	
	D	Contaminated	Contaminated	Contaminated	Contaminated	Contaminated	—	

* A: in typical inflow (no./ℓ); B: in typical outflow (no./ℓ); C: removal (%); D: final sludge.
† After Seachem *et al.* (1980).

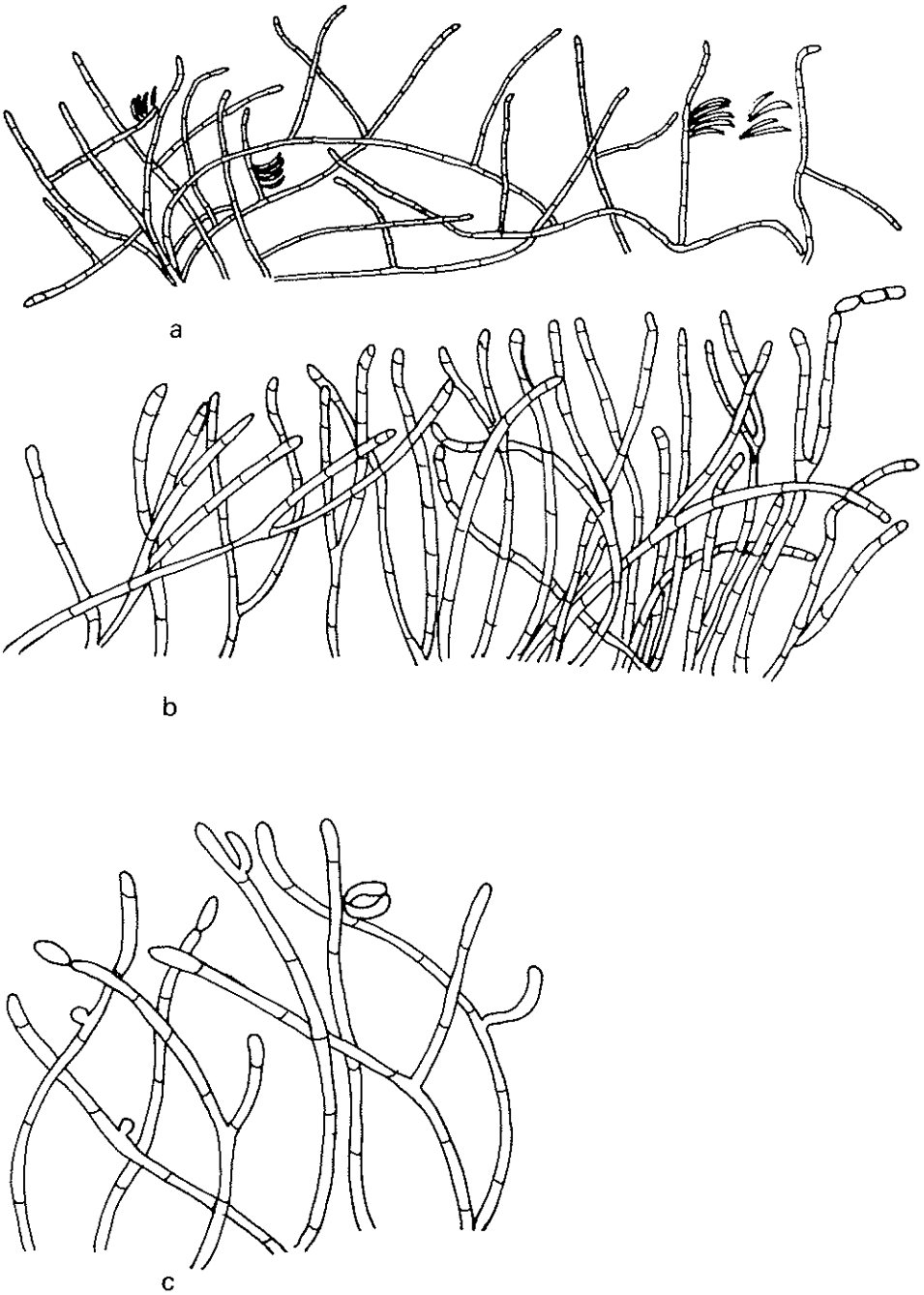

Figure 10. The most common fungi in effluent treatment processes. (a) *Fusarium aquaeductuum* (hyphal diameter 5 μm); (b) *Geotrichum candidum* (hyphal diameter 10 μm); (c) *Subbaromyces splendens* (hyphal diameter 10–12 μm).

and *Ascoidea* (Tomlinson, 1942; Cook, 1954; Painter, 1954; Wheatley, Mitra and Hawkes, 1982). The combination and dominance of the organisms which become established depends on local conditions, but in general the filamentous bacteria and fungi are more tolerant than the flocculated bacteria (Tomlinson and Williams, 1975). Thus, changes from a neutral pH, or less than optimum C:N:P ratios (i.e. 100:5:1), or poor concentrations of DO (<2·0 mg/ℓ) have the effect of encouraging the filamentous groups. This is of little consequence in biological filtration but seriously interferes with settling in the activated sludge process. If the activated sludge floc contains a significant proportion of filamentous material, then the density of the sludge is reduced and there are major problems with regard to settling and returning the bios (Pipes, 1977; Chambers and Tomlinson, 1982) (*Figures 10 and 11*); this condition is known as 'bulking'.

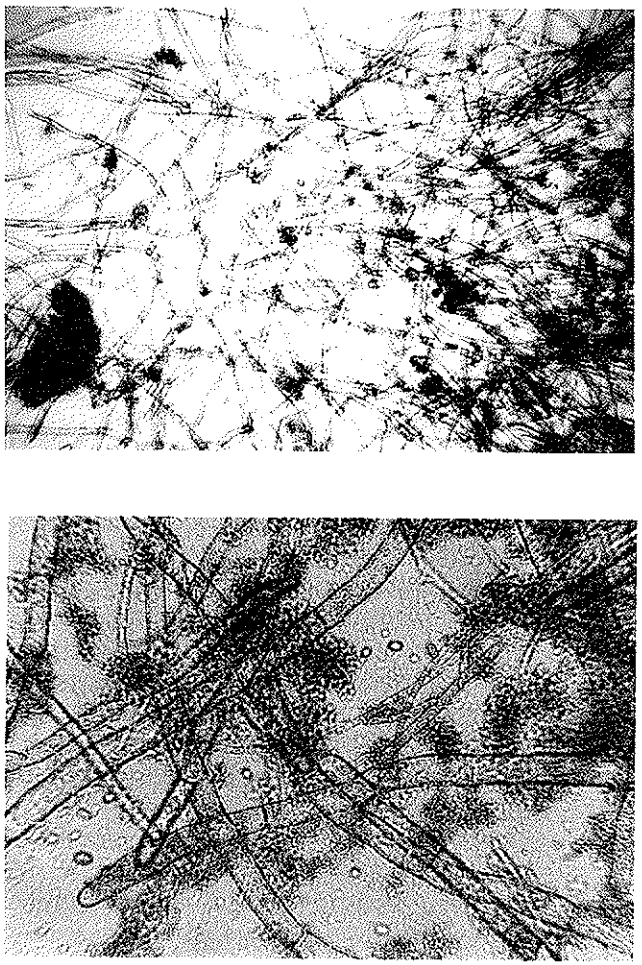

Figure 11. *Geotrichum* spp. in effluent (hyphal diameter 10 μm). (a) Fungi growing in high-rate biofilter film; (b) *Geotrichum* sp. at 5 × magnification of (a).

For the successive stages in the complete oxidation and mineralization of the organic waste, a number of other organisms also become established. The heterotrophic bacteria (*Figures 4 and 5*) are responsible for primary removal of organic matter. They are followed by a number of secondary communities feeding off the primary population and its breakdown products. One of the important groups are the autotrophic nitrifying bacteria *Nitrosomonas* and *Nitrobacter* which have been studied in some detail because of their importance in removing ammonia. Nitrifying bacteria are slow growing and sensitive to low concentrations of dissolved oxygen ($<2{\cdot}0\,mg/\ell$) (Painter, 1970; Bruce, Merkens and Haynes, 1975). In the activated sludge process, unlike biological filtration, the autotrophic bacteria occupy the same physical position in the floc as the heterotrophs and although not competing for substrate they do compete for oxygen. This means that if an activated sludge plant is to produce a well-nitrified effluent, residence times and oxygen concentrations have to be higher than those required simply to remove BOD. The algae are the other major autotrophic group present in waste-water treatment systems. They are normally restricted by the light available, and are not of primary importance in the UK. Further stages in treatment, such as oxidation ponds or lagooning, would generate large algal and diatom populations. The algae are always present in biological filters and the most commonly identified are the blue-green algae (Cyanophyceae), *Phormidium* and *Oscillatoria*, together with *Stigeoclonium*, *Ulothrix* and *Chlorella* from the Chlorophyceae (*Figures 8 and 12*).

Secondary grazers rapidly become established in waste-treatment reactors with the holozoic protozoa, rotifera and nematode worms occupying the second trophic level. The species and diversity of the ciliates also depends on the degree of treatment, with a succession to the stalked peritrichous protozoa from the holotrichia and flagellates (Baines *et al.*, 1953; Curds and Cockburn, 1970) (*Figure 13*). It seems likely that most of the protozoa are scavengers feeding both saprobically (on organic detritus) and holozoically (on living organisms). Experiments conducted by Curds, Cockburn and Vandyke (1968) have shown that the protozoa play an important part in the treatment process, with a reduction in protozoa coinciding with a more turbid effluent with a higher BOD.

In biological filters there is also a wide range of Metazoa, absent from the truly aquatic activated sludge. *Table 8* shows the range present in a typical

Table 8. Common Metazoa in biological filters

Oligochaeta:	Diptera:
Lumbricillus	Psychoda
Enchytraeus	Chironomids
Eisenia	Anisopus
Dendobenea	Scatella
Collembola:	Acarina
Hypogastura	Platyseius
Crustacea:	
Daphnia	
Canthocamptus	

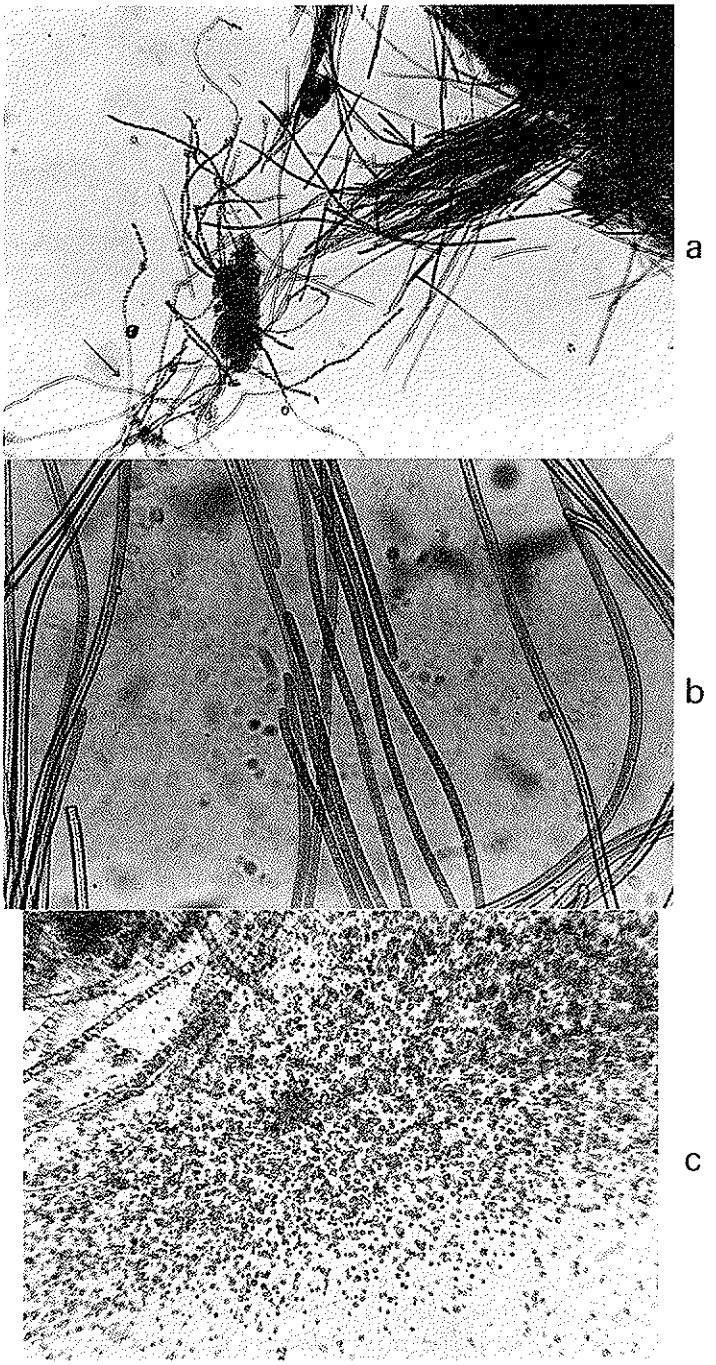

Figure 12. Algae in effluent treatment processes. (a) Algae growing in biological filter film, showing (arrowed) *Ulothrix* (Chlorophyceae) (with filament diameter 5 μm) and straight rods of *Phormidium* (Cyanophyceae) (5μm in diameter); (b) *Phormidium* at 4× the magnification in (a); (c) *Chlorella* (3–5 μm in diameter)

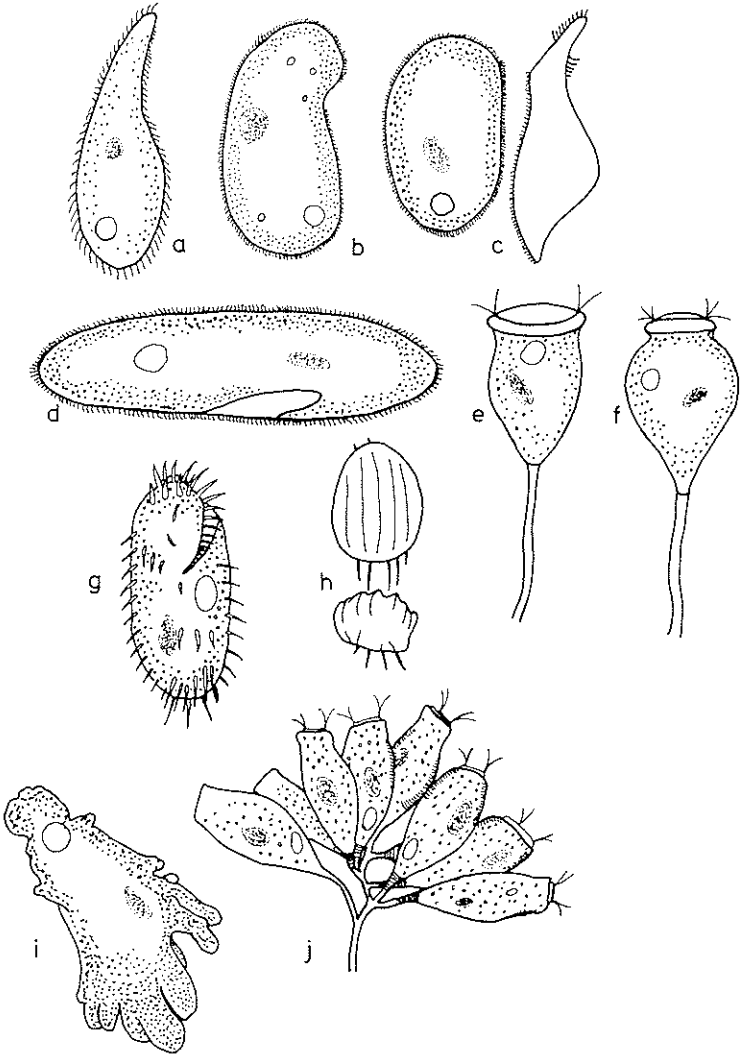

Figure 13. Some of the most important protozoa in waste treatment processes. (a) *Hemiophrys* sp. (length 150–200 μm); (b) *Colpidium colpoda* (100–120 μm); (c) *Chilodonella* sp. (100–120 μm); (d) *Paramoecium caudatum* (150–180 μm); (e) *Vorticella alba* (cell (not stalk) length 60–80 μm); (f) *Vorticella microstoma* (cell length 50–90 μm); (g) *Stylonychia* sp. (150–200 μm); (h) *Aspidisca costata* (25–40 μm); (i) *Amoeba proteus* (approx. 10–20 μm); (j) *Opercularia coarctata* (head, not stalk, each 45–50 μm).

filter; these organisms originate from mud flats. Strong wastes or high irrigation rates tend to limit the species diversity. The Psychoda flies are the most common and are present even in very high-rate filters. The flies can be a nuisance but fortunately, with one rare exception, are non-biting.

Because of their importance in controlling film accumulation, the flies and worms have been subject to a considerable amount of investigation. Lloyd (1945) has published a series of papers on the origins and the role of the flies, and Hawkes (1957), Terry (1956) and Hawkes and Shephard (1972) have given

details about the preferences of each species. Reynoldson (1941, 1943, 1947, 1948) has published a similar account of the worms. Williams, Solbe and Edwards (1969) and Solbe (1971) have conducted work on the aspects of distribution, life history and amount of film metabolized by the worms. The grazing organisms are responsible for a major shedding of bios in the spring of each year, coincident with a consistent rise in temperature in late April and May. Sludge production can double during this period but fortunately the solids produced are easily settleable. Biological filters accumulate solids during the colder months from a combination of waste solids and partly autolysed film debris.

RECENT DEVELOPMENTS IN TECHNOLOGY

An important advance in biological filtration took place in 1970 with the introduction of plastic media. This has extended the range of applications to include high-concentration wastes typical of industrial effluents. Conventional mineral media become clogged with too much biomass when treating strong industrial wastes. Plastics are light compared with stone and it is now possible to build space-saving tall filters without the usual substantial retaining walls (*Figure 14*). The high rates of treatment possible with the plastic media (*Table 4*) have enabled major savings to be made in the cost of effluent disposal and its use to treat industrial wastes has become widespread. There are now about 1000 such plants in Europe.

Two characteristics of media which exert an important influence on the efficiency of biological treatment are the specific area available for biological growth and the void space between the pieces allowing ventilation, discharge of solids and drainage (Chipperfield, 1967). Providing a large specific surface while retaining adequate voidage has, in the past, been the problem in selecting suitable mineral media. Plastic materials can be fabricated into shapes with optimum surface area and voidage but they also have to be capable of promoting uniform utilization of the greater surface area. The modular synthetic media rely on a high irrigation rate through recirculation to ensure adequate wetting of the available surface, but at lower rates of load a random pack medium has to be used to give an even flow pattern through the filter depth.

Another innovation introduced into the UK in 1973 was the Rotating Biological Contacter (RBC). A honeycomb of plastic sheets is slowly rotated on a shaft through a tank containing the waste water. About 40% of the surface is submerged and a typical unit 7·5 m in length and 3·5 m in diameter will have a surface area of 9500 m^2 for biological growth. RBCs have characteristics similar to those of biological filters, with a large surface area for fixed biological culture which is alternately in contact with the waste water and air. Rotation of the media ensures that uniform concentrations of dissolved oxygen and substrate are available to the total biomass. The effluent tank may be baffled to produce a plug flow of waste and in this way high BOD removals of over 95% and nitrification can be achieved in the final compartments. There are about 4000 such units operating throughout the world, mostly as treatment systems for small communities or factories (Iggleden, 1981). Pike *et al.* (1982) have carried

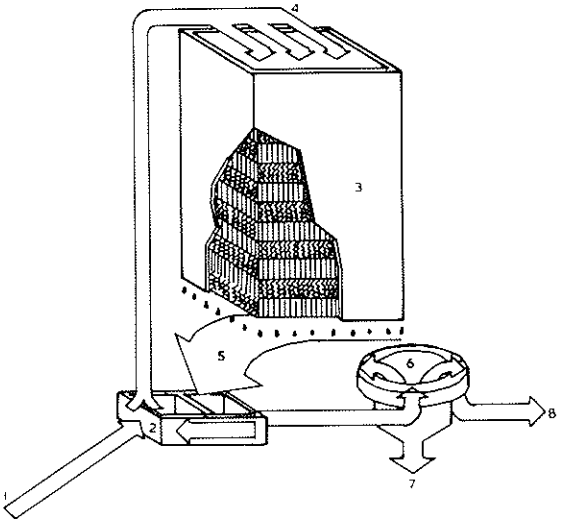

Figure 14. High rate biotower. 1. Incoming waste; 2. Recycle sump; 3. Biotower; 4. Distribution system; 5. Effluent; 6. Settlement tank; 7. Sludge; 8. Clarified effluent.

out a performance survey of some RBC treating domestic sewage.

Recent developments have also been made in the activated sludge process: the first was the use of pure oxygen in completely enclosed tanks in 1972. The system was based on the availability of cheap oxygen from pressure-swing adsorption equipment. The effluent treatment plant is very similar to conventional activated sludge but is able to operate at higher biomass concentrations and lower residence times without a loss in efficiency (*Figure 15*). The higher dissolved oxygen concentrations are reported to overcome the problem of bulking sludges suffered by many strong industrial wastes (Fuggle, 1981). About 150 plants have now been built world-wide, with five in the UK.

An innovative application to effluent treatment was the use of a deep air-lift fermenter in 1974 by Imperial Chemical Industries (Hemming *et al.*, 1977) (*Figure 16*). The concentric tube arrangement, the 'deep shaft', can be 50–100 m deep and 0·5–5 m in diameter. The shaft is partitioned into a downflow section, into which the incoming waste, returned active sludge and the process air are injected, and an upflow section. In the upflow section, bubbles of dissolved gases are released as the pressure decreases, thus reducing the density of the circulating water. The difference in density between the downflow and the upflow liquids above the point of air injection produces the net driving force for the shaft. The velocity generated is much higher than the rise rate of air bubbles in the downflow portion and the air is carried down. Dissolved oxygen and turbulence in the shaft are high, which permits a high concentration of very active biomass to be maintained. To avoid flotation, the effluent is vacuum degassed before settlement. The system is expected to be more economic than conventional activated sludge by virtue of its low residence time and low running cost. There are about five full-scale plants, one of which is in the UK.

A combination of fixed film and activated sludge, the fluidized bed, was

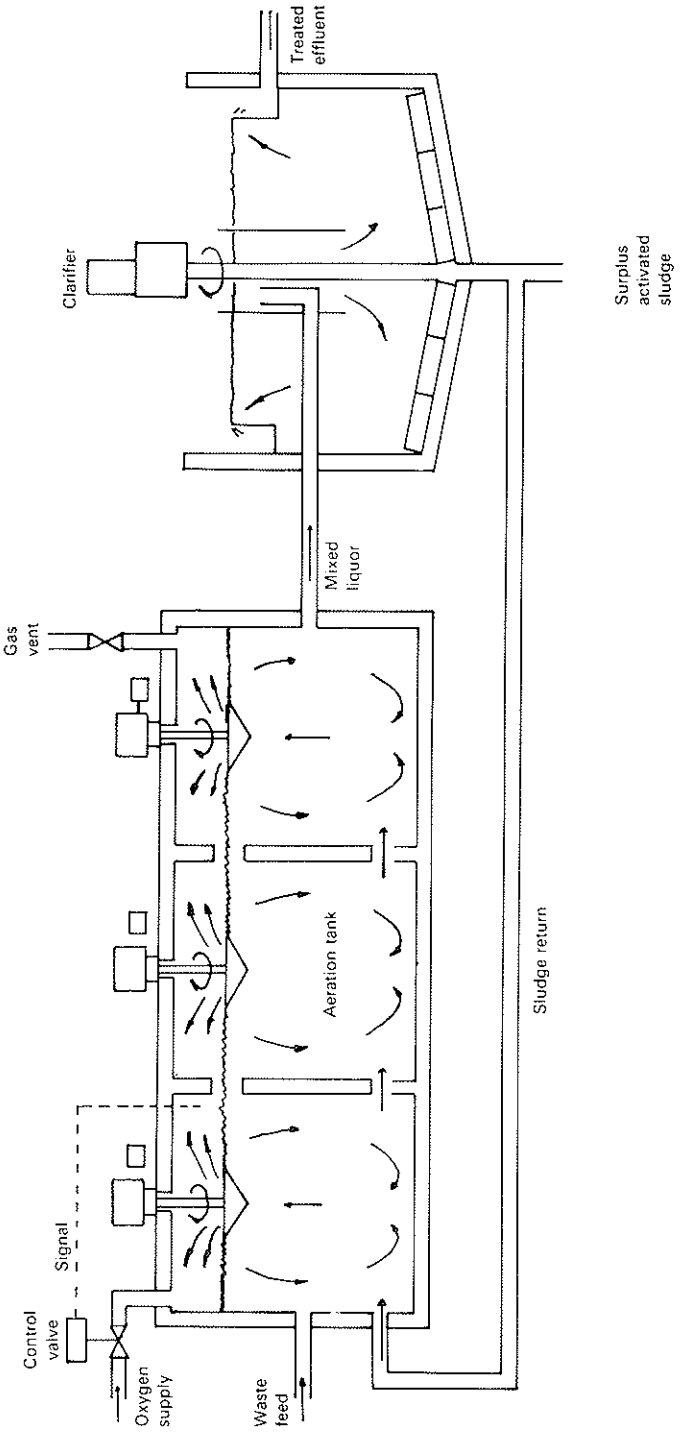

Figure 15. Schematic diagram of an oxygen-activated sludge plant.

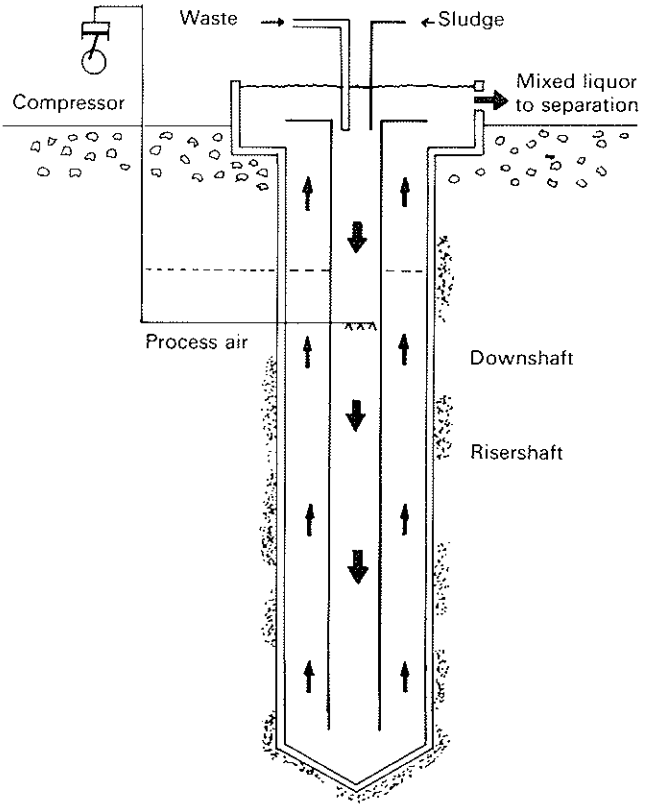

Figure 16. Diagram of the deep shaft.

introduced in 1980. The biological fluidized bed, like the deep shaft and pure-oxygen-activated sludge, is able to retain a high concentration of micro-organisms—about five times the concentration of conventional plant with consequent savings in capital cost. There is a further saving in capital because the plant does not require final settlement. The waste sludge from the reactor is highly concentrated and the effluent is reported to be suitable for direct discharge. There are two basic types: the Simon Hartley Captor with plastic support particles and compressed air for fluidization (Walker and Austin, 1981) and the Dorr-Oliver Oxitron, with sand support particles and oxygen injection for fluidization (Sutton *et al.*, 1981).

In the Oxitron system, the sand overflows the reactor and is recycled after cleaning in an agitator or vibrating screen (*Figure 17*). In the Captor system, the particles are retained in the reactor by a mesh but a portion is periodically cleaned by squeezing. They are then returned to the bottom of the reactor by an air lift (*Figure 17*).

An unwanted consequence of intensifying the aerobic treatment processes has been an increase in the amount of surplus biomass (sludge) to be disposed of.

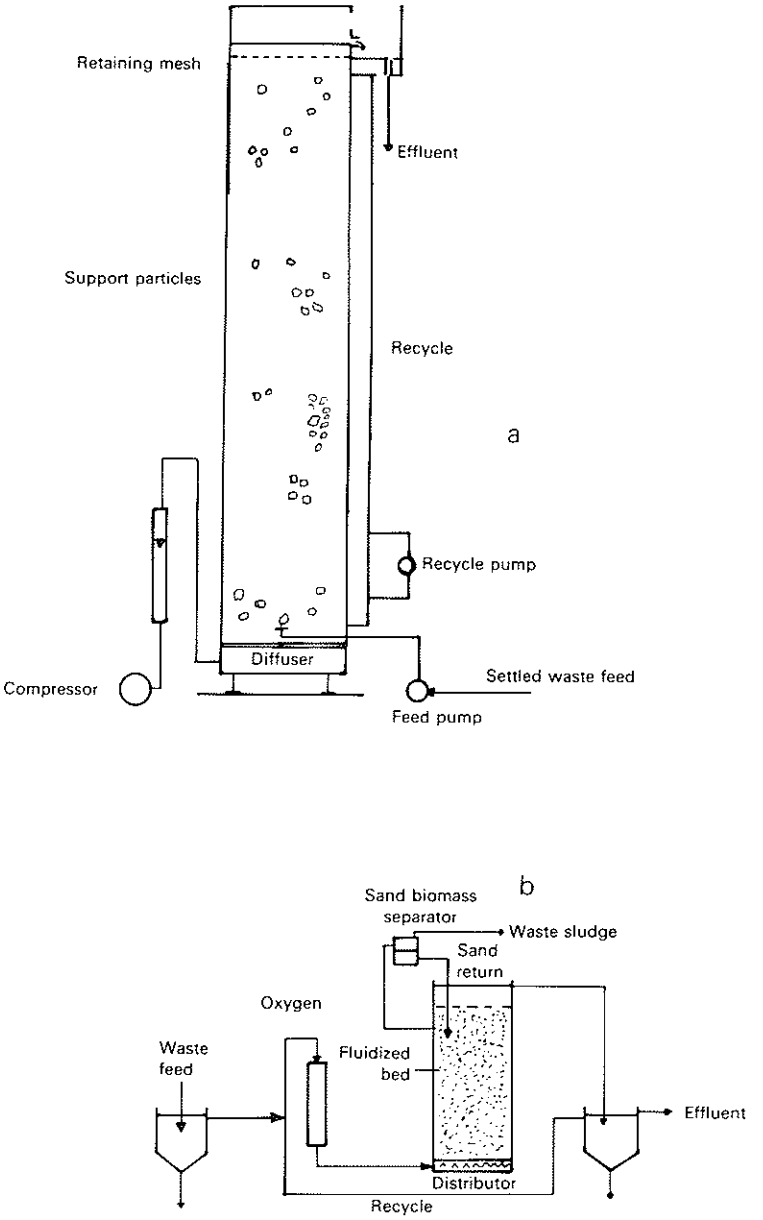

Figure 17. Fluidized-bed process. (a) Captor system; (b) Oxitron system.

Sludge disposal can represent 50% of effluent treatment costs (Porch, Bayley and Bruce, 1977). Thus one of the most important possibilities to be derived from a greater understanding of the metabolism of micro-organisms would be to uncouple microbial anabolism and catabolism. This would ensure an inefficient conversion of substrate to biomass. Empirical work already conducted (Hawkes and Shephard, 1972) indicates that certain trace nutrient deficiencies and intermittent feeding may have this effect.

By-product recovery

One of the largest potential sources of organic pollution in the UK is the biologically based industries. The scale of fermenters, the water consumption and the biological nature of the processes used tends to generate large volumes of strong wastes. *Table 9* shows some strengths of a few typical wastes from

Table 9. Strengths of common wastes from the food, drink and fermentation industries (approximate figures; actual values vary from site to site depending on the process).

Industry	Strength of waste (COD in mg/ℓ)
Pharmaceutical:	
Citric acid	20 000
Antibiotics	10 000
Distillery waste	25 000
Confectionery	15 000
Cheese, butter, cream	5000
Brewery	5000

the food, drink and fermentation industries. Most of these wastes are spent broths or wash waters which are very variable in composition. Traditionally, the only suitable treatment has been biological oxidation admixed with domestic sewage. Typically, the waste from antibiotics or citric acid fermentation, for example, can have a COD of 10 000–20 000 mg/ℓ, and although easily biodegradable, this type of waste is 20 times the strength of domestic sewage. Thus an antibiotics plant producing tetracylines with an effluent flow of 250 m^3/day and a COD of 10 000 mg/ℓ has a population equivalent of 36 000 and would cost about £200 000–300 000 a year in water charges to dispose of to sewers (*Table 10*). If the biologically based industry is considered as a whole, then a relatively minor improvement in the biotechnology of waste treatment would have a substantial financial impact. There is therefore considerable interest in reducing the volume or utilizing these wastes. There are two approaches to the recovery of materials from wastes: (1) direct recovery or concentration of valuable materials, and (2) transformation of wastes into useful materials. There are three widely used large-scale product-recovery biotechnologies concerned with, (1) water; (2) biomass and (3) biomethanation.

Table 10. Mogden formula for trade effluent charges (North West Area, 1979).

$$C = R + V + \frac{O_t}{O_s} B + \frac{S_t}{S_s} S$$

where:

C = daily cost of trade effluent (pence*/m^3)
R = reception and conveyance cost of sewage (1·11 p/m^3)
V = volumetric and primary treatment cost of sewage (1·37 p/m^3)
O_t = COD of trade effluent after 1 hours' settlement
O_s = the average COD of settled sewage
B = unit biological oxidation cost for settled sewage (2·537 p/m^3)
S_t = the total suspended solids in trade effluent (mg/ℓ)
S_s = total suspended solids in crude sewage (mg/ℓ)
S = treatment and disposal cost of primary sludges (1·463 p/m^3)

* One new penny = £0·001.

WATER RECLAMATION

A major problem with reclaiming organically polluted water to a high standard, suitable for reuse, is the capital cost of the equipment compared with the cost of mains water. Mains water costs 75–100 p/tonne and there are, in most cases, sufficient supplies of the required quality and price for most industrial needs. Recycling industrial water is economic only if a water quality inferior to that available from the supply is acceptable. This is often the case in the heavy industries such as power generation, steel making and coal preparation; water treatment can then be kept to a minimum—usually settlement and/or cooling.

In the water supply industry about 30% of raw water is obtained from recycled effluent (Water Data Unit, 1979) principally in areas without access to supplies of upland water, for example South-East England and the southern counties. The biologically treated effluent is discharged to lowland rivers which then serve as aqueducts and sources of water supply. The biological activity in the river further improves the quality of the recycled water (*Figure 18*). The rivers Thames and Lee can be 75% recycled sewage effluent in summer (Packham, 1983). Supplies of upland water are now scarce and it is envisaged that any expansion in supply will have to include a substantial quantity of reclaimed water (Water Resources Board, 1973). There are potential problems associated with this type of supply from the recycling of recalcitrant pollutants. All raw waters are normally treated by a combination of biological and physico-chemical processes. Solids are removed by chemically assisted precipitation; the residual liquid is filtered through sand and is then chemically sterilized before being pumped into the mains. Additional treatment with carbon, membranes or deionizing plant may be necessary if the raw water quality is low. The sand filter is partly biological—a layer of micro-organisms which grows on the surface of the sand particles, degrades organic matter and reduces some of the nitrate, phosphate and carbonate present in the water. In slow sand filtration this biologically active layer is known as the schmutzdecke and is responsible for the principal filtering action.

It is likely that there will be improvements in recycled water supply technology

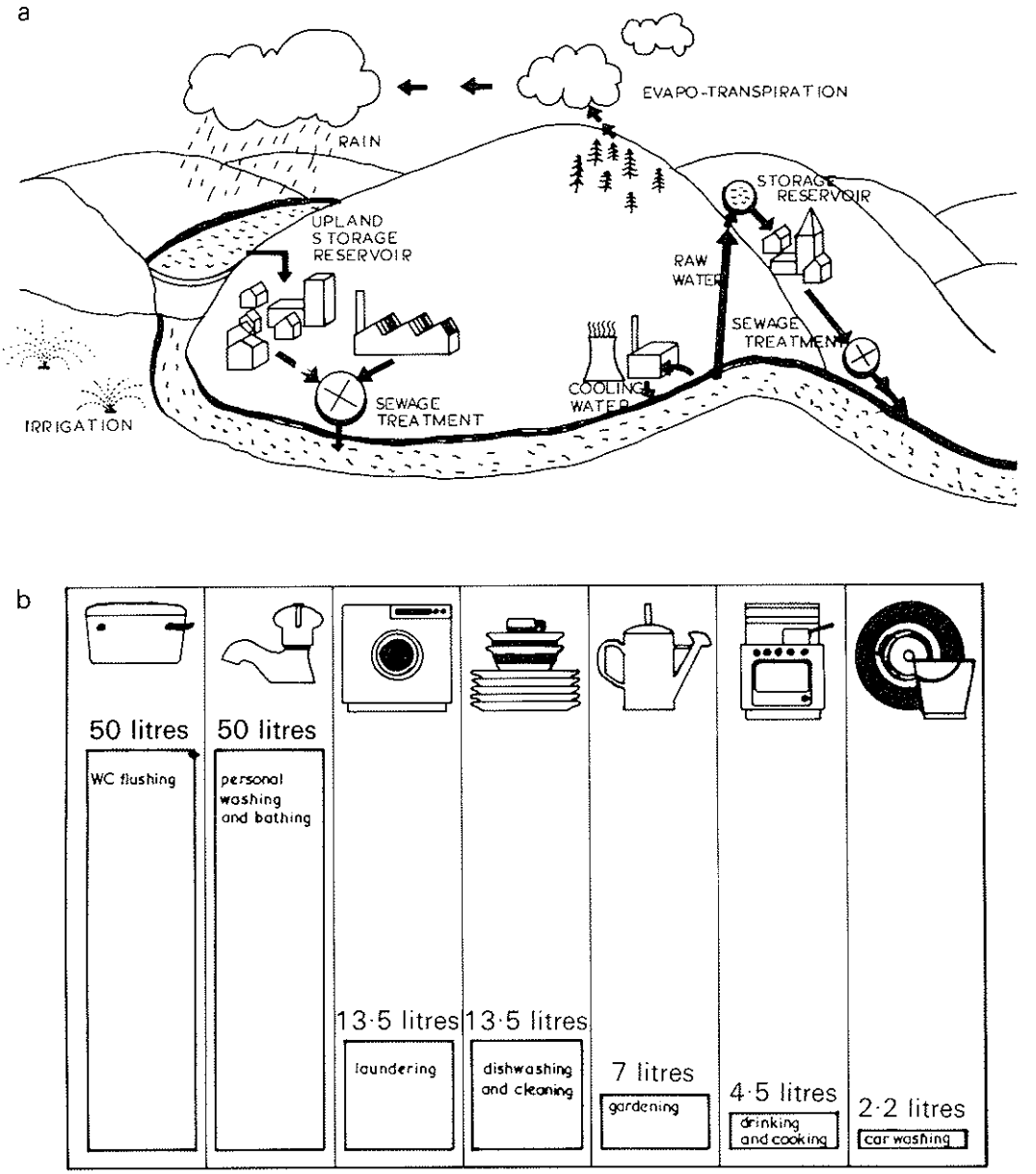

Figure 18. (a) The water cycle; (b) Average daily domestic water consumption.

as a result of the application of micro-organisms for the breakdown and scavenging of recalcitrants.

Recalcitrant compounds

According to Storck (1979) the total world production of synthetic organic chemicals (xenobiotics) is now estimated at 150×10^6 tonnes/year. One hundred and fifty chemicals are produced in quantities in excess of 50 000 tonnes/year and the use and losses of these compounds are fairly well known (Schmidt-Bleek, 1980). There is, however, little information on the fate of a much wider range of chemicals produced in smaller quantities. A large number of these synthetic compounds are recalcitrant or persistent, and considerable efforts are being made to understand how micro-organisms might acquire the metabolic potential to degrade these recalcitrant xenobiotics. The high affinity of biological catalysts for their substrates makes biodegradation particularly useful at low concentrations of the xenobiotic. This makes biodegradation complementary to the traditional methods for disposing of bulk waste products (oxidation, incineration and precipitation). Another advantage is that the xenobiotic is converted into natural products.

Most biodegradation research has been classic, investigating the metabolism of pure cultures; this reduces the complexity of the system and ensures reproducible results. The two basic methods used are enrichment culture and gene manipulation. In enrichment culture the compound to be degraded is added in progressively larger doses during an acclimatization period until the xenobiotic then becomes a source of essential nutrient. The starting inoculum is important and should be taken from an area where the organisms are most likely to have evolved, a habitat which has been exposed to the compound of interest. Batch culture is usually used to screen for suitable organisms, and continuous culture is used to exert continuous selection pressure for the desired organisms. Important additional information on the biochemistry of the microbial degradation, such as the rate-limiting steps, catabolic sequences and the substrate specificity, can also be obtained from these cultures. Harder (1981) in a recent review of enrichment techniques emphasized the role of mixed cultures. Previous work had shown that an interdependent microbial community was often necessary to metabolize the complex sequence of intermediates which resulted from the breakdown of xenobiotics. This biochemical approach can be complemented by work on the genetics of degradative pathways. Work has been reported on the analysis of plasmids, gene cloning, and transposon mutagenesis. Williams (1981), for example, has extended original work by Chakrabarty (1980) on the structural analysis of plasmids in *Pseudomonas*. The plasmids contain information for the breakdown of alkanes, toluene and xylene. Another group (Franklin, Bagdasarian and Timmis, 1981) have studied the use of gene cloning and transposon mutagenesis for the cleavage of a wide range of aromatic compounds.

The list of recalcitrants currently under investigation (Leisinger, 1983) include the azo dyes, stilbenes (optical brighteners), chloroaromatics, *S*-triazines, chlorinated hydrocarbons, DDT and lignin.

Although the results of microbiological, biochemical and genetic research on biodegradation are accumulating there are, as yet, no practical processes on an industrial scale. Nevertheless, three important areas of application are envisaged:

1. Controlled degradation of specific wastes with specialized cultures at the source of the waste. Cook *et al.* (1983) have described a specific example for the treatment of parathion insecticide wastes.
2. Improvement of waste treatment systems by inoculation with adapted laboratory strains. Cook (1983) has reported commercially available specialized organisms for improving BOD removal, for eliminating filamentous growths in 'bulking', and for improving methane generation in anaerobic treatment. Five companies (Biolyte, London; Bactozyme, Worksop, Nottingham; Agrico, Stafford; Ubichem, Middlesex; and Interbio, London) are now actively selling this type of preparation for supplementing existing effluent treatment systems, but no controlled experiments have been reported so far.
3. The clean-up of spills and decontamination of soils using specialized cultures.

Specific reactors for the breakdown of recalcitrants would be the easiest to operate and control because the waste stream could be better defined and applied to existing reactor designs. To supplement existing waste treatment systems, or to be effective on spills, the organisms and their enzymes will have to survive and function in the suboptimal conditions of a complex ecosystem.

Removal by adsorption

It has been observed for some time that biological effluent treatment effectively removes metals from sewage (Brown and Lester, 1979). The metals are precipitated with the sludge or surplus biomass and can reach a concentration sufficient to interfere with the subsequent processing of the sludge. Research at UMIST (Kiff and Brown, 1981) was directed at understanding the mechanism of this removal. Investigations on an acetate rayon waste which contained zinc in high concentrations (50 mg/litre) showed that the biomass could contain up to 12% by weight of zinc without interfering with BOD removal. Diffraction and microscopical examination showed that the zinc was bound in the extracellular slime of the zoogloeal growth. The zinc and other metals present in sewages could be elutriated by oxidative acid hydrolysis, a process which has been established by Simon Engineering and Richland Resources Ltd. Hydrochloric acid and hydrogen peroxide at pH 1·5 are used in conjunction with a polyelectrolyte to release the water. Recovery of the metal is 70–95% but the process is not economically viable unless subsidized by a high negative cost from the disposal of a metals-contaminated sludge. Examination of the biofilm (*Figure 7b*) shows that a large proportion of the bios is extracellular bacterial polymer rather than actual bacterial cells. This polymer has important metabolic functions with regard to the absorption and transfer of nutrient from solution to bacterial cells. Field data from operating plants have indicated that the

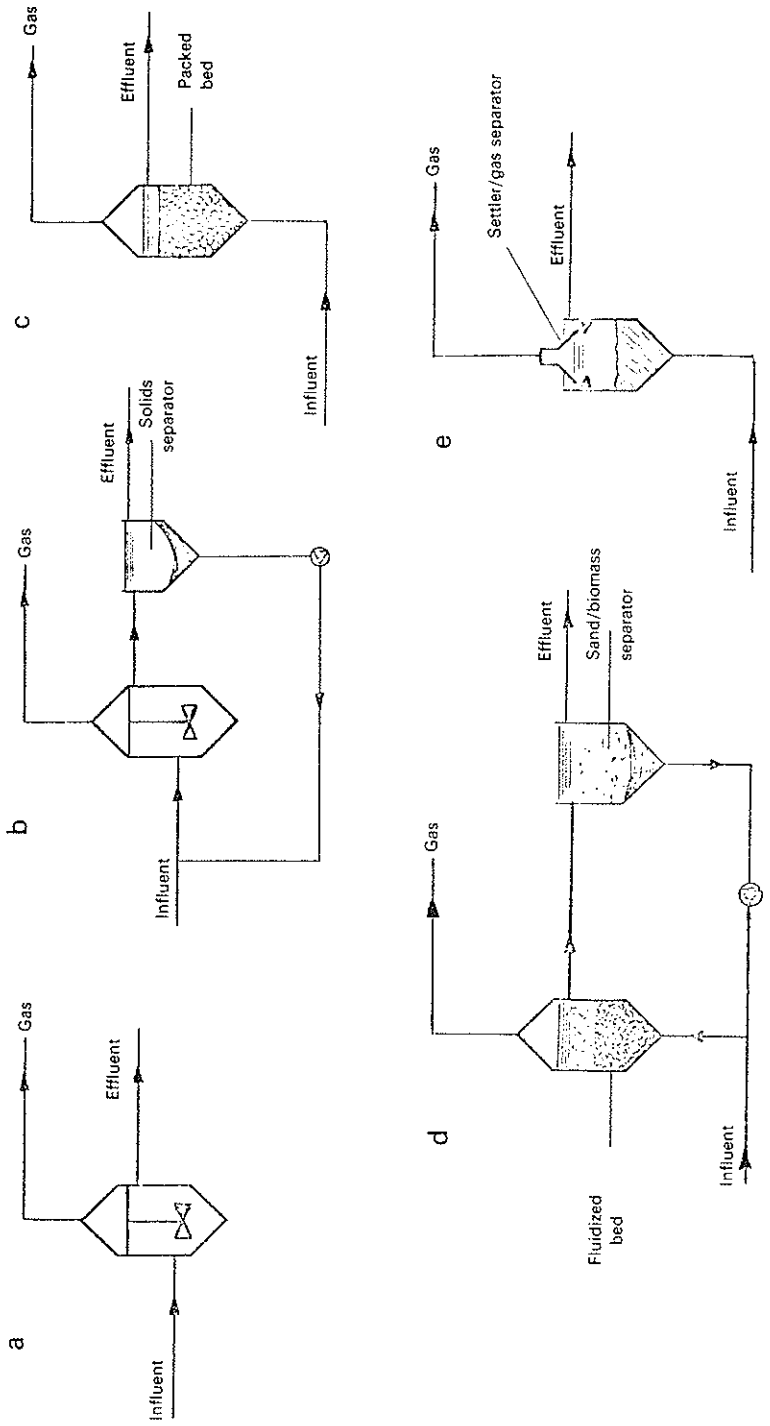

Figure 19. Schematic diagram of various anaerobic treatment processes. (a) Conventional digester; (b) Anaerobic contact process; (c) Anaerobic filter; (d) Anaerobic fluidized or expanded bed; (e) Upflow anaerobic sludge blanket. (*See* pages 300–302)

structural characteristics and quantity of polymer vary according to the substrate.

Carbohydrate wastes, particularly the oligosaccharides, generate the most polymer at similar concentrations and metabolic rates. Preformed polymers such as dextrans, starch and polyacrylamides also produce large quantities of extracellular polymer.

Little information has been derived from these early experiments on the chemical structure and nature of the polymer. Further work is required to determine its structure and how it effects adsorption. It is known to be a complex co-polymer held together by fibrils and to contain large amounts of bound water. It is also rich in a number of bound extracellular enzymes for the specific uptake of required nutrients. Long-term research is directed at altering the chemical structure of the polymer to recover a wide range of useful materials. Mercury tolerance and accumulation has also been isolated in a strain of the fungus *Chrysosporium* which could be adapted to detoxify mercury-bearing wastes. The mercury is adsorbed and fixed to the hyphal walls of the fungus (Williams and Pugh, 1975).

Bioscrubbing

Traditional odour and waste gas control processes, such as incineration, dispersion, catalytic oxidation, scrubbing and adsorption, are all very expensive, are best suited to large volumes of well-defined waste gases and are not able to adjust to varying complex mixtures of materials. Malodour problems particularly are normally caused by complex varying mixtures at very low concentrations: many of the mercaptans, for example, have odour thresholds below 1 ppb. Biological control of this type of problem, like that of the recalcitrants, may offer a simpler alternative treatment. Other advantages of the biological nature of the process include combined liquid and gas treatment, no chemical costs and low energy requirements, which are of general interest in all gas scrubbing. Patents for biological control systems were filed in 1930 but there are still few working systems. It seems likely that the idea arose from the observation that biological growths occurred on many water-scrubbing systems. The types of apparatus currently used resemble physico-chemical scrubbing units combined with a biological filter. The design requirements of bioscrubbing systems are similar to those of physico-chemical scrubbing units, the principal requirement being the best surface possible for gas–liquid–biomass interchange. Le Roux and Mehta (1980) have reported on the design of biological systems for the oxidation of malodour, using both a bioscrubber and percolation through a soil bed. Later, in 1982, Le Roux discussed a full-scale application for the treatment of malodours from a high-rate biological filter. The initial removal mechanism, as in a biological filter, is likely to be biophysical adsorption and solubilization of the material. In this form it will then be available for microbial breakdown. The packed bed serves both to provide a large gas–liquid surface area for adsorption and to immobilize the biomass.

Excessive amounts of biomass could occlude some of the surface area and the concentration of organic matter in the effluent should be controlled to main-

tain optimal efficiency. It is probable that the prototype bioscrubbing systems will be part of effluent treatment plants because this is the most likely source of substrate for biological growth. Specific inoculation would not, therefore, be required but later plants for the removal of xenobiotic materials from gases would require specific cultures and a controlled substrate. Completely novel chemicals may require a prolonged acclimatization period, but seeding of subsequent systems handling similar materials would then be simplified. Biological control of gases is as yet an under-researched area, although applications both for oxidizing biofilm, (for mercaptans, hydrogen sulphide and cyanide) and for reducing anaerobic biofilms (for sulphur dioxide and chlorinated hydrocarbons) can be envisaged.

RECOVERY OF BIOMASS

Biomass and single-cell protein recovery (on a small scale) from wastes is now very well documented with reviews in 1959 by Prescott and Dunn and in 1968 by Peppler. James and Addyman summarized the state of the technology in 1974 and described the most common recovery processes from molasses, whey and starch using pure culture to ensure product consistency. These well-defined materials are now, however, rarely wastes but are valuable commodities as substrates for other higher-value microbial products (Tomlinson, 1976a). The production of protein from the more common dilute wastes has met with little success despite a number of investigations in both Europe and the United States. The two most common groups of micro-organism investigated have been the yeasts and the Fungi Imperfecti. Important work on the yeasts has included that by Holderby and Moggio (1960) on paper mill wastes, by Wassermann, Hampson and Alvare (1961) on milk wastes, by Thanh and Simard (1973) on domestic sewage, by Righelato, Imrie and Vlitos (1976) and Tomlinson (1976a) on food processing wastes, by Smith and Bull (1976) on coconut waste water and by Braun *et al.* (1976) on citric acid wastes. The filamentous fungi are easier to separate and dry than the yeasts, and they also have a better food texture than other micro-organisms. Work by Church, Erikson and Widmer (1973) was conducted on full-scale plant trials using *Trichoderma* and *Geotrichum* for the treatment of vegetable processing wastes, Munden and King (1973) reported on the use of *Fusarium* for treatment of carbohydrate wastes; Quinn and Marchant (1980) described the use of *Geotrichum* for distillery wastes and Wheatley, Mitra and Hawkes (1982) used *Geotrichum* and *Fusarium* for the treatment of milk wastes. The utilization of bacteria (Grau, 1980), algae (McGarry, 1971), and water hyacinths (Cornwall *et al.*, 1977), have also been tested.

In general, it has been concluded that waste waters are not suitable for large-scale single-cell protein (SCP) production because of the difficulties of producing a biomass of consistent nutritive value and a material free from toxicological uncertainties or microbial contamination. Production of SCP becomes even less attractive when the cost of the fermenters and drying equipment are considered. Tomlinson (1976b) and Wheatley, Mitra and Hawkes (1982) report that protein recovery can be justified only if contamination-free wastes from the food, drinks and fermentation industry are used, if effluent treatment efficiency is unimpaired and if high sludge-treatment costs are reduced. The recovered SCP can then

Table 11. Comparison of costs of protein production* by fermentation† and biofiltration‡ in 1975.

Fermentation		Biofiltration	
Capital costs			
Civil Engineering	£45 000	Civil Engineering Tower	£11 000
Mechanical plant	£15 000	Base slab	£5000
Separation and drying costs	£50 000	Pumps and pipework	£10 000
		Sedimentation tank	£5000
		Plastic medium	£17 000
		Separation and drying costs	£20 000
Total	£110 000	Total	£68 000
Extras (10%)	£11 000	Extras (10%)	£7000
	£121 000		£75 000
Running costs			
Power required		4 pumps at 4 hp	
At 2·25 p/kWh	£3500	At 2·25 p/kWh	£1000
Manpower maintenance	£10 000	Manpower maintenance	£7500
Chemicals/nutrient	£3500	Chemicals/nutrient	£3000
Total	£17 000	Total	£11 500
Capital over 10 years at 15% interest	£24 200	Capital over 10 years at 15% interest	£15 000
Total annual costs	£41 000	Total annual costs	£26 500
Protein yield 106 t/year		Protein yield 50 t/year	

* Waste details: maltings liquor, hydraulic load 454 m^3/day; BOD load 454 kg/day; BOD concentration 1000 mg/ℓ; final required BOD 75 mg/ℓ; BOD removal required 92% (925 mg/ℓ).
† Batch process 30 h (including let down); BOD removal 630 kg/batch; oxygen required 0.7 kg/kg BOD removed ∴ 441 kg O_2 per batch aeration; capacity 681 m^3; biomass production 295 kg/day.
‡ Three-stage treatment load 1·5 kg/m^3; 310 m^3 plastic medium; biomass production 170 kg/day.

be used as a protein additive to a mixed animal feed: *Table 11* shows some anticipated costings. Forage (1978) has described the full-scale recovery of *Candida* yeasts from confectionery wastes.

There has also been interest in the possibility of energy recovery from surplus biomass. So far, interest has been restricted to energy recovery from short-rotation forestry and from gasohol (ethanol) (Nyns and Naveau, 1983) but there has also been work on utilizing biomass, principally water hyacinths and grass, grown on effluent (Reddy, Hueston and McKim, 1983; Schwegler and Cynoweth, 1983). It has also recently been concluded that the European agricultural surpluses could be converted economically into energy (Verstraete, 1983). Processes have also been suggested for the production of more valuable materials such as ethanol and ketones (Amberg, Aspitante and Cormack, 1969) and the volatile acids and alcohol (Coombs, 1981 and Chapter 11 of this volume).

BIOGAS

The most common anaerobic treatment is the digestion of sewage sludge. This process has been successfully applied since 1901, to reduce sludge volume, eliminate pathogens, prevent smell nuisance and generate methane as a by-product. This is well established and widely publicized technology, but there are difficulties which have prevented its more widespread use and development. Most of these problems have been associated with the slow growth rate of the obligate anaerobic methanogenic bacteria, which makes the process susceptible

to a wide range of interferences and less resilient to sudden changes in load. Until recently, the conversion of 50% of the organic matter into inorganic salts typically took 30 days (Ministry of Housing and Local Government, 1954; Stanbridge, 1976) and the process is, consequently, expensive to build.

The Water Pollution Research Laboratory has published the results of a national survey on digestion (Swanwick, Shurben, and Jackson, 1969), indicating that inhibition of the metabolic processes, for example by detergents, metals and chlorinated hydrocarbons, is not as common as was believed; most difficulties were attributed to poor design and operation. Recent work by Brade, Noone and colleagues (Brade and Noone, 1981; Brade *et al.*, 1982) has shown that the cost of building anaerobic digestion plants accounted for 20% of the capital of new works and represented £20–40 per capita served. They concluded that similar problems were encountered in all types of sludge treatment and that the cost of digestion could be reduced by some changes in the traditional civil engineering.

The rising costs of aerobic waste treatment and energy have revived interest in anaerobic treatment as an alternative and this has led to important developments in the microbiology and engineering of anaerobic treatment. The University of Louvain has recently completed a survey of anaerobic processes which includes data on over 400 operating plants (Demuynck, Naveau and Nyns, 1983).

Microbiology

Like aerobic waste treatment, the anaerobic digestion of wastes is based on a complex interdependent microbial community. Three different but interdependent groups of bacteria are involved (Zehnder, 1978; Mosey, 1978, 1982). The first group are the hydrolytic fermentative bacteria which hydrolyse complex polymeric substrates to organic acids, alcohols, esters and sugars, generating carbon dioxide and hydrogen. The second group are the hydrogen-producing and acetogenic bacteria; they convert the fermentation products of the first group into hydrogen, acetate and carbon dioxide. The third group are the methanogens which then convert the acetate and hydrogen into methane and carbon dioxide.

An important discovery, leading to a major advance in anaerobic treatment, was made by Bryant *et al.* (1967) who observed that the concentration of hydrogen exerted a crucial control on the anaerobic process. For the successful breakdown of carbohydrate under anaerobic conditions it was suggested that the partial pressure of hydrogen had to be less than 10^{-3} atm ($\approx$ 1 kPa). It is only at such low hydrogen concentrations that NADH formed by the breakdown of organic matter can be reoxidized by the release of hydrogen. These low hydrogen concentrations result from the activity of the hydrogenotrophic bacteria which constantly scavenge the hydrogen. Two groups—the methanogens (utilizing hydrogen and bicarbonate to form methane and water) and the sulphate reducers (forming hydrogen sulphide and water)—are the most important (*Table 12*). The hydrogenotrophic methanogens are thought to have doubling times of about 8–10 h (Schauer and Ferry, 1980) and are much slower growing than the acid-forming hydrolytic bacteria (doubling time about 30 minutes). Thus the hydro-

Table 12. Anaerobic digestion of waste and microbial groups involved.

Organic matter	Process	Organisms
1. Complex polymer wastes		
↓		
2. Monomer	1. Hydrolytic organisms	*Clostridium* *Eubacterium* Bacteroids
↓		also *Peptococcus* *Propionibacterium*
Simple volatile fatty acids and/or lactic acid, ethanol	2. Acetogenic bacteria	*Syntrophobacter* *Syntrophomonas* *Desulfovibrio*
↓		
3. Acetate, hydrogen and other acids		Hydrogenotrophic *Methanobacterium* *Methanobrevibacterium*
↓	3. Methanogens	
Methane, carbon dioxide, hydrogen sulphide, simple salts		Acetoclastic *Methanothrix* *Methanosarcina* *Methanospirillum*

genotrophic methanogens are susceptible to stress as a result of sudden surges in load and an accumulation of hydrogen from the increased activity of the acid-forming hydrolytic bacteria. The acid-forming bacteria respond to the increased hydrogen concentration by altering their metabolism to form propionic, butyric, valeric, caproic and lactic acids instead of acetic. This decreases the formation of hydrogen:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$
$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$$
$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$

So far no methane bacteria capable of metabolizing propionic or butyric acids have been discovered and it is assumed that these acids have to be converted to acetic to form methane (McInerney, Bryant and Stafford, 1980). Although bacteria capable of this conversion have not yet been isolated, enrichment culture studies by Lawrence and McCarty (1969) have shown that the process does exist, although it is very slow. The reactions as explained by Heyes and Hall (1981) are energetically difficult (i.e. of low energy benefit) and theoretical doubling times of 1·5–4 days were suggested.

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$$
$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$$

For benefit to be derived from these reactions the partial pressure of hydrogen must not exceed about 10^{-6} atm. ($\approx$ 1 Pa) (Sahm, 1983). The hydrogen-utilizing methane bacteria are therefore thought to regulate the formation of acetic acid, which is the vital substrate for the most economically important group—the acetoclastic methanogens. The acetoclastic methanogens are responsible for about 70% of the methane produced by the anaerobic digestion

Table 13. Characteristics of methanogens isolated in pure culture (Sahm, 1983).

Species	Morphology	Substrate	Cell wall
Methanobacterium spp.	Long rods or filaments	H_2, formate	Pseudomurein
M. formicium		H_2	
M. thermoautotrophicum		H_2	
(*Methanothix*) *soehngenii*		acetate	
Methanobrevibacter spp.	Lancet-shaped cocci or short rods	H_2	Pseudomurein
M. ruminantium		H_2, formate	
M. smithii		H_2, formate	
M. arboriphilus		H_2	
Methanococcus vannielii	Motile irregular small cocci	H_2, formate	Polypeptide subunits
M. voltae		H_2, formate	
M. thermolithotrophicus		H_2, formate	
M. mazei	Pseudosarcina	H_2, methanol, acetate	
Methanomicrobium mobile	Motile short rods	H_2, formate	Polypeptide subunits
Methanogenium carciaci	Motile irregular small cocci	H_2, formate	Polypeptide subunits
M. marisnigri		H_2, formate	
Methanospirillum hungatei	Motile regular curved rods	H_2, formate	Polypeptide
Methanosarcina barkeri	Irregular cocci as single cells or packets	H_2, acetate, methanol, methylamines	Heteropolysaccharide

process. They have a doubling time of 2–3 days. They are strict anaerobes and require a lower redox potential (≈ -330 mV) than most anaerobes, relying on the hydrolytic organisms to maintain this low redox potential. In comparison with the hydrogen-utilizing methanogens, relatively few species are known to degrade acetate (*Table 13*). Biochemical studies of the methanogenic bacteria have shown that they differ from other bacterial groups in several important respects. Their cell walls contain no muramic acid and they exhibit resistance to antibiotics such as the penicillins and cephalosporins which affect the cell wall. Their lipids also differ chemically from those of classic bacteria, and several new coenzymes and factors have been discovered in association with their metabolism; coenzyme F420 has been recommended by Nyns and co-workers (Binot, Naveau and Nyns, 1982) as a method of recognizing methane bacteria in mixed culture. The methanogens have also been shown to require several trace elements such as iron, manganese, molybdenum, zinc, copper, cobalt, selenium, tungsten and nickel: Sahm (1983) has demonstrated that *Methanosarcina bakeri* requires 1·0 nmol cobalt, 150 nmol nickel and 0·5 nmol molybdenum; the cobalt is required for corrinoid Factor 3 and the nickel for a nickel tetrapyrolle cofactor.

These discoveries have enabled changes to be made in the design and control

of anaerobic systems. Any sudden large changes in load to the reactor must be avoided because these would encourage the hydrolytic and acid-forming bacteria (doubling times 30 min and 1–4 h respectively) to metabolize much more rapidly than the acetoclastic methanogens (doubling time 2–3 days). Any overload will be expressed by an increase in the hydrogen concentration in the gas, together with more propionic and butyric acids in solution. The concentration of hydrogen and types of volatile acid can therefore be monitored to control the feed rate.

Design of Anaerobic Systems

The second major advance in anaerobic treatment has been in reactor design. Traditionally, because of the recalcitrance of particulate organic matter and the slow growth rate of anaerobic bacteria, reactors have been designed as stirred tanks with very long residence times. Unlike sludges, the organic materials in waste waters are often in solution and thus are more amenable to treatment. In a completely mixed system such as the traditional sludge digester, hydraulic retention time (HRT) and solids retention time (SRT) are the same and the minimum HRT is defined by microbial growth rate. To overcome the long generation time (several days) special reactors have been developed to retain most of the organisms inside the reactor or to recycle the bacteria after separation. Solids retention time is thus uncoupled from HRT and high bacterial concentrations can be obtained. There are four basic designs (*see Figure 19* on page 293): (1) the anaerobic contact process; (2) the Upflow Anaerobic Sludge Blanket (UASB) in which the biomass is held in the reactor by flocculation; (3) fluidized or expanded beds; (4) anaerobic filtration (by upflow or downflow). In types (3) and (4) the biomass is attached to an internal support medium. Three companies (Biotechnics, Sweden; ESMIL, Holland; Agip, Italy) have installed more than 10 large-scale plants for industrial waste treatment. The most common application is for the treatment of vegetable processing waters, particularly from sugar production; the volume of such reactors currently ranges from 2000 m^3 to 20 000 m^3.

Dorr-Oliver (USA) have built three full-scale fluidized-bed plants; American Celanese (USA) have built three upflow anaerobic filter plants, and Bacardi Rum have constructed one downflow filter plant of 1300 m^3. There are four full-scale anaerobic plants in the UK: two are completely mixed, one has external biomass separation, and one has an upflow anaerobic filter. In all cases, anaerobic treatment has been designed as a high-rate pre-treatment stage to remove large amounts of polluting load and to recover energy. The most successful applications have been for the treatment of warm strong soluble wastes from the food and drinks industry. Under the best conditions, loads with a COD of 25–30 kg/m^3/day can be applied (Verstraete, 1983, Wheatley and Cassell, 1983). The quantity of methane produced can be related to the quantity of COD degraded, based on the stoichiometry of conversion of organic matter into carbon dioxide and methane (Andrews and Graef, 1971). The equation demonstrates a disproportionate breakdown of organic materials: in theory, therefore, the methane content of the biogas is correlated with the chemical composition of the substrate;

when alcohols are converted, then the methane content of the biogas is about 75%, but when carbohydrates are metabolized the methane content is only about 50%. Thus,

$$C_cH_hO_oN_nS_s + \tfrac{1}{4}(4c - h - 2o + 3n + 2s)H_2O \rightarrow$$
$$\tfrac{1}{8}(4c - h + 2o + 3n + 2s)CO_2 + \tfrac{1}{8}(4c + h - 2o - 3n - 2s)CH_4$$
$$+ nNH_3 + sH_2S$$

where c, h, o, n, s, represent the number of atoms of carbon, hydrogen, oxygen, nitrogen and sulphur, respectively. In practice, each kilogram of COD (mixed substrate) will yield 350 ℓ of methane and this gives a useful estimate of the efficiency of the process. *Table 14* shows some results obtained from three different types of waste treated by anaerobic upflow filtration.

Table 14. Comparative summary of results from anaerobic filtration.

Type of waste	Date	COD load applied (kg/m³/day)	COD removed (%)	Gas ratio (m³/kg COD removed)	Methane (%)
Maltings	May–July	4	65	0·23	68
	Aug.–Oct.	6	72	0·16	70
	Nov.–Jan.	8	70	0·10	65
Confectionery	May	5·0	40	0·23	74
	June	7·5	72	0·13	70
	July	5·4	78	0·10	59
	August	4·6	78	0·15	61
	September	4·2	75	0·18	58
	October	10·5	32	0·31	62
Distillery	Jan.–March	13·5	45	0·3	57
	April–June	22·1	69	0·6	57
	July–Sept.	23·1	63	0·5	59
	Oct.–Dec.	25·6	56	0·5	59

Most investigations into anaerobic treatment have encountered problems associated with pH control. The difficulty arises from differences in the growth rates and optimal pH of the synergistic bacterial populations in anaerobic fermentation. Once a stable population of the groups has been established, then the mixed culture is able to operate without external pH control but the differential rates of growth of the bacterial populations makes the system susceptible to overload, especially when treating industrial wastes which have insufficient alkalinity or additional nutrients. Three methods of controlling this problem have been tried (Henze and Harremoes, 1982): these are (1) high recycle rates; (2) chemical buffering, and (3) buffering using domestic sewage. High rates of recycle are an integral part of some anaerobic reactor designs such as fluidized beds and downflow filters (Szendrey, 1983).

On the basis of the energy recovered and the savings in discharge costs, there is a good economic case for the anaerobic treatment of strong wastes, and a rapid expansion of the technology is probable. Some basic research and development is still required, to improve reliability and start-up procedures and to reduce capital costs.

Future work

There is now a better understanding of the characteristics of the micro-organisms involved in the treatment of effluent and of the interrelationships between them. High biomass concentrations can now be retained in anaerobic reactors and loading rates of COD up to 30 kg/m^3/day can be achieved, with the maximum expected amounts of gas produced. Better conversions of direct substrates such as acetates have been reported (Verstraete, 1983) and it may be possible to increase the methanogenic activity by conventional strain selection or genetic manipulation.

Further work may also assist the control of toxicity; problems with ammonia, sulphate and calcium are very common. Wastes containing large quantities of organic nitrogen and of a high pH may produce toxic concentrations of ammonia (ammonia concentration should be below 100 mg/ℓ). High concentrations of sulphate (> 500 mg/ℓ) reduce methane production and generate large quantities of H_2S in the biogas (up to 4%). Most investigators (Sahm, 1983) have found that sulphide starts to inhibit methane formation at about 3 mmol/ℓ. Very hard waters (Ca > 6 mmol/ℓ) can give rise to calcium carbonate and calcium phosphate precipitates which may then cause blockage and reduced activity.

Microbial methane production occurs over a wide temperature range (0–97°C) but two distinct optima exist, one at 35°C and one at 60°C. Most work has been carried out on the mesophilic group and more work is required on the thermophilic and psychrophilic methane bacteria, particularly to test whether a temperature increase improves performance. Psychrophilic methanogens have not been isolated nor have mixed cultures shown a clear psychrophilic optimum; much more work is required to confirm that the process could be used for cold wastes. The start-up of anaerobic treatment systems is still a problem: at present a 30–50% inoculum of actively digesting sludge is used and techniques need to be developed to reduce this volume. Most authors agree that the process is slow (possibly taking up to 6 months) but the differences in acclimatization period necessary for different combinations of the various substrates and the trace nutrients required, are not well understood.

Conclusions

Aerobic effluent treatment is the largest controlled use of micro-organisms in the biotechnological industries. There is no obvious alternative to this type of treatment and it is likely that the size of this market will continue to stimulate the development of more efficient processes.

Of the new technologies discussed, only biomethanation has a promising economic future. The market forces are already sufficient to ensure that this technology is applied and continues to develop. Confidence in the reliability of the process is growing and it is predicted that the majority of new effluent plant installed in the biological industry will be of this type. The research and development priorities have been identified and there is a high level of development activity in the United States and Europe, to improve the process and to expand the range of substrates to which it is applied.

The use of bioprocesses for the breakdown of aqueous and atmospheric recalcitrants has yet to be realized and most research is still at a fundamental stage. There is strong competition from the non-biological methods which do not need such large investments in research and development. This type of process could, however, make a significant contribution to removing recalcitrants from the environment and there is a case for centralized or concerted action by national and international institutions to fund development work.

Experience has shown that the recovery and marketing costs of large-scale biomass recovery cannot, at present, be justified by the value of the product. More valuable products such as important metals, special oils, peptides and vitamins need to be recoverable from specific wastes if this technology is to expand.

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