Pollution of Aquatic Systems: Pollution Through Eutrophication, Fecal Materials, and Oil Spills

7

Abstract

Natural bodies of water will purify themselves and remove added materials given sufficient time. Pollution therefore occurs when the self-purifying powers of a body of water have not had enough time to remove the pollutant and return itself to its original state. Pollutants in water include bacteria, chemicals, heat and by the addition of organic or inorganic nutrients or eutrophication. Eutrophication causes excesses growth of cyanobacteria or blooms.

Pollution by fecal matter is determined principally by the identification of *E. coli* in water. Water bodies are expected to meet standards of the maximum content of microbial and chemical pollutants set by governments all over the world. The Total Maximum Daily Load (TDML) is a calculation of the maximum amount of a pollutant that a water body can contain and still meet water quality standards set by the regulating authority for a particular water use. Since not all *E coli* is necessarily of human origin, its source is determined through methods of microbial source tracking.

Oil spills are major sources of marine pollution. They are remediated physically by skimmers which collect the oil, adsorption onto suitable materials, the use of dispersants or in-situ burning. Biological methods include addition of nutrients to stimulate the growth of oil-degrading microorganisms, the use of surfactants to emulsify the oil and increase contact between oil and microorganisms, and the introduction of organisms specially adapted to growth on oil.

Keywords

Self-purification • Eutrophication • "Algal blooms" • Fecal pollution • Indicator organisms in pollution • Oil spills • Remediation of oil spills • Standard water analysis • Total Daily Maximum load (TDML) • Microbial source tracking (MST)

7.1 Nature of Pollution

Pollution of water may be defined as the introduction into a body of water of any material or condition which may be injurious to human health or offend aesthetic sensibilities and thus limit the use of water for drinking, recreation, fishing, and other activities to which water is normally put.

It should be borne in mind that the idea of pollution in the wider sense is anthropocentric (i.e., centered around man's needs). Seen in this light, pollution should be understood as a relative term, which depends

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on the specific use to which a particular body of water is put. A good example relates to the bacterium, *Sphaerotilus natans*. This bacterium grows profusely into strands when there is eutrophication (i.e., the availability of more nutrients than is usual), especially by the introduction of sewage. On account of the bacterium's abundant growth accompanied by its formation of strands when eutrophication occurs through the introduction of sewage, the trivial name of the bacterium is "sewage fungus." The sewage fungus is objectionable from the point of view of man's use of water, especially as it is reminiscent of sewage, although the excess nutrient causing its growth may not in fact come from sewage. Despite being ordinarily objectionable, *S. natans* is sometimes deliberately cultivated in the rivers of some countries. This is because the larvae of certain insects (*Chironomidae*) are encouraged to develop by the bacterium. The larvae in turn are fed upon by fish (trout), which is then farmed. When it is associated with fish farming, the sewage fungus is certainly not a pollutant!

7.1.1 The Concept of the Self-purification of Water as Basis for the Understanding of Pollution

When a large body of water either flowing, such as a river, or fairly static, such as a reservoir or lake, suffers eutrophication (the addition of nutrients and/or microorganisms), the organic materials and microorganisms introduced thereby will gradually disappear and the water will return to the state it was before the eutrophication. The process, which brings about this return to the original state is known as *self-purification*. Self-purification takes place in all bodies of water, including seas and oceans. The processes underlying self-purification can be grouped into physicochemical and biological. These will be discussed below to provide a basis for a better understanding of pollution.

1. *Physicochemical mechanisms in the self-purification of water*
   (a) Flocculation
   The materials introduced into water sediment at rates which depend on their weight; they remove themselves from general circulation as they sediment. Equally important is that, during this sedimentation, microorganisms, which bind to heavier organic materials as well as clay particles are dragged down as they sediment, and are removed from circulation.
   (b) Light
   Sunlight contains ultraviolet light, which has germicidal effects. Aquatic bacteria exposed to light are therefore killed, although the effect of light is limited to the uppermost portion of water.
   (c) Aeration
   Aeration is brought about by both the physical diffusion of air and the release of oxygen by the photosynthetic activity of algae and cyanobacteria. Aeration encourages the growth of aerobic bacteria, which participate in the self-purification of water by breaking down organic matter introduced into water. Additionally, certain compounds, for example, those of manganese and iron, are oxidized in the presence of oxygen and hence are precipitated, contributing to the self-purification of water.
   (d) Dilution
   Materials added to natural bodies of water are usually less in volume than the water body. As a consequence, the added materials are necessarily diluted when introduced. The dilution of added organic material helps reduce its concentration at the point of entry in the case of nonflowing waters such as lakes. In the case of flowing waters such as streams, the concentration of added organic matter is reduced both at the point of introduction and downstream as the water flow moves the added material downstream. Provided that the load of added organic matter is not so heavy that it cannot be sufficiently diluted, the process of dilution helps reduce the onset of pollution.
2. *Biological factors*
   Biological factors are by far the most important in the self-purification of water. Biological factors act through the breakdown (stabilization) of added materials by microorganisms, mainly by bacteria and occasionally by fungi. By far, the bulk of the breakdown is brought about by bacteria and among these, aerobic bacteria are most important. The activities of anaerobic bacteria lead to only partial breakdown of organic matter, and take much longer time. The factors, which affect the breakdown of materials added to water are discussed below.

   Factors affecting the biological breakdown of materials added to water

(a) The chemical nature of the added material
The nature of the material is crucial in deciding whether or not, and how fast, the added material is degraded or stabilized. For instance, sugars, starch, amino acids, and proteins are easily broken down, whereas cellulose is not as easily degraded. Organic chemicals from industrial activity are often only slowly degraded. In some cases they are not broken down at all unless microorganisms specially developed by enriching the materials to be degraded, are introduced.

(b) Predation by Protozoa
The bacteria, which breakdown organic materials, are themselves consumed by Protozoa, particularly the ciliates. Most of the bacteria, which are introduced in the process of eutrophication, for example, with sewage, are removed in this way. Among the ciliates, which are encountered in eutrophic waters are *Paramecium*, *Colpoda*, and *Carchesium.*

(c) Bacteriophages
Because bacteriophages attack and destroy specific bacteria and are so commonly found in polluted waters, some authors have suggested that they should be used as indicators of the presence of fecal bacteria.

(d) *Bdellovibrio* spp.
Bacteria belonging to this group are small (0.3–0.4-μm wide), Gram-negative, polarly flagellated, and comma-shaped rods. They are found in high numbers in eutrophic waters, especially those polluted with sewage effluent. They attack Gram-negative, but not Gram-positive, bacteria including *Escherichia coli*, *Pseudomonas*, *Erwinia*, *Salmonella*, and *Shigella*.

(e) Antibiotic activity
Antagonistic activity between some bacteria against other bacteria and between some algae against some bacteria has been reported. Thus, *Pseudomonas aeruginosa* and other bacteria have been reported to produce anti-coliform factors against *E. coli.* The production of similar materials has also been reported from *Bacillus coagulans* and *B. licheniformis*. Algae are well known as producers of antibacterial substances. The green alga *Scendemus obliquus*, for example, has been shown to be active against *Salmonella*, while the diatom *Skelotonoma costatum* has been shown to be active against *Vibrio* and *Pseudomonas* (Okafor 1985).

7.1.2 Definition of Pollution

As described above, organic materials and microorganisms added to water are removed by the self-purifying power of water acting through physicochemical and biological processes. The self-purifying power of water requires time to be effective. The definition of pollution to be given here relies on the idea that *given sufficient time* the self-purifying powers of any body of water will restore that body of water to the state it was before the added material entered the water, even with the addition of the most recalcitrant items.

Given sufficient time, even the most recalcitrant among degradable added materials will be removed because microorganisms able to degrade it will develop. In the process of the degradation through the development of the appropriate organisms, sufficient time will have occurred for the component parts of self-purification to occur.

Based on this idea, pollution occurs when the self-purifying powers of a body of water are not able to remove the materials added to a body of water because the component parts of the self-purifying process, including sufficient time, have not been able to achieve the removal. When the added materials have not been removed by the processes of self-purification, they may render the body of water unsuitable for normal uses to which a body of water is put, such as drinking, washing, irrigation, or for recreation such as swimming.

A 1971 United Nations report defined ocean pollution as: "The introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities, including fishing, impairment of quality for use of sea water and reduction of amenities." The definition given in this book tallies well with the United Nations definition as *given sufficient time*, "substances or energy (introduced into the) marine (or any aquatic environment) will be removed and will have no chance to "result in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities, including fishing, impairment of quality for use of sea water and reduction of amenities" (Okafor 1985).

7.1.3 Kinds of Pollutants

In general terms, a pollutant is anything, which when added to a body of water, directly or indirectly limits man's legitimate use or appreciation of that body of water because sufficient time has not occurred for the self-purifying powers of the water to remove the added materials or conditions. As mentioned earlier, the use of water can be found in the domestic, agricultural, transportation, and recreational arenas. A material, of whatever nature, which when added to water does violence to the observer's aesthetic appreciation would also be regarded as a pollutant. When this broader view of definition is adopted, one may classify pollutants into the following four categories: pollution by eutrophication, pollution by the addition of harmful or undesirable organisms, pollution by toxic materials and industrial effluents, and thermal pollution. The bulk of the discussion will be on pollution by eutrophication; the other three will be discussed very briefly before the comparatively extensive discussion on eutrophication.

1. *Pollution by the addition of harmful or undesirable organisms*
   This type of pollution typically occurs when sewage is the cause of the eutrophication. It can also occur when effluents rich in easily degradable organic materials are added to water, such from a food processing factory. When sewage derives from a population harboring enteric pathogens, water pollution caused by such sewage will naturally contain the pathogens of that community.
2. *Toxic materials and industrial effluents*
   These include industrial heavy metals, radioactive materials, and pesticides, which can be harmful to man in one way or the other. In this group also would be placed effluents from the chemical industry and pollution by crude oil when oil-tankers run aground. Pollution by crude oil during spills and methods of remediating the situation will be discussed later in this chapter.
3. *Thermal pollution*
   Industrialization uses power. When such power is derived from a nuclear plant, water is needed for cooling. Such cooling water becomes hot in the process and is usually discharged into rivers or estuaries. Cooling is also done in other industries outside nuclear establishments, for example, in metal smelting. As a result of the addition of hot water to a natural body of water the balance of the population in the ecosystem may alter, giving rise to a preponderance of thermophilic organisms. Heated water also renders bodies of water unusable for some of man's legitimate undertakings such as fishing.
4. *Pollution by eutrophication*
   Pollution by eutrophication is a regular occurrence in rivers and lakes and will be discussed in the following section.

   Oil spills important in oil producing countries and when accidents occur in the oceans with oil tankers will be examined at the end of this chapter.

7.1.4 Pollution by Eutrophication

Eutrophication, also known as nutrient pollution, is a process whereby water bodies, such as lakes, estuaries, or slow-moving streams, receive excess nutrients that stimulate excessive growth of microorganisms including algae and cyanobacteria. This enhanced growth often results in a bloom (see below).

Point and Nonpoint Eutrophication

Pollution by eutrophication may be *point* or *nonpoint. Point* pollution occurs when pollutants enter directly into a body of water from identifiable sources. The well-known Exxon Valdez oil spill best illustrates a point source water pollution. *Nonpoint* sources deliver pollutants indirectly through environmental changes, and cannot always be indentified directly. An example of this type of water pollution is when fertilizer from a field is carried into a stream by rain, in the form of runoff, which in turn affects aquatic life. Pollution arising from nonpoint sources accounts for a majority of the contaminants in streams and lakes.

The Role of Oxygen in Eutrophication

Dissolved oxygen (DO) is a most important factor in aquatic pollution by eutrophication. The excessive growth of aerobic bacteria (and to a lesser extent fungi), which are the primary stabilizers of added material results in greater demand for oxygen. Under normal conditions, the nutrient load of a stream is low and hence the bacterial and fungal populations are also low. With heavy eutrophication and the consequent increased microbial growth, the depletion of oxygen may be so severe that the dissolved oxygen is finally exhausted and anaerobiosis sets in; consequently, fish, which also depend on dissolved O_2, may die, followed by deaths among crustaceans, rotifers, and protozoa.

Under anoxic conditions (i.e., conditions of oxygen shortage), anaerobic bacteria develop. Their metabolism gives rise to the foul odors of H_2S and methane, as well as the black color of the mud, due to sulfide production by the anaerobic bacteria.

Furthermore, under anaerobic conditions the silting up of rivers and lakes occurs because the organic materials are not completely broken down, and hence the partially decomposed organic matter accumulates.

Reoxygenation of Water

Oxygenation of water helps reverse the effects of eutrophication by encouraging the growth of aerobic microorganisms. The reoxygenation of water bodies is brought about in two ways: (a) by the activities of aquatic photosynthetic cyanobacteria and algae and (b) by physical reaeration.

(a) *Photosynthetic organisms* in water use CO_2 released by the aerobic bacteria to produce carbohydrates and release O_2 as a by-product. Reoxygenation by photosynthetic organisms may be prevented or reduced if the water contains large amounts of suspended particles, which prevent light penetration.

(b) *Physical reaeration*: The temperature of water and the speed of its flow are two factors affecting the rate at which oxygen is physically reintroduced into water. The higher the temperature, the less the amount of oxygen or any other gas dissolved in water.

Regarding the speed of water, in lotic (swift-moving) waters there is turbulent movement of water; hence, there is regular change of water at the air/water interface where O_2 exchange actually takes place, leading to rapid reoxygenation of water. In slow-moving (or limnetic) waters, oxygen permeates into water from the fixed air–liquid interfaces by simple diffusion. Since this is slow, the lower layers of the water column will be more oxygenated in river under lotic conditions than when the same river is under limnetic conditions. Because the breakdown of organic materials in water is brought about by aerobic bacteria, whose growth is encouraged by the availability of oxygen, pollution by eutrophication (i.e., nondisappearance of added materials) is more likely to occur in limnetic waters, for example, lakes where the slower movement of water leads to less available oxygen for the activity of aerobic bacteria, than in rapidly moving or lotic waters (Okafor 1985).

7.1.4.1 "Algal Blooms" and Eutrophication

"Algal bloom" is a term used to describe an abundance of blue-green algae (Cyanobacteria) (and occasionally, proper algae) at the surface of lakes or reservoirs, which abundance is such as to confer on the water the color of the predominant organism (s) in the bloom. Blooms are also known as "water blooms," "flowering of waters," or "flos-aquae." Blooms can form and disperse again within a matter of hours and are found the world over but in temperate countries they form mainly in summer while in the tropics they may form at any time of the year (e.g., in Lake George, Uganda and Lake Kilotes, Ethiopia). As a rule, blooms form only in waters rich in dissolved nutrients, especially phophorus.

Blooms are formed mainly by cyanobacteria and those in which blooms have been found are listed in Table 7.1.

Blooms are sometimes regarded as useful since they provide additional food for fish and hence may increase the productivity of freshwater fisheries. Cyanobacteria of the genus *Spirulina* form blooms, which are consumed directly as food in Chad Republic in central Africa and probably other countries. It is used as a food supplement in Europe and the USA, because it is believed to impact positively on the health of the consumers (see Anonymous 2010a).

Blooms are, however, regarded as nuisance in many parts of the world, for the following reasons:

(a) In water-treatment plants they sometimes clog the filters.

Table 7.1 Cyanobacteria which form blooms (From http://www.cdc.gov/hab/cyanobacteria/table1.htm; Credit: Center for Disease Control [CDC]; Anonymous 2010a)

Order	Family	Genus
Chrococales	Chrococcaceai	*Coelosphaerium*
		Gomphosphaeria
		Microsystis
Nostocales	Oscilatoriaceae	*Oscillaloria*
		Spirulina
		Trichodesmium
	Nostocaceae	*Anabaena*
		Anabaenopsis
		Aphanizomenon
	Rivulariacea	*Gloetrichia*

(b) Many species produce unpleasant odors and tastes in drinking water.
(c) Some blooms produce toxic materials, which kill fish and domestic animals.
(d) Blooms make recreational lakes unavailable for fishing and other water sports.

Blooms are restricted mainly to blue-green algae (cyanobacteria) because most of them are able to float at some stage in their lives, whereas other organisms are always heavier than water and hence sink to the bottom; not all blue-green algae can, however, form blooms. Buoyancy is conferred on those, which form blooms by gas vacuoles present in the protoplasm of these bacteria.

Factors Encouraging Bloom Formation

The following factors affect the development of algal blooms:

(a) Availability of inoculum: In tropical lakes, which regularly form blooms, the collapsed cells and resistant structures sink to the bottom and may be lodged in the mud from where they develop when conditions are favorable.
(b) Sudden influx of organic nutrients: One of the major predisposing factors of bloom formation is a sudden influx of organic material such as is found in sewage. Since cyanobacteria are photo-autotrophic and can manufacture their own food, they would appear to absorb the added nutrients mostly in the dark. It is also possible that the addition of sewage introduces trace elements, which are required by the bacteria. Furthermore, eutrophication with organic matter induces the growth of aerobic bacteria, which not only use up the oxygen and create anaerobiosis, but also release CO_2. The CO_2 would encourage greater photosynthesis by the cyanobacteria, while anaerobiosis is known to favor nitrogen fixation in them, producing nitrogenous compounds, which encourage the growth of aerobic bacteria; the net result of these activities is the growth of the cyanobacteria and the aerobic decomposers.
(c) Availability of light and a suitable temperature: Light is required for photosynthesis by cyanobacteria. Suitably warm temperatures dictate that blooms are formed in the summer and autumn in the temperate countries and year-round in the tropics.
(d) Availability of suitable pH: Blooms tend to form more in hard waters (which contains Mg^{2+} and Ca^{2+} carbonates) than in soft waters. Furthermore, they will not form in water with a pH value less than 6. Indeed, most cyanobacteria grow best in the pH range 7.9–9.0. The Mg^{2+} and Ca^{2+} carbonates would appear to act as buffers, which prevent the pH from changing drastically.
(e) The availability of phosphorous and nitrogen: These two nutrients, especially phosphorous, are critical and must be available in suitable quantities before blooms can form.

Factors Adversely Affecting Blooms

Blooms may be suppressed by a number of biological agents within a body of water. These include fungi of the order *Chytridiales* and *Blastocladiales*, which attack cyanobacteria. Some viruses ("cyanophages") attack cyanobacteria, while some protozoa, for example, *Pelomyxa* (amoeboid) and *Ophryoglena* (ciliate) graze them; finally, fish, for example, *Tilapia* and *Haplochromis* consume large quantities of cyanobacteria.

When blooms are a definite nuisance such as in reservoirs, they may also be destroyed by vigorous shaking.

7.1.5 Biological Indicators of Pollution by Eutrophication

Pollution by eutrophication is difficult to measure by the chemical analysis of the components of water. This is because it would involve an unthinkable amount of work if the various organic and inorganic compounds, which cause pollution were to be determined by this means, even if they were all identifiable. For this reason, the method used for determining the decomposable organic matter added to water is the biochemical oxygen demand (BOD), which determines the amount of oxygen consumed by aerobic bacteria in the decomposition of organic matter in water (see Chap. 11).

However, a method simpler than the BOD technique can be employed. This method is based on knowledge of the change in the flora and fauna of natural waters as a natural water changes from a eutrophic to postpollution status. It consists of examining the various organisms found in water. Although not favored by all aquatic biologists the method recommends itself to the environment of developing countries where equipment and reagents for the most simple analyses are often lacking. Among biological indicators of water pollution, the following have been used: fish, algae, sponges, and benthic invertebrates. However, bacteria and protozoa are the principal organisms used.

(a) *Use of bacteria*
The use of bacteria to detect pollution is based on the principle that when there is a high nutrient concentration the biological population is high and diverse (i.e., a large number of a wide range of bacteria are present) unless toxic materials are present in the water or there is a shortage of some key nutrients. This high bacterial population (apart from using up the nutrients) uses up the oxygen and hence creates anaerobic or near-anaerobic conditions. The result is that many bacteria die out, and only a few types will remain. This is even accentuated by the grazing activity of protozoa.

In order to detect pollution changes it is necessary to make observations at least from two different points along the stream or to do so at some point at two different occasions. By taking plate counts on a variety of media and both under aerobic and anaerobic conditions, it is possible to follow the progress of pollution.

(b) *Use of protozoa and other organisms*
The types of organisms present besides the bacteria will indicate whether the water is just receiving pollutants or is just recovering from them by natural self-purification. If bacteria, protozoa of group Sarcodina, flagellates, and free-swimming ciliates are seen in that order of abundance, then the pollution is fresh. On the other hand, if stalked ciliates, free-swimming ciliates, and bacteria are seen in that order of abundance, then the water is recovering from pollution. A predominance of insects and rotifers indicates that the pollution is over as these organisms are encountered in bodies of water low in organic matter.

7.2 Pollution of Water with Reference to Human Health: Bacterial Indicators of Fecal Pollution

Many parameters are used by different authorities as indicators of water pollution. These parameters include biological oxygen demand (BOD), dissolved oxygen (DO), pH, specific conductance, water temperature, and chemical constituents including nitrates and phosphates. BOD and DO will be discussed in Chap. 11.

The most widely used parameters are, however, bacterial indicators of fecal pollution. The indicators have been undertaken in order to protect human health. They have been in use for over a 100 years and the procedures involved have become highly standardized.

Bacterial indicators are used to indicate if:

(a) Drinking water sources are fit for drinking.
(b) Treatment of drinking water has been adequate.
(c) Drinking water in the distribution system continues to be protected.
(d) Recreational and shellfish waters are microbiologically safe.

7.2.1 Microbiological Examination of Water for Fecal Contamination

The purpose of examining water microbiologically is to help in the determination of its sanitary quality and its suitability for general use. The sanitary quality of water may be defined as the relative extent of the absence of suspended matter, color, taste, unwanted dissolved chemical, bacteria indicative of fecal presence, and other "aesthetically offensive" objects or properties. In short, the sanitary quality of water depends on its acceptability for internal consumption and other uses in which water comes directly or indirectly in contact with man.

The current bacterial indicator approaches have become standardized, are relatively easy and inexpensive to use, and constitute the cornerstone of many monitoring and regulatory programs. Due to an increased understanding of the diversity of waterborne pathogens, including their sources, physiology, and ecology, a growing understanding has occurred that the use of bacterial indicators may not be as universally protective as was once thought. Thus, the greater environmental survival of pathogenic viruses and protozoa, when compared with indicator bacteria (coliforms) has raised serious questions about the suitability of relying on relatively short-lived organisms as an indicator of the microbiological quality of water (Anonymous 2004, 2005).

The implication of this situation is that while the presence of coliforms could still be taken as a sign of fecal contamination, the absence of coliforms can no longer be taken as assurance that the water is uncontaminated. Thus, existing bacterial indicators and indicator approaches do not, in all circumstances, identify all potential waterborne pathogens. Furthermore, recent advances in microbiology, molecular biology, and analytical chemistry make it necessary to reassess the current reliance on traditional bacterial indicators for waterborne pathogens.

Nevertheless, indicator approaches will still be required for the near future for two reasons. Firstly, it will be impracticable to monitor all known waterborne pathogens directly. Secondly, pathogens usually occur in very low numbers and even where it is possible to culture them, this may present difficulties on account of their low numbers.

Organisms to be used as indicators of pollution should have certain attributes. Although the attributes of the ideal bacterial have been refined, they are still based on those described by Bonde (1966) (see Tables 7.2 and 7.3).

7.2.1.1 Principle of Indicator Organisms

The greatest hazard associated with drinking water is that it may recently have been contaminated with sewage or by human (or even animal) excrement. Water recently contaminated by feces from patients or carriers of waterborne pathogens, for example, cholera, *Salmonella*, and *Shigella* may carry the live pathogens and thus be a source of fresh outbreaks. It is, however, not practicable to isolate and identify these pathogenic organisms as a routine practice. When pathogens are present in sewage or feces, they are, however, usually out-numbered by bacteria normally present in the alimentary canal and hence in the feces. These normal inhabitants are easier to detect. If they are not found in water it can be inferred in general that the water is free of pathogens, but it should be borne in mind that viruses may well be present.

Table 7.2 Criteria for an ideal indicator (Bonde 1966. With permission)

An ideal indicator should
1. Be present whenever the pathogens are present
2. Be present only when the presence of the pathogens is an imminent danger (i.e., they must not proliferate to any greater extent in the aqueous medium)
3. Occur in much greater numbers than the pathogens
4. Be more resistant to disinfection and to the aqueous environment than the pathogen.
5. Grow readily on simple media
6. Yield characteristic and strong reactions enabling as far as possible an unambiguous identification of the group
7. Be randomly distributed in the sample to be examined, or it should be possible to obtain a uniform distribution by simple homogenization procedures, and
8. Grow widely independent of other organisms present when inoculated into artificial media (i.e., indicator bacteria should not be seriously inhibited in their growth by the presence of other bacteria)

Table 7.3 Suggested refinements of biological attributes of the fecal indicator and its detection methods (Bonde 1966. With permission)

Desirable biological attributes of indicators
1. Correlated to health risk: the indicator should be present whenever the pathogen is present
2. Indicator should have similar or (greater) survival than pathogen to adverse conditions:
• Ultraviolet exposure
• Temperature
• Salinity
• Predation by indigenous flora
• Desiccation
• Freezing
• Response to disinfectants
• Biologic survival mechanisms (where available) would advantageous
Sporulation
Cyst and other latency mechanisms
Arrested metabolism (viable, but nonculturable)
Shock proteins and other survival strategies
3. Similar or greater transport than pathogens during
• Filtration
• Sedimentation
• Adsorption to particles
4. Indicator should always be present in greater numbers than pathogen
5. Indicator should be specific to fecal source or identifiable as to source of origin
Desirable attributes of detection methods for fecal indicators
1. Specificity to desired target organism which is
• Independent of matrix effects
2. Broad applicability
3. Precision: method should be precise
4. Rapidity of results
5. Quantifiable
6. Measures viability or infectivity: method should measure only living organisms
7. Logistical feasibility
• Training and personnel requirements should be easy and accessible
• Utility in field
• Cost
• Volume requirements

The organisms which are used as indicators of fecal contamination are the following:

E. coli
Streptococcus fecalis
Clostridium welchii (*C. perfringens*)
Bifidobacteria

Among these the most commonly used indicators are *E. coli* and the coliform group as a whole. *E. coli* is of fecal origin and *E. coli* Type I (Eijkmann positive), which is of human (and warm-blooded animal) origin grows at 44.5°C. Hence, the presence of fecal *E. coli* is a definite indication of fecal pollution. All coliforms in general are not necessarily of fecal origin; nevertheless, since they are not *indigenous* to water their presence in drinking water should cast suspicion on the water and should indicate pollution in the widest sense.

Coliform bacteria are defined as Gram-negative, nonspore-forming rods capable of fermenting lactose, aerobically or facultatively, with production of acid and gas at 35°C in less than 48 h.

S. fecalis occurs regularly in feces in numbers that usually probably disappear at the same rate as *E. coli*, but quicker than other coliforms. When, therefore, it is found in water in which *E. coli* cannot be detected, this is an important confirmatory evidence of fecal pollution.

C. welchii is also regularly present in human feces but fewer than *E. coli.* Spores survive longer in water and usually resist chlorination. Its presence suggests fecal contamination and the absence of *E. coli* suggests contamination in the distant past.

7.2.1.2 Procedure for the Determination of Fecal Contamination

1. *Collection of samples*
   Samples of water for bacteriological examination should be collected in clean sterile bottles, and should not be less than 100 ml. If the water to be examined is chlorinated, then a dechlorination agent should be added to the bottle before sterilizing. Sodium thiosulfate is usually used for the purpose, the amount employed depending on the amount of residual chlorine in the water (0.1 ml of 10% solution of sodium thiosulfate will neutralize a 250-ml sample of water containing 15 mg of residual chlorine per liter). For samples containing metals such as tin or copper, a chorinating agent such as EDTA should be added. If the water is collected from a tap it should be allowed to run for 2–3 min before the bottle is filled. If from a river, stream, lake, reservoir, etc. the aim should be to collect the water in the same way as for consumers. In samples suspected to be highly contaminated, dilutions of up to 10^4 may be required. The colonies should, as in routine plate counting, lie between 30 and 300 (Anonymous 2006).
2. *Media used for enumerating indicator organisms of fecal pollution*
   Various media are used as indicator organisms. In effect they are selective media, which eliminate other organisms while encouraging the development of the indicators. The active components of the more common media are discussed briefly.
   (a) Coliform media
   Among the short Gram-negative rods the ability to ferment lactose appears to be limited to the family *Enterobacteriaceae* within which the coliforms are found. Anaerobic spore formers notably *C. welchii*, which are also of fecal origin also ferment lactose, as do some aerobic spore formers. In order to inhibit these Gram-positive organisms during the initial (presumptive) tests for coliforms, a number of inhibitors are employed; some of the Gram-positive inhibitors' activities promote the growth of coliforms. The inhibitors act by lowering the surface tension of the medium thereby making the medium more favorable for the growth of intestinal organisms, which are already adapted to the low surface tension (due to bile salts) of the lower alimentary canal. Sometimes dyes and other chemicals, which selectively inhibit Gram-positive bacteria are used. Some surface tension-reducing salt compounds appear to act by both methods.

   The surface tension-reducing agents, which have been used include the following: Ox bile, bile salt (or sodium taurocholate), lauryl sulfate, and sodium ricinoleate (i.e., soap made from castor oil). The dyes, which have been used are the triphenyl methane dyes which in low concentrations inhibit Gram-positive organisms. In much higher concentrations they inhibit even Gram-positives. Thus, brilliant green inhibits *C. welchii* at 1:30,000, but coliforms grow at 1:350. Gentian (crystal) violet inhibits the anaerobe at 1:9,000, but at 1:100,000 begins to inhibit the coliforms. Rosolic acid, a dye in this family, has also been used.

   After the initial (presumptive and confirmatory) tests, which are described latter in this chapter, the media used in the subsequent completed tests do not need to contain Gram-positive inhibitors. The media used on both sides of the Atlantic vary slightly, but the principles dictating which medium is used at which stage are

Table 7.4 Some bacteriological media used for the presumptive and confirmed tests in water examination (Compiled from *Standard Methods for the Examination of Water and Wastewater*; Anonymous 2006)

Medium	Dye for inhibiting Gram-positive bacteria	Reducing agent	Buffer	Nitrogen source	pH indicator	Remarks
Media for presumptive test						
Brilliant green lactose bile (BGLB)	Brilliant green	Ox bile	Peptone	Peptone	Brilliant green	–
Lauryl sulfate tryptose broth (LST)	–	Sodium lauryl sulfate/bile salts	Na/K_2HPO_4, KH_2PO_4	Tryptose	–	–
McConkey broth	–	Bile salts	Peptone	Peptone	Neutral red	
Media for confirmed test						
Eosin methylene blue (EMB) agar	–	–	Peptone, K_2HPO_4	Peptone	Eosin	Metallic sheen produced by *Escherichia coli*
Endo agar	–	–	Peptone	Peptone	Basic fuchsin	

the same. Table 7.4 lists some of the media, which have been used. They all contain lactose, a buffer, a nitrogen source, while those used for the presumptive test contain besides the above, a pH indicator, Gram-positive inhibitor, and/or a surface-tension reducer.

One of these media, E.M.B. agar, characteristically shows *E. coli* colonies as having a metallic sheen, whereas other coliforms, particularly *Enterobacter* and *Klebsiella,* are not only larger on this agar, but do not show this metallic sheen The metallic sheen typical of *E. coli* depends on the action of the eosin and methylene blue to form a methylene blue eosinate, which acts as an acidic dye. Absorption of the eosinate by the *E. coli* cells is facilitated by the lowering of the pH by the acid produced by the fermentation of the lactose.

(b) Streptococcus medium
Several media are available for selectively growing *Streptococcus*, including azide dextrose broth, Ethyl violet azide broth, and KF streptococcus, the compositions of which are found in *Standard Methods.* They all have in common the presence of sodium azide, which inhibits Gram-negative bacteria. They also contain buffers, nitrogen source, and other requirements of a bacteriological medium.

(c) *C. perfringens* (welchii) medium
C. perfringens produces a large number of gas bubbles in litmus milk. The clot of protein produced by the acid from the fermentation of the milk lactose is broken up by the gas bubbles to produce the so-called stormy clot.

7.2.1.3 Methods Used in the Enumeration of Indicator Organisms in Water

Two general methods are used for enumerating fecal contaminants in water (and indeed any liquid): the multiple tube method and the membrane-filter method. With either method it is customary to relate the numbers to 100 ml of water. Both methods may be used with all three indicator organisms.

1. *The multiple-tube fermentation method*
The multiple tube method is basically the dilution count (or *dilution to extinction*), which is made more accurate by the use of several tubes. In the ordinary dilution count, a serial tenfold dilution of the liquid whose bacterial load is to be counted is made. A loopful is introduced from each dilution to a suitable liquid medium in a tube, which contains a Durham tube to trap any gas produced. This method can only be used if no growth occurs in one of the dilutions introduced into the broth (hence the alternate name of "dilution to extinction" (see Fig. 2.2). In the ordinary dilution, the number of organisms in the original liquid is assumed to be the reciprocal of the dilution just before the negative one, that is, the highest dilution. Thus, if of a series of ten tubes in serial tenfold dilution, the highest dilution in which growth occurred is 10^3, the number of organisms in the original tube is taken as 10^3 or 100 (or more accurately more than 10^3 but less than 10^4. The rationale behind this is illustrated in Fig. 2.2.

The basic reason for the development of the most probable number (MPN) method can be seen from Fig. 2.2. Suppose several replicates were taken of the 10^3 dilution, then growth might occur in one at

least if the number was less than ten. The MPN tables were developed to take care of this possibility by using statistical tables.

In the multiple tube method several replicates are used in accordance with probability tables to determine the most probable numbers (for details of the procedures, *Standard Methods* should be consulted). Table 7.5 shows a table of MPN numbers. The number of organisms is recorded in terms of most probable numbers (MPN). Figures obtained in MPN determinations are indices of numbers which, more probably than any others, would give the results shown by laboratory examination. It should be emphasized that they are not actual numbers, which, when determined with the plate count are usually much lower.

Membrane filtration method

This method consists in filtering a measured volume of the sample through a membrane composed of cellulose esters and other materials. All the bacteria present are retained on the surface of the membrane and, by incubating the membrane face upward on a suitable medium and temperature, colonies develop on the membrane, which can then be counted. The volume of liquid chosen will depend on the expected bacterial density of the water and should be such that colonies developing on the membrane lie between 10 and 100.

The great advantage of the filtration method over the multiple-tube method is its rapidity. Thus, it is possible to obtain direct counts of coliforms and *E. coli* in 18 h without the use of probability tables. Secondly, labor, media, and glassware are also saved. Thirdly, neither spore-bearing anaerobes nor mixtures of organisms, which may give false presumptive reactions in McConkey broth cause false-positive results on membranes. Finally, a sample may be filtered on the spot or in a nearby laboratory with limited facilities instead of taking the liquid to the laboratory.

However, membranes have the following disadvantages:

(a) They are unsuitable for waters of high turbidities in which the required indicator organisms are low in number, since they are blocked before enough organisms have been collected.
(b) When non-coliforms predominate over coliforms, the former may overgrow the membrane and make counting of coliforms difficult.
(c) If nongas-producing lactose fermenters predominate in the water, false results will be obtained.

Both methods do not give the same results. Parallel tests should, therefore, be used to determine corresponding results but using the multiple-tube method as the standard.

2. *Examination of water for general coliforms*

Coliforms may be enumerated by two methods: the multiple-tube and the membrane filtration methods.

(a) Multiple-tube method

In the examination of water for coliforms the procedure is carried out as the *Presumptive Test*, the *Confirmed Test*, and the *Completed Test.* The schematic outline indicating the appropriate media and conditions for these tests is set out in Table 7.6. The details of the various methods are available in *Standard Methods* (21st edition) (Anonymous 2006) and only an outline will be given here. Not all the tests need to be employed all the time. What is used depends on the nature of water and the use to which it is to be put. Thus, the presumptive test alone is used for a polluted water, whose consideration for direct potability without further treatment is not in question. The Confirmed Test alone is applied to potable water in the process of purification, chlorinated sewage effluents and bathing waters, and the finished water samples. The Completed Test is used for drinking water.

In computing the MPN numbers for presumptive, confirmed, or completed test, in the multiple-tube method of determining numbers, the combination of sample sizes to be used should be obtained by a knowledge of the quality of the water as indicated below. Furthermore, the appropriate combination of water sample sizes to be used should be chosen by examining the MPN. Different sets of tables are available in *Standard Methods for the Examination of Water and Waste Water*. One such set of tables is given in Table 7.5. The combination of positive and negative tubes is read off the table against the sample sizes to determine the MPN.

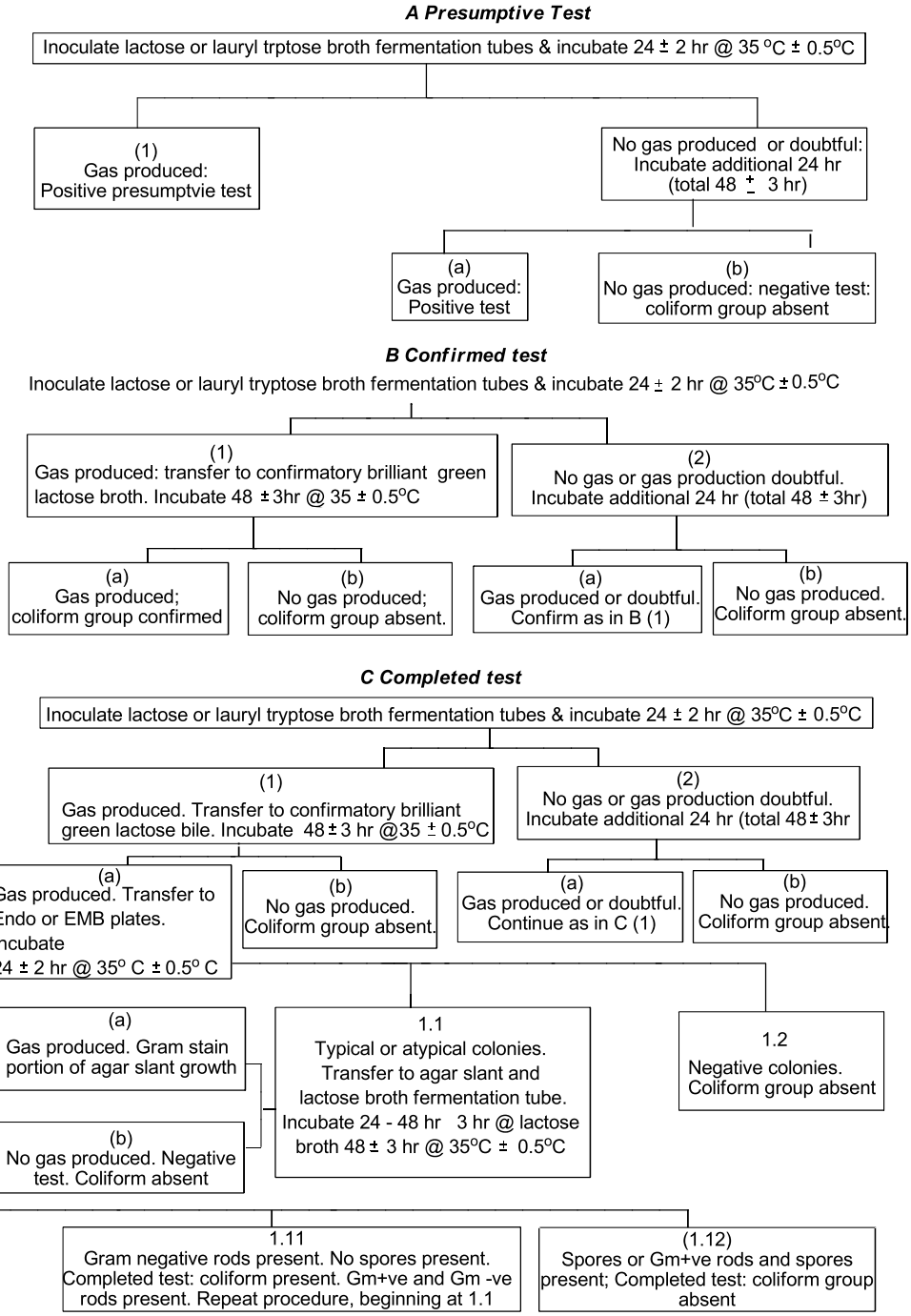
The combination of sample sizes used in Table 7.5 are 5 of 10 ml, each, 5 of 1 ml each, and 5 of 0.1 ml each. It is possible, however, to use larger or smaller portions. Thus, samples of 100, 10, or 1 ml could be used when the MPN is 0.1 times the values. Similarly, 1.0, 0.1, and 001 ml may be used when the MPN numbers should be ten times those on the table.

Table 7.5 Sample MPN table (From Anonymous 2006)

MPN index and 95% confidence limits for various combinations of positive results when five tubes are used per (10 ml, 1.0 ml, 0.1 ml)[a]							
Combination of positives	MPN index/ 100 ml	Confidence limits		Combination of positives	MPN index/ 100 ml	Confidence limits	
		Low	High			Low	High
0-0-0	<1.8	-	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.090	6.9	4-1-1	21	6.8	42
0-1-1	3~6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	.4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	10	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1,100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1,100
3-4-1	24	9.8	70	5-5-2	540	150	1,700
3-5-0	25	9.8	70	5-5-3	920	220	2,600
4-0-0	13	4.1	35	5-5-4	1,600	400	4,600
4-0-1	17	5.9	36	5-5-5	>1,600	700	
4-0-2	21	6.8	40				

[a]Results to two significant figures

Table 7.6 Procedure for the standard bacteriological analysis of water (Modified from *Standard Methods for the Examination of Water and Wastewater*; Anonymous 2006)



At least 100 ml of water are required for the MPN test. The volumes to be used in tests in liquid media will depend on the quality of the water to be examined, and the experience of the worker. When the water is expected to be of good quality one 50-ml volume and five 10-ml

volumes are suitable. If it is of doubtful quality, one 50 ml, five 10 ml, and five 1-ml quantities could be used. When the water is heavily polluted the original water may have to be diluted by a factor of 100 or more in order to obtain some negative results in the series put up, and thus obtain a finite figure for the MPN. Whatever the series used, the volumes of water in the individual tubes and the number of tubes containing each volume of water should be such that an estimate of the MPN number of organisms present in 100 ml of the original water can be obtained from the table.

In the procedure outlined in *Standard Methods* for carrying out the presumptive test, water is inoculated into multiple tubes (chosen as indicated above) of lactose or lauryl tryptose broth and incubated at 35°C. The production of acid and gas within 48 h indicates a positive presumptive test.

In the confirmatory test, one to three loopfuls of broth are transferred from positive tubes to brilliant green lactose broth (BGLB) or streaked on endo or EMB agar (although the latter is not recommended). Gas formation after 48 h at 35°C indicates a positive Confirmed Test using the liquid medium.

On EMB agar, a typical positive colony of *E. coli* is nucleated with or without a metallic sheen. An atypical colony is opaque unnucleated, mucoid, and pink after 24 h in the incubator.

In the Completed Test, a positive growth from BGLB broth is streaked on endo or EMB agar. If plates were used in the confirmatory test typical and atypical colonies are transferred to lactose or lauryl tryptose broth, as well as to nutrient agar. Formation of gas in secondary fermentation and the occurrence of Gram-negative rods on staining is a positive Completed Test and indicates that *E. coli* is present. It ought to be pointed out that the media and some aspects of the techniques set out in Table 7.4 and which are available in *Standard Methods* are not universally adopted. On the European continent, the media used are different.

(b) Membrane filtration method

After filtration the pad is picked up with sterile forceps and placed on an absorbent pad soaked in lauryl tryptose broth (or agar) if enrichment is desired or directly on to M-Endo soaked pad or M-Endo agar. The result is expressed as coliform density (total coliform colonies per 100 ml). This is calculated as follows:

$$\frac{\text{Coliform colonies counted} \times 100}{\text{ml sample filtered}}$$

3. *Fecal coliforms*

The above tests are for general coliforms; they do not distinguish coli forms of animal origin from others. They are used for the examination of potable water since no coliform of any kind should be tolerated in treated water. For investigations of stream pollution, raw water sources, sewage treatment systems, bathing water, and general water-quality monitoring, it is important to know whether or not the coliform is of fecal origin, that is, whether or not from the intestine of warm-blooded animals.

The procedure for detecting fecal coliform is similar to the above in both the multiple tube or the membrane technique. In each the confirmatory test is carried out at an elevated temperature of 44.5°C for 24 h (i.e., the Eijkmann test). Gas production indicates coliform of warm-blooded animals. Prior to the confirmatory test, however, the coliform must be enriched in the lactose broth used in the presumptive test. A loopful is then transferred from all positive tubes to the confirmatory broth (Brilliant Green Lactose Bile Broth at 44.5°C for 24 h or Boric Acid Lactose Broth at 45°C for 48 h). When the membrane filter method is used, the incubation of the filters at 44.5°C may be done directly after the filtration of water.

Fecal Streptococci

(a) Multiple-tube method

Multiple portions of water are inoculated into tubes of glucose azide broth and incubated at 37°C for 72 h. When acidity is observed a heavy inoculation is introduced into further tubes of glucose azide broth and incubated at 44.5°C for 48 h. Tubes showing acidity at this temperature contain fecal streptococci.

Multiple portions of water may also be inoculated into tubes of buffered azide-glucose-glycerol broth (BAGG) and incubated at 44.5°C for 48 h. Acid production is indicative of fecal streptococcal presence, but the broth should be checked with Gram stain to confirm the presence of Gram-negative rods.

(b) Membrane filter technique
After filtration, the filter is placed on a plate of glucose-azide agar. It is then incubated at 37°C for 4 h and then at 44°C for 44 h. Red or maroon colonies are fecal streptococci.

4. *C. perfringens*
Multiple portions of water previously heated at 75°C for 10 min to kill nonspore formers are put into well-filled screw-cap bottles containing Differential Reinforced Clostridiral Medium (DRCM) and incubated at 37°C for 48°h. A positive reaction is shown by a blackening of the medium due to a reduction of sulfite and precipitation of ferrous sulfate. Any clostridium will show this reaction, but when inoculations are made from positive tubes into litmus milk a "stormy clot" is produced, that is, gas bubbles break up the clot of milk.

7.2.1.4 Standard Water Analysis

The procedure for standard water analysis is summarized in Table 7.6 and discussed below. It is divided into the presumptive test, the confirmed test, and the completed test (Anonymous 2006).

The Presumptive Test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample to be tested. The series of tubes may consist of three or four groups of three, five, or more tubes. The more tubes utilized, the more sensitive the test. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes (see Table 7.5).

The Confirmed Test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. However, it is possible that the formation of gas may not be due to the presence of coliforms. In order to confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram-positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. *E. coli* and *E. aerogenes* can be distinguished from one another by the size and color of the colonies. *E. coli* colonies are small and have a green metallic sheen, whereas *E. aerogenes* forms large pinkish colonies.

If only *E. coli* or both *E. coli* and *E. aerogenes* appear on the EMB plate, the test is considered positive. If only *E. aerogenes* appears on the EMB plate, the test is considered negative. The reason for this interpretation is that, as previously stated, *E. coli* is an indicator of fecal contamination, since it is not normally found in water or soil, whereas *E. aerogenes* is widely distributed in nature and occurs outside the intestinal tract.

The Completed Test

The completed test is made using the organisms which grew on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 h at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample (Table 7.6).

7.2.1.5 Total Maximum Daily Loads and Microbial Source Tracking in Water Pollution

The US Clean Water Act (the actual official title is The Federal Water Pollution Control Act, 1972), in section 303(d)(1)(C) established the water quality standards and the Total Maximum Daily Loads (TMDL) programs thus (Anonymous 2007): (see Fig. 7.1).

> (c) Each State shall establish for the waters identified in paragraph (1)(A) of this subsection, and in accordance with the priority ranking, the total maximum daily load, for those pollutants which the Administrator identifies under section 304(a)(2) as suitable for such calculation. Such load shall be established at a level necessary to implement the applicable water quality standards with seasonal variations and a margin of safety which takes into account any lack of knowledge concerning the relationship between effluent limitations and water quality.

A Total Maximum Daily Load (TMDL) is thus a calculation of the maximum amount of a pollutant that a water body can receive, from point and nonpoint sources, and still meet water quality standards, and an allocation of that amount to the pollutant's sources. The calculation must include a margin of safety to ensure that the water body can be used for the purposes the State has designated, account for seasonal variation in water quality, and natural background conditions.

General Pollutant	Number of TMDLs Approved	Percent of Reported
METALS (OTHER THAN MERCURY)	5263	20.93
PATHOGENS	5073	20.17
NUTRIENTS	3146	12.51
SEDIMENT	2340	9.31
OXYGEN DEPLETION	1383	5.50
PH	1160	4.61
TEMPERATURE	1020	4.06
AMMONIA	909	3.61
PESTICIDES	845	3.36
MERCURY	780	3.10
SULFATES	769	3.06
TURBIDITY	583	2.32
SALINITY/TDS/CHLORIDES	538	2.14
CHLORINE	322	1.28
PCBS	242	.96
TOXIC INORGANICS	168	.67
OTHER TOXIC ORGANICS	134	.53
CAUSE UNKNOWN - BIOLOGICAL INTEGRITY	132	.52
OTHER CAUSE	72	.29
ALGAL GROWTH	65	.26
TOTAL TOXICITY	60	.24
FLOATABLES	46	.18
HABITAT ALTERATION	24	.10
NOXIOUS AQUATIC PLANTS	21	.08
DIOXINS	17	.07
RADIATION	17	.07
OIL AND GREASE	12	.05
TASTE, COLOR AND ODOR	5	.02
FLOW ALTERATION	1	.00

Fig. 7.1 Total Nationwide Number of TMDLs Approved since October 1, 1995 reported to EPA: 25,147 (Source: Anonymous 2007)

The Clean Water Act, requires States, Territories, and authorized Tribes every 2 years to prepare a list of impaired waters (i.e., water bodies for which water quality standards set for them by the States will not be met after application of technology-based controls), and establish priorities for action among the listed water bodies. These impaired waters do not meet water quality standards that states, territories, and authorized tribes have set for them for activities such as drinking water supply, contact recreation (swimming), and aquatic life support (fishing), and the scientific criteria to support that use. The States must then establish total maximum daily loads (TMDLs) for each listed water body, which are the sum of waste load allocations for point sources, load allocations for nonpoint sources, natural background contributions, and a margin of safety.

Until recently many states, territories, authorized tribes, and EPA had not developed many TDMLs' although they had been required by the Clean Water Act since 1972. Citizen organizations began bringing legal actions against EPA seeking the listing of waters and development of TMDLs. Legal actions have been instituted by these organizations in nearly 80% of the states and EPA is under court order or consent decrees in many states to ensure that TMDLs are established, either by the state or by EPA.

The processes for establishing a TDML are as follows:

1. Identify waters that do not meet water quality standards. In this process, the state identifies the particular pollutant(s) causing the water not to meet standards.
2. Prioritize waters that do not meet standards for TMDL development (e.g., waters with high naturally occurring "pollution" will fall to the bottom of the list).
3. Establish TMDLs (set the amount of pollutant that needs to be reduced and assign responsibilities) for priority waters to meet state water quality standards. A separate TMDL is set to address each pollutant with concentrations over the standards.
4. Develop strategies for reducing water pollution and assess progress made during implementation of the strategy. This is when a watershed partnership, including citizen groups, most likely will want to get involved. If the partnership has already developed a plan of action, it should be shared with the state. In fact, several states have incorporated watershed partnership plans in the state's strategy for specific TMDLs. The advantage is that local citizen groups usually know the topography and aspects of a watershed (i.e., the catchment area), which feeds water into a stream, river, lake or other body of water.

Microbial Source Tracking

Approximately 13% of surface waters in the USA do not meet designated use criteria as determined by high densities of fecal indicator bacteria. Although some of the contamination is attributed to point sources such as confined animal feeding operation and wastewater treatment plant effluents, nonpoint sources are believed to contribute substantially to water pollution (Anonymous 2005; Stockel and Harwood 2007).

The Clean Water Act in Section 101 (sub-sections 5 and 7) states that policy be developed in each state "to assure adequate control of *sources of pollutants* in each State"; and programs "for the control of nonpoint *sources of pollution* be developed and implemented in an expeditious manner so as to enable the goals of this Act to be met through the control of both point and nonpoint sources of pollution."

One of the requirements of the Clean Water Act is the identification of sources of pollution so as to adequately and rationally control their introduction into water. Accordingly, much effort has gone over the past years to develop methods for tracking the sources of pollutants. With regards to pathogens, the indicators of fecal pollution are used for reasons discussed above.

When a body of water has been listed as impaired by fecal bacteria, a TMDL study must be conducted to determine how the impairment can be remedied so the water body will meet appropriate water quality standards. It is required that the source of the pollutant bacteria be determined. Microbial source tracking (MST) methods have recently been used to help identify nonpoint sources responsible for the fecal pollution of water systems. MST tools are now being applied in the development of TMDLs as part of Clean Water Act requirements and in the evaluation of the effectiveness of best management practices.

Microbial source tracking (MST) includes a group of methodologies that are aimed at identifying, and in some cases quantifying, the dominant source(s) of fecal contamination in resource waters, including drinking, ground, recreational, and wildlife habitat waters. MST is transitioning from the realm of research to that of application. It is being discussed so that the student is aware of its existence and understands it as

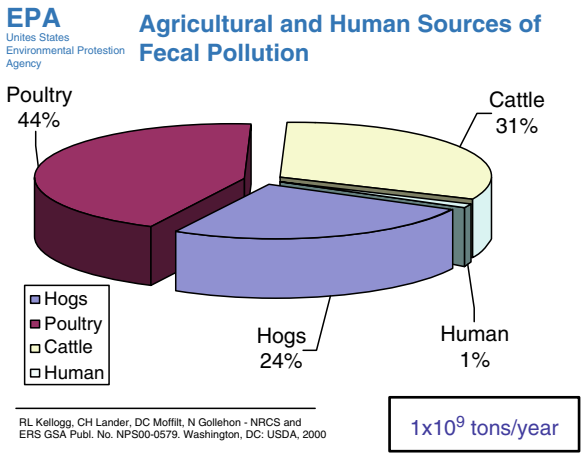


Fig. 7.2 Feces production by different animals and man: total, 1×10^{12} kg/annum (From Shanks 2005)

some of current procedures may become routine, while others are dropped (Long and Plumme 2004).

Methodologies Employed in MST

Present-day bacterial indicators of fecal pollution in water are total coliforms, fecal coliforms, *E. coli*, and enterococci. These are normally prevalent in the intestines and feces of warm-blooded mammals, including wildlife, livestock, and humans. It is not possible to tell which is from humans or from nonhuman sources. On the other hand, nonhuman feces spread pathogens such as *Salmonella* spp., *E. coli* O157, *Campylobacter jejuni*, *Leptospira interrogans*, *Giardia* spp., *Cryptosporidium parvum*, and hepatitis E virus. Figure 7.2 shows the annual feces production of humans and various animals in the USA. Humans produce only 0.7% of the total indicating the possibility that most of the fecal microorganisms in water could be from nonhuman sources, and indicating for adequate methodologies to accurately identify human fecal pollution (Stockel et al. 2004; Stewart-Pullaro et al. 2006).

Previously, fecal coliform/fecal streptococci (FC/FS) ratios were used to assess the general source of nonpoint fecal pollution: with FC/FS higher than 4 indicated human fecal pollution; FC/FS between 0.1 and 0.6 indicated domestic animals, and FC/FS less than 0.1 indicated fecal pollution from wild animals. This approach has, however, been abandoned because the FC/FS ratio was difficult to use in agricultural settings.

Current methods for MST fall into three groups: molecular, biochemical, and chemical. The molecular or genotypic methods are based on the genetic make-up of different strains of fecal bacteria. The biochemical methods can be referred to as the phenotypic in contrast to the molecular methods, because these are based on the activities of the genes in secreting biochemical compounds. The chemical methods do not assess the microorganisms; rather they are based on finding in wastewaters chemical compounds associated with humans and are used to determine if the source of the pollution is human. A fourth method, the *immunological source tracking* method is being studied by some workers. It is based on identifying the unique proteins (antigens) peculiar to each animal and which are shed into feces. When developed this technique should enable the proper identification of the animal from which a fecal bacterium has come. Just as with chemical methods, immunological methods would not require a library, but it is yet under development.

MST methods can also be divided into two: library-dependent and library-independent methods. Library-dependent methods are culture based and rely on isolate-by-isolate typing of bacteria cultured from various fecal sources and from water samples. The isolates are then matched to their corresponding source categories by direct subtype matching or by statistical means. On the other hand, library-independent methods are based on sample-level detection of a specific, host-associated genetic marker in a DNA extract by PCR. All cultivation-independent methods are library independent as are chemical and immunological methods (when perfected). Some cultivation-dependent methods such as the use of phages are also library independent.

1. *Molecular methods*

   The molecular methods of MST are based on DNA patterns that are unique to each source due to variables such as the food consumption and health of the individual. For molecular methods, fecal bacteria are isolated from water samples and a DNA pattern or "fingerprint" is obtained. The pattern is then compared to the library of patterns from specific sources to identify the source(s) of isolates in a sample. Many molecular methods are being used and most require known-source libraries, although a few do not. It is thought that the distinctions between fecal bacteria from different animals (including humans) occur because the intestinal environments (selective pressures) are not the same, and fecal bacteria develop with detectable differences that can be related to sources. The key to

molecular MST methods is finding these differences against a large background of similarity. Molecular methods are believed to be more accurate for individual sources (e.g., deer, muskrat, chickens, and horses). However, the molecular methods are also considerably more expensive and slower to perform in the laboratory. Only a selected number of molecular methods will be discussed and only the outline will be given.

(a) Repetitive PCR (Rep PCR)
Rep-PCR genomic fingerprinting makes use of DNA primers complementary to naturally occurring, highly conserved, repetitive DNA sequences, present in multiple copies in the genomes of most Gram-negative and several Gram-positive bacteria. DNA between adjacent repetitive extragenic elements is amplified using PCR to produce various size DNA fragments. The PCR products are then run on agarose-gel electrophoresis to produce specific DNA fingerprint patterns, which can then be analyzed using a pattern recognition computer software. The rep-PCR genomic fingerprints generated from bacterial isolates permit differentiation to the species, subspecies, and strain level.

(b) Random amplification of polymorphic DNA (RAPD)
This is a type of PCR, but the segments of DNA that are amplified are random. To perform RAPD creates several arbitrary, short primers (8–12 nucleotides) are produced, then PCR is performed using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from an RAPD reaction.

No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had previous attention, or in a system in which relatively few DNA sequences are compared (it is not suitable for forming a DNA databank). Due to the fact that it relies on a large, intact DNA template sequence, it has some limitations in the use of degraded DNA samples. This method requires screening primers (there are over 1,200 commercially available) to find sets of polymorphisms (i.e., differences in nucleotide sequence among individuals) that are either unique to fecal bacteria from a given source or occur in a given source to a large and predictable degree. Once such sets of polymorphisms have been found, fecal bacteria can be "sourced" by comparison. It has been used in differentiating human and nonhuman *E. coli* sources.

(c) Ribotyping
Ribotyping, also referred as molecular finger printing, involves the bacterial genes that code for 16S ribosomal RNA. Because such genes are highly conserved (i.e., change very slowly evolutionarily) in microorganisms, ribotyping has been widely accepted for microbial identification. Ribotyping involves cutting the total genomic bacterial DNA with different DNAases, or restriction enzymes, followed by gel electrophoresis. Following electrophoresis, Southern blotting is performed to blot the DNA bands onto nylon membranes from the gels. DNA probes must be prepared for bacterial 16S and 23S rRNA and labeled with some type of detection system, such as fluorescent dye or radioactively). Membrane hybridization is then performed to hybridize the probes with the appropriate DNA bands on the nylon filter. Difference in the size and location of the ribosomal RNA bands on the filters can then be used to differentiate between the sources of the fecal bacteria.

Strains of *E. coli* are adapted to their own specific environment (intestines of host species), and as a result differ from other strains found in other host species. To use this MST method, collections of potential source material (fecal samples of all potential sources in the environment) must be collected and subtyped. The genetic fingerprints of the bacterial isolates from the water samples can then be compared to those of the suspected source.

Ribotyping has been widely used in microbial source tracking studies. It has been found that database size, geographic distribution of the isolated bacteria, and the presence of replicate isolates in the bacterial source library impact the ability of ribotyping to differentiate among bacteria at the host species level

(d) Pulse-field gel electrophoresis (PFGE)
PFGE is a DNA "fingerprinting" technique that uses restriction enzymes on the entire DNA

genome. The large genomic fragments are placed in a unique gel apparatus where electric current is passed through a gel in different directions at low voltage for 10–12 h to achieve the best level of band separation possible. PFGE is similar to ribotyping, but instead of analyzing rRNA, it uses the whole DNA genome.

In another system of PFGE, the bacterial DNA is embedded in an agarose plug and digested while in the plug; the plugs are placed in hollow gel combs and become part of the gel as the gel is cast around the combs. The gels are stained and photographed after electrophoresis. It is better for discriminating host sources compared to the other fecal coliforms.

(e) Amplified fragment length polymorphism (AFLP-PCR)

Amplified Fragment Length Polymorphism (AFLP) is a polymerase chain reaction (PCR) based genetic fingerprinting technique. AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of complementary double stranded adaptors to the ends of the restriction fragments. A subset of the restriction fragments are then amplified using two primers complementary to the adaptor and restriction site fragments. The fragments are visualized on denaturing polyacrylamide gels either through autoradiographic or fluorescence methodologies. AFLP-PCR is a highly sensitive method for detecting polymorphisms in DNA. The procedure of this technique is divided into three steps:

- Digestion of total cellular DNA with one or more restriction enzymes and ligation of restriction half-site specific adaptors to all restriction fragments.
- Selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site-specific sequences.
- Electrophoretic separation of amplicons on a gel matrix, followed by visualization of the band pattern.

(f) Gene-specific PCR

Gene-specific PCR methods are based on the discovery that certain enterotoxin genes are carried almost exclusively by *E. coli* that infect individual species of warm-blooded mammals including humans, cattle, and swine. *E. coli* samples are isolated from water and the DNA extracted. The samples are amplified and probed with the genes specific for man and other animals. The procedure is relatively simple and can be performed within two working days. The advantages of the gene-specific methods are that they are highly specific and library independent.

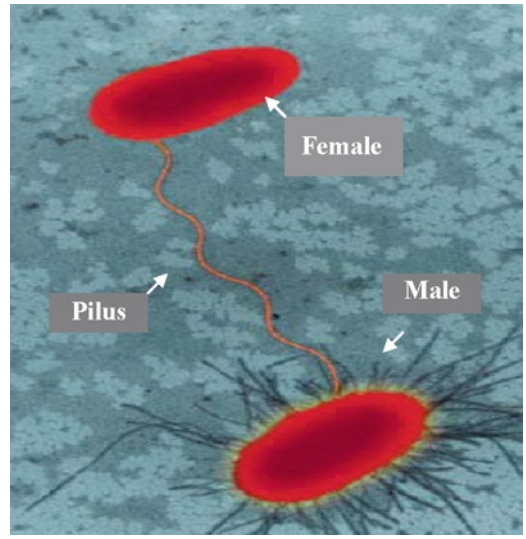


Fig. 7.3 Pilus linking a male to a female *Escherichia coli* cell in conjugation (Image reproduced with permission)

(g) The use of bacteriophages

Bacteriophages (viruses) that infect *E. coli* and possibly other closely related coliform bacteria are called coliphages (Stewart-Pullaro et al. 2006). Coliphages were first proposed as indicators of the presence of *E. coli* bacteria and are taxonomically very diverse, covering the following six virus families: three families of double-stranded DNA viruses (*Myoviridae*, *Styloviriae*, *Podoviridae*), two families of single-stranded DNA phages (*Microviridae* and *Inoviridae*), and one family of single-stranded RNA viruses (*Leviviridae*). Coliphages that infect via the host cell wall of *E. coli* are called somatic coliphages (including families *Myoviridae*, *Styloviriae*, *Podoviridae*, and *Microviridae*). Male-specific (also called F+) coliphages (*Inoviridae* and *Leviviridae*) infect by attaching to hair-like appendages called F-pili protruding from the host bacterium surface (Fig. 7.3).

Male-specific coliphages have been studied extensively as fecal indicators and for water/

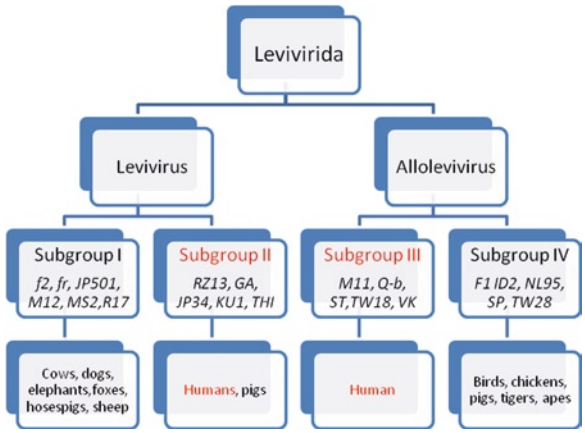


Fig. 7.4 Spectrum of F+ coliphages (Bacteriophage Family *Leviviridae*) (Modified from Smith 2006)

wastewater treatment/disinfection efficacy (Stewart-Pullaro et al. 2006). F^+ RNA coliphages can be distinguished genetically (via nucleic acid detection methods) or antigenically (via immunological methods) into four distinct subgroups: I, II, III, and IV. Groups II and III are associated primarily with human fecal waste and Groups I and IV are associated primarily with animal fecal waste (see Fig. 7.4).

Strengths of the use of coliphages as indicators

The advantages of F^+ coliphages as fecal indicators include their

- Presence in relatively high concentrations in sewage
- Relatively high persistence through wastewater treatment plants, compared to typical bacterial indicators like *E. coli* and fecal coliforms (coliphages may behave similarly to human viruses during wastewater treatment)
- Ability to be detected in relatively small (100 ml) to medium (1,000 ml) volumes of fecally contaminated water

The use of coliphage typing for microbial source tracking is library independent, but can only be used to broadly distinguish human and animal fecal contamination. However, there is a problem with separation between human serotypes and serotypes associated with pigs, which also contain group II. Furthermore, not all animals have FRNA coliphage associated with their respective *E. coli*. The coliphage is persistent in the environment for less than a week and survival is a function of sunlight and water temperature. Ultraviolet light denatures the virus and below 25°C, F-pilus synthesis ceases. The coliphage does not replicate in the environment, only in the presence of F-pilus *E. coli*, and is not found in sediments, just in the water column.

2 *The biochemical methods*

(a) Antibiotic resistance analysis (ARA)

ARA is a method that is based on patterns of antibiotic resistance of bacteria from human and animal sources. The premise behind this method is that human fecal bacteria will have greater resistance to specific antibiotics followed by livestock and wildlife, and that livestock will have greater resistance to some antibiotics not used in humans. These differences occur because humans are exposed to different antibiotics as against cattle or pigs, poultry, or wildlife. Human fecal bacteria will be expected to have the greatest resistance to antibiotics normally used for humans and that domestic and wildlife animal fecal bacteria will have significantly less resistance (but still different) to the battery of antibiotics and concentrations used. Isolates of fecal streptococci and/or *E. coli* are taken from various sources (human, livestock, and wildlife), and these isolates are grown on a variety of antibiotics. Following incubation, isolates are scored as "growth/no growth" for each concentration of an antibiotic. The resistance pattern of an organism is used to identify its source. A database of antibiotic resistant patterns from known sources within a watershed is prepared as a basis for comparing and identifying isolates in that watershed.

Three approaches have been followed in the use of antibiotic resistance in MST: antibiotic resistance analysis (ARA), multiple antibiotic resistance (MAR), and the filter paper disc (or Kirby-Bauer). In ARA studies, different concentrations of each antibiotic being tested are used; in MAR studies, bacteria are tested for resistance to one concentration of different antibiotics. In the filter paper disc approach, small filter disks impregnated with antibiotics are placed on the test organism on agar; the zone of growth inhibition around the disks is used to

quantify resistance. Some workers believe that ARA provides the most information of the three antibiotic-based approaches. The extent of the accuracy of this method is measured by the average rate of correct classification (ARCC). With wild animals the ARCC is between 98% and 100%, whereas with livestock it is between 34% and 89%. The methods are low cost and easy to perform.

(b) Carbon utilization profiles (CUP)
The CUP is based on differences among bacteria in their use of a wide range of carbon and nitrogen sources. The method compares differences in the utilization of several carbon and nitrogen substrates by different bacterial isolates. The working hypothesis behind CUP is that various animal populations have different diets; therefore, fecal bacteria have evolved in the various guts to utilize different food sources. Substrate utilization can be rapidly scored by the formation of a purple color due to the reduction of a tetrazolium dye included with the substrates and automatically detected using a microplate reader. The method is rapid, simple, and requires little technical expertise, and has been simplified by the availability of commercial microwell plates containing substrates, one of the most commonly used being Biolog microplates. The Biolog system allows the user to rapidly perform, score, and tabulate 96 carbon source utilization tests per isolate and is widely used in the medical field for identification of clinical isolates. The bacterial isolates are first grown in liquid culture and suspension of cells at a standardized turbidity is used to inoculate the microplates. After incubation at 37°C for 24 h, presence or absence of growth is indicated by purple dye formation and is assessed manually or automatically using a plate reader.

3. *Chemical methods*
The use of chemical targets has been suggested as an alternate approach to biological markers based on the premise that certain chemicals are only found in fecal samples. Chemical methods do not detect fecal bacteria; they detect chemical compounds that are associated with humans. If the compound(s) are found in water body, then there is a likely human source. Different chemical compounds have been recently used as tools to predict sources of human fecal pollution. Most chemical markers have been used primarily to trace human contamination. For example, caffeine, fragrance materials, and fluorescent whitening agents (laundry detergent brighteners) have been under investigation due to their exclusive use by humans. Fecal sterols and fecal stanols found in humans are also promising sewage pollution markers. The main problem is that the long-term fate of these organic chemicals in environmental waters is yet unknown.

(a) Optical brighteners
This method detects the optical brighteners that are in all laundry detergents. They are persistent in the environment and are detected using mass spectroscopy. Sample collection is accomplished by placing optical brightener-free cotton in a wire mesh trap and placing the trap in the stream for a few days. After the trap is recovered the cotton is examined with a black light to see if it glows. The fluorescent cotton can then be examined with mass spectroscopy to verify the presence of the compounds. If they can be detected, then there must be a human source. Laundry detergents such as fluorescent whitening agents, sodium tripolyphosphate, and linear alkyl benzenes have been used to predict human impact; however, these chemicals cannot reliably be traced to sewage or fecal pollution and can only be attributed to general human or industrial sources.

(b) Caffeine detection
Caffeine is present in several beverages, including coffee, tea, soft drinks, and in many pharmaceutical products. It is excreted in the urine of individuals who have ingested the substance, and subsequently, it has been suggested that the presence of caffeine in the environment would indicate the presence of human sewage. Levels of caffeine in domestic wastewater have been measured to be between 20 and 300 g/l (68). Caffeine could thus be used as an indicator chemical. A major problem is that it is expensive trying to detect caffeine in the environment. Furthermore, some other plants such as watermelon have significant levels of caffeine and could obscure the results. Finally, caffeine is easily degraded by soil microbes; hence, the detectable quantities could be greatly reduced.

(c) Coprostanol
Coprostanol is a fecal stanol that is formed during catabolism of cholesterol by indigenous bacteria present in the gut of humans and higher animals and is the primary stanol detected in domestic wastewater. Coprostanol has been found to make up about 60% of the total stanols in human, whereas feces from pigs and cats contained coprostanol at a ten-fold lower quantity. Other fecal stanols, such as 24-ethyl-coprostanol, were found to be predominant in herbivores, such as cows, horses, and sheep, suggesting the potential use of these chemicals for MST.

Choice of the MST Method to Use

Although the impetus for MST comes from the TDML requirements of the Clean Water Act (1972) in the USA, tracing the source of water contamination is now a worldwide event, and is practiced in countries with developed economies such as those of the European Union and Japan.

At present, the various methods for MST are being examined in these countries and have not yet been selected. The purpose of this chapter is to expose the students to the various methods, most especially their scientific underpinning, so as to understand any methods, which are eventually selected in any one country, state within a country, or a water authority.

Table 7.7 gives the abbreviated procedure and the advantages and disadvantages of each method. Some of the factors, which will weigh in the minds of those deciding on which method to use are the following:

1. MST methods are still very new, and no one method will do for all situations; the method(s) to be used will depend of the fecal material be dealt with.
2. As many methods suitable for a given watershed as possible should be used. If similar results are obtained from different methods, then this helps reinforce their credibility.
3. MST methods have been used primarily with *E. coli* and the fecal streptococci, and to a lesser degree with bifidobacteria, *Bacteroides*, and coliphages. Although *E. coli* is widely used, other indicator organisms have shown good results, and are sometimes even more reliable than *E. coli*, in some circumstances. Wherever possible, therefore, the range of indicator organisms should be used (Fig. 7.5).

7.3 Pollution by Petroleum in Oceans and Seas: Role of Microorganisms in Oil Degradation and Remediation

7.3.1 Composition of Crude Oil

Crude petroleum is a complex combination of hydrocarbons. It consists predominantly of aliphatic, alicyclic, and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen, and sulfur compounds. This category encompasses light, medium, and heavy petroleums, as well as the oils extracted from tar sands (Anonymous 2003). Hydrocarbonaceous materials requiring major chemical changes for their recovery or conversion to petroleum refinery feedstocks such as crude shale oils, upgrade shale oils, and liquid coal fuels are not included in this definition of crude petroleum.

Crude oil contains hydrocarbons in the carbon number range from C1 to C60+. It also contains organometallic complexes, notably of sulfur and vanadium, and dissolved gases such as hydrogen sulfide. Crude oils range from thin, light-colored oils consisting mainly of gasoline quality stock to heavy, thick tar-like materials. An "average" crude oil has the following general composition: carbon 84%, hydrogen 14%, sulfur 1–3%, nitrogen 1%, and oxygen 1%. Minerals and salts make up 0.1%. The chemical composition of crude oils can vary tremendously from different producing regions (see Table 7.8) and even from within a particular formation (Anonymous 2003).

Crude oils are made up of a wide spectrum of hydrocarbons. They vary greatly in appearance depending on their composition. They are usually black or dark brown but may be yellowish greenish. In the ground crude oil is sandwiched between natural gas on top and salt water at the bottom. It may sometimes be in a semisolid form mixed with sand and is known as bitumen.

7.3.1.1 Categorization of Crude Petroleum

Crude petroleum may be further categorized thus as follows:

Paraffinic versus Naphthenic

Crude oils contain both paraffinic and naphthenic hydrocarbons but if there is a preponderance of paraffinic hydrocarbons present, the crude oil is referred to as paraffinic crude. These crudes would be rich in straight and branched chain paraffins. Conversely, a crude in which naphthenic hydrocarbons are predominant is

Table 7.7 Advantages and disadvantages of the various microbial source tracking (MST) methods (From *Microbial Source Tracking Guide Document*, Center for Disease Control [CDC]; Anonymous 2005)

Method	Advantages	Disadvantages
Antibiotic resistance	• Rapid; easy to perform	• Require reference library
		• Requires cultivation of target organism
	• Requires limited training	• Libraries geographically specific
	• May be useful to differentiate host source	• Libraries temporally specific
		• Variations in methods in different studies
Carbon utilization profiles (CUP)	• Rapid; easy to perform	• Require reference library
	• Requires limited training	• Requires cultivation of target organism
		• Libraries geographically specific
		• Libraries temporally specific
		• Variations in methods in different studies
		• Results often inconsistent
Repetitive PCR (rep-PCR)	• Highly reproducible	• Requires reference library
	• Rapid; easy to perform	• Requires cultivation of target organism
	• Requires limited training	• Libraries may be geographically specific
	• May be useful to differentiate host source	• Libraries may be temporally specific
Random amplification of polymorphic DNA (RAPD)	• Rapid; easy to perform	• Requires reference library
	• May be useful to differentiate host source	• Requires cultivation of target organism
		• Libraries may be geographically specific
		• Libraries may be temporally specific
		• Has not been used extensively for source tracking
AFLP	• Highly reproducible	• Labor-intensive
	• May be useful to differentiate host source	• Requires cultivation of target organism
		• Requires reference library
		• Requires specialized training of personnel
		• Libraries may be geographically specific
		• Libraries may be temporally specific
		• Variations in methods used in different studies
PFGE	• Highly reproducible	• Labor-intensive
	• May be useful to differentiate host source	• Requires cultivation of target organism
		• Requires specialized training of personnel
		• Requires reference library
		• Libraries may be geographically specific
		• Libraries may be temporally specific
Ribotyping	• Highly reproducible	• Labor-intensive (unless automated system used)
	• Can be automated	
	• May be useful to differentiate host source	• Requires cultivation of target organism
		• Requires reference library
		• Requires specialized training of personnel
		• Libraries may be geographically specific
		• Libraries may be temporally specific
F+ RNA coliphage	• Distinguishes human from animals	• Requires cultivation of coliphages
	• Subtypes are stable characteristics	• Subtypes do not exhibit absolute host specificity
	• Easy to perform	• Low in numbers in some environments
	• Does not require a reference library	

(continued)

Table 7.7 (continued)

Method	Advantages	Disadvantages
Gene-specific PCR	• Can be adapted to quantify gene copy number	• Require enrichment of target organism
	• Virulence genes may be targeted; providing direct evidence that potentially harmful organisms present	• Sufficient quantity of target genes may not be available requiring enrichment or large quantity of sample
	• Does not require reference library	• Requires training of personnel
		• Primers currently not available for all relevant hosts
Host-specific PCR	• Does not require cultivation of target organism	• Little is known about survival and distribution in water systems
	• Rapid; easy to perform	• Primers currently not available for all relevant hosts
	• Does not require a reference library	
Virus specific PCR	• Host specific	• Low in numbers, requires large sample size
	• Easy to perform	• Not always present even when humans present
	• Does not require reference library	

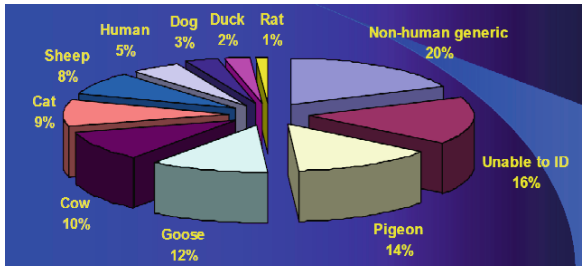


Fig. 7.5 Typical MST results (From Callaghan 2005)

referred to as a naphthenic crude. These crudes contain mainly naphthenic and aromatic hydrocarbons.

Sweet versus Sour

Crude oils may be referred to as either sweet or sour depending upon the level of hydrogen sulfide present. A sweet crude has very little H_2S, whereas a sour crude has larger quantities of H_2S present.

Light versus Heavy

Crude oil may be divided into light and heavy on the basis of their gravity. The API (American Petroleum Institute) gravity is determined as:

$$\text{API} = \frac{141.5}{\text{Specific gravity}} - 131.5$$

or

$$\text{API} = \left(141.5 / \text{Specific gravity}\right) - 131.5$$

Crude oils with gravity > 33°API are considered as light crudes. Such crudes with a high percentage composition of hydrogen are usually more suitable for processing for gasoline production. Heavy crudes, that is, those with gravity < 28°API tend to contain more asphaltenes and are usually rich in aromatics. These heavy crudes require more steps in their processing (Anonymous 2003).

The major components of crude oil are hydrocarbons, ranging from very volatile, light materials such as propane and benzene to more complex heavy compounds such as bitumens, asphaltenes, resins, and waxes. They are separable into four fractions: the saturates, the aromatics, the resins (pyridines, quinolines, carbozoles, sulfoxudes, and amides), and asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins).

Saturates are hydrocarbons containing no double bonds. They are further classified according to their chemical structures into alkanes (paraffins) and cycloalkanes (naphthenes). Alkanes have either a branched or unbranched (normal) carbon chain(s), and have the general formula C_nH_{2n+2}. Cycloalkanes have one or more rings of carbon atoms (mainly cyclopentanes and cyclohexanes), and have the general formula C_nH_{2n}. Most of the cycloalkanes in crude oil have an alkyl substituent(s) (Fig. 7.6).

Aromatics have one or more aromatic rings with or without an alkyl substituent(s). Benzene is the simplest one (Fig. 7.6), but alkyl-substituted aromatics generally exceed the nonsubstituted types in crude oil. In contrast to the saturated and aromatic fractions, both the resin and asphaltene fractions contain non-hydrocarbon

Table 7.8 Gross compositions of crude oils from different parts of the world (From http://www.petroleumhpv.org/docs/crude_oil/111503_crude_robsumm_final.pdf. Reproduced courtesy of the American Petroleum Institute from *Robust Summary of Information on Crude Oil*, 2003; Anonymous 2003)

	Paraffin	Naphthalenes	Aromatics	Sulfur	API gravity
Country or origin of crude	vol%	vol%	vol%	wt%	(°API)
Light crudes					
Saudi Light	63	18	19	20	34
South Louisiana	79	45	19	0	35
Nigerian Light	37	54	9	0.1	36
North Sea Brent	50	34	16	0.4	37
Beryl	47	34	19	0.4	32
Lost Hill	Nonaromatics	50	50	0.9	
Mid-range crudes					
Kuwait	63	26	24	24	31
Venezuela Light	52	34	14	1.5	30
USA West Texas Sour	46	32	22	1.9	32
Heavy crudes					
Prudhoe Bay	27	36	15	2.1	28
Saudi Heavy	60	20	15	2.1	28
Venezuela Heavy	35	53	12	23	24
Belridge Heavy	Nonaromatics	37	63	1.1	–

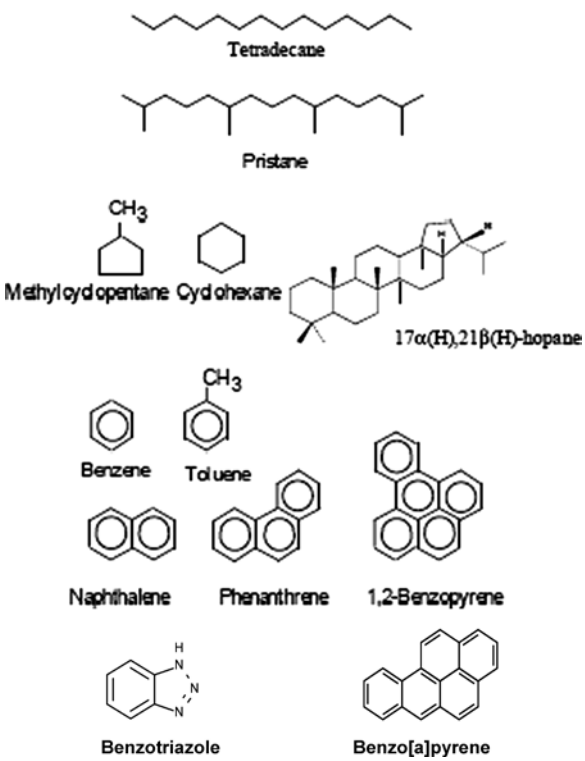


Fig. 7.6 Various hydrocarbons (From Harayama et al. 1999)

polar compounds. Their elements contain, in addition to carbon and hydrogen, trace amounts of nitrogen, sulfur, and/or oxygen. These compounds often form complexes with heavy metals.

Asphaltenes consist of high-molecular-weight compounds, which are not soluble in a solvent such as *n*-heptane, while resins are *n*-heptane-soluble polar molecules.

Resins contain heterocyclic compounds, acids, and sulfoxides. The components of petroleum in crude oil have been analyzed mainly by using gas chromatography in combination with mass spectrometry (GC/MS). Consequently, the chemical structures of the higher-molecular-weight components (the heavy fractions) that cannot be identified by GC are mostly unknown. Furthermore, the compositions of many branched alkanes and alkyl cycloalkanes have not been determined because their isomers are numerous and cannot be resolved by gas chromatography. Many compounds in crude oil are yet to be identified.

During refining, many products are obtained from crude oil by fractional distillation, yielding different fractions or cuts. Alkenes, unsaturated hydrocarbons including ethylene, are not found in crude oil, but are produced during the refining or cracking of crude oil.

Various hydrocarbons and fractions of crude oil are given in Fig. 7.6.

7.3.2 Oil Spills

Petroleum has a wide range of applications in the form of vehicle fuel, heating source for homes and industry, for electricity generation, and in basic industrial operations; there is, therefore, an enormous demand for the production and movement of oil. Due to its high-energy density, easy transportability, and relative abundance, crude petroleum has become the world's most important source of energy since the mid-1950s (American Petroleum Institute 2010).

About 16% of the petroleum produced is used as raw material for many chemical products, including pharmaceuticals, solvents, fertilizers, pesticides, and plastics. Oil is thus being continuously pumped from the ground, refined, transported, and stored, sometimes resulting in spilling during "operations" or "accidents."

World oil production peaked in 2008 at 81.73 million barrels/day (mbd) including crude oil, lease condensate, oil sands, and natural gas plant liquids. If natural gas plant liquids are excluded, then the production peak remains in 2008 but at 73.79 mbd. About half of this is transported by sea. The international transport of petroleum by tankers is frequent and the potential for spills is therefore great. Tanker accidents such as the well-known one of the T/V Exxon Valdez in Prince William Sound, Alaska, severely affect the local marine environment. Tankers take on ballast water, which contaminates the marine environment when it is subsequently discharged. Off-shore drilling is another source of petroleum marine pollution. The largest source of marine contamination by petroleum appears to be the runoff from land. Annually, more than two million tons of petroleum are estimated to end up in the sea. This section will discuss oil spills and their occurrence, the fate of oil in spills (see Table 7.9), and some aspects of remediation in spills.

Oil spills have been a major cause of concern as they pose a danger to public health, devastate natural resources, and disrupt the economy. Marine life can be affected by both the physical and chemical properties of the oil spilled, the main threat posed by the residues on the contaminated sea surface. A short-term exposure can render unpleasant tastes and smells to aquatic life, but a prolonged exposure can impair the ability of marine organisms to reproduce, grow, feed, or perform other functions. Beaches and shorelines may be adversely affected from the aesthetic and recreational points of view (Anonymous 2003).

In freshwaters, oil contamination can result in severe impacts on the habitat because the movement associated with water is minimal, as compared to marine environment. Stagnant water bodies cause the oil to remain in the environment for long, resulting in prolonged exposure of the plants and animals. In the case of flowing streams and rivers, the oil not only tends to collect on plants and grasses growing on the banks, but also interacts with sediments, thereby affecting the organisms.

Oil spills are usually thought of as occurring in takers in oceans only. However, they can be due to accidents within the industry, or to natural causes. Accidents occur within the industry during the processes of generating and moving oil in tankers, barges, pipelines, refineries, and storage facilities. Natural causes of oil spillage include hurricanes, which affect tankers. Furthermore, oil lying in the ground under the sea may seep out and pollute sea and ocean water. Some of the causes of oil spills from 1970 to 2009 are given in Table 7.10.

The spills in oceans and seas are more dramatic and are usually carried by the news media. There have been numerous oil spills and three of the largest will be mentioned briefly. The world's largest so far (January 2011) is generally agreed to be the *Gulf War Oil Spill* (January 23, 1991), which occurred during the Iraq–Kuwait war. Most oil spills are accidents, but this was no accident at all. Rather, the Iraqi military had released oil from several tankers to prevent US forces from attacking. The Lakeview Gusher Oil Spill (March 14, 1910) is regarded as the largest recorded US oil well gusher, in the Sunset oil field in Kern County, California. It took 18 months to control and it is believed to be due to the infancy of oil technology at the time. The Ixtoc I oil Spill (June 3, 1979) was an exploratory oil well in the bay of Campeche in the Gulf of Mexico. It is the third largest oil spill and second largest accidental spill in the history. In recent times, the Gulf of Mexico oil spill (also known as Deepwater Horizon oil spill and the BP oil spill) resulted from a seafloor oil gusher and flowed for 3 months before being capped on July 15, 2010, but not before 4.9 million barrels or 205.8 million gallons of crude oil had gushed from it. With 627,000 t of crude oil, this would appear to be the largest spill in history, second only to the Lakeview Gusher, California, USA, March 1910–September 1911, which spilled 1,230,000 t. Table 7.11 gives some major oil spills in oceans and seas (see also Table 11.1).

Table 7.9 Degradation of benzene-extractable material from Colgate Creek sediment by microorganisms indigenous to the sediment (From Walker and Colwell 1977. With permission)

	Weight (mg) in:		Amount of hydrocarbons wt% degraded expressed as:	
Class of hydrocarbon	Un-degraded control	Degraded sample	%total hydrocarbon	% individual hydrocarbon
Alkanes	312.0	96.3	94.4	69.1
1-Ring cycloalkanes	417.7	134.2	92.3	67.9
2-Ring cycloalkanes	145.6	41.9	97.6	71.2
3-Ring cycloalkanes	112.7	31.8	98.2	71.8
Alkylbenzenes	350.1	77.2	95.5	77.9
Benzcycloparraffins	152.5	45.4	97.4	70.2
Benzdicycloparraffins	110.0	35.8	97.9	67.7
Naphthalenes	74.5	23.2	98.7	68.9
Acenaphthalenes	26.0	9.6	99.5	63.1
Fluorenes	13.9	4.0	99.8	71.2
Phenanthrenes	10.4	2.0	99.9	71.0
Cyclopentanaphenalenes	6.9	2.0	99.9	71.0

Table 7.10 Causes of oil spills, 1970–2009 (From http://www.itopf.com/information-services/data-and-statistics/statistics/; Anonymous 2010b. With permission)

	<7 t	7–700 t	>700 t	Total
Operations				
Loading/discharging	3,155	383	36	3,574
Bunkering	560	32	0	593
Other operations	1,221	62	5	1,305
Accidents				
Collisions	176	334	129	640
Groundings	236	265	161	662
Hull failures	205	57	55	316
Equipment failures	206	39	4	249
Fire and explosions	87	33	32	152
Other/unknown	1,983	44	22	2,049
Total	7,829	1,249	444	9,522

7.3.2.1 Behavior of Oil in an Oil Spill

Complex physical, chemical, and biological factors collectively known as weathering, determine the fate and behavior of oil during a spill leading to its dispersion and the degradation of its components and its disappearance. Below are the weathering phenomena associated with spilled oil (Anonymous 2009).

1. *Spreading of the oil*
   Oil floats on the water in a marine spill and quickly spreads. In as short as 10 min, a spill of 1 t of oil can disperse over a radius of 50 m, forming a slick 10-mm thick. The slick gets thinner (<1 mm) as oil continues to spread, covering an area of up to 12 km^2. When oil is spilled into the sea evaporation plays an important part in its disappearance and during the first several days after the spill, the volatile components of the oil evaporate. The rate of this evaporation depends on the composition of the crude oil. Brent (North Sea) and Nigerian oil, which are known for their high content of the lighter components, would have, within a few days, lost about two-thirds of its content on being spilled into the sea. Venezuelan oil, on the other hand, would have lost only two-fifths because it has a larger quantity of the heavier components of crude oil. Furthermore, apart from the water-soluble components a considerable part of oil transforms into the gaseous phase. Besides volatile components, the slick rapidly loses water-soluble hydrocarbons. The rest – the more viscous fractions – slow down the slick spreading. The spread of the slick follows the direction of the wind, waves, and current. As the slick thins down to a thickness of about 0.1 mm, it disintegrates into separate fragments that spread over larger and more distant areas. It soon forms into droplets, which are transported to distances far removed the site of the spill.
2. *Solubility of some components*
   Many components are water soluble to a certain degree, especially low-molecular-weight aliphatic and aromatic hydrocarbons. Some compounds formed as a result of oxidation of some oil fractions in the marine environment also dissolve in seawater. In comparison with evaporation, dissolution is slower.
3. *Emulsification*
   The emulsification of oil in the marine environment depends on oil composition and the degree of turbulence in the water. The most stable water-in-oil

Table 7.11 Major oil spills, 1910–2010 (in chronological order) (Modified from The International Tankers Owners Pollution Federation (ITOPF), http://www.itopf.com/information-services/data-and-statistics/statistics/ and http://en.wikipedia.org/wiki/List_of_oil_spills; Anonymous 2010b, c. With permission)

S. No.	Dates	Vessel name or name of spill or cause of spill	Location	Spill size (t)
1.	January 21, 2011	India, Mumbai, Arabian Sea	Mumbai–Uran pipeline spill	55
2.	January 11, 2011	Italy, Sardinia, Porto Torres	Fiume Santo power station	15
3.	August, 2010	Mumbai oil spill	Mumbai, India, Arabian Sea	400
4.	July 26, 2010	Talmadge Creek oil spill	Kalamazoo River, Michigan, USA	3,250
5.	July 16, 2010	Xingang Port oil spill	Yellow Sea, China	90,000
6.	June 16, 2010	Jebel al-Zayt oil spill	Egypt, Red Sea	Unknown
7.	June 11, 2010	Red Butte Creek oil spill	Salt Lake City, Utah, USA	107
8.	May 25, 2010	Trans-Alaska Pipeline spill	Anchorage, Alaska, USA	1,200
9.	May 25, 2010	MT *Bunga Kelana 3*	Singapore Strait, Singapore	2,500
10.	May 1, 2010	ExxonMobil oil spill	Niger Delta, Nigeria	95,500
11.	April 20, 2010	*Deepwater Horizon*	Gulf of Mexico, USA	627,000
12.	January 23, 2010	Port Arthur oil spill	Port Arthur, Texas, USA	1,500
13.	January 5, 2010	Yellow River oil spill	China, Chishui River (Shaanxi)	130
14.	August 21, 2009	Montara oil spill	Timor Sea, Australia	30,000
15.	July 31, 2009	*Full City* oil spill	Rognsfjorden near Langesund, Norway	200
16.	April 8, 2009	2009 Lüderitz oil spill	Southern coast, Namibia	Unknown
17.	March 10, 2009	Queensland oil spill	Queensland, Australia	260
18.	February 2009	West Cork oil spill	Southern coast, Ireland	300
19.	July 28, 2008	2008 New Orleans oil spill	New Orleans, Louisiana, USA	8,800
20.	December 12, 2007	2007 *Statfjord* oil spill	Norwegian Sea, Norway	4,000
21.	December 7, 2007	2007 Korea oil spill	Yellow Sea, South Korea	10,800
22.	November 11, 2007	Kerch Strait oil spill	Strait of Kerch, Ukraine	1,000
23.	November 7, 2007	*COSCO Busan* oil spill	San Francisco, California, USA	188
24.	October 23, 2007	*Kab 101*	Bay of Campeche, Mexico	1,869
25.	August 11, 2006	Guimaras oil spill	Philippines	1,540
26.	July 14, 2006	Jiyeh power station oil spill	Lebanon	30,000
27.	June 19, 2006	Citgo refinery oil spill	Lake Charles, Louisiana, USA	6,500
28.	March 2, 2006	Prudhoe Bay oil spill	Alaska North Slope, Alaska, USA	689
29.	August 30, 2005	Bass Enterprises (Hurricane Katrina)	Cox Bay, Louisiana, USA	12,000
30.	August 30, 2005	Shell (Hurricane Katrina)	Pilottown, Louisiana, USA	3,400
31.	August 30, 2005	Chevron (Hurricane Katrina)	Empire, Louisiana, USA	3,200
32.	August 30, 2005	Murphy Oil USA refinery spill (Hurricane Katrina)	Meraux and Chalmette, Louisiana, USA	3,410
33.	August 30, 2005	Bass Enterprises (Hurricane Katrina)	Pointe à la Hache, Louisiana, USA	1,500
34.	August 30, 2005	Chevron (Hurricane Katrina)	Port Fourchon, Louisiana, USA	170
35.	December 8, 2004	MV *Selendang Ayu*	Unalaska Island, Alaska, USA	1,560
36.	November 26, 2004	*Athos 1*	Delaware River, New Jersey, USA	860
37.	September 16, 2004	MP-80 Delta 20" pipeline (Hurricane Ivan)	Louisiana, USA	963

(continued)

Table 7.11 (continued)

S. No.	Dates	Vessel name or name of spill or cause of spill	Location	Spill size (t)
38.	September 16, 2004	Nakika 18" pipeline (Hurricane Ivan)	Louisian, USA	618
39.	September 16, 2004	Chevron-Texaco tank collapse (Hurricane Ivan)	Louisiana, USA	423
40.	July 28, 2003	*Tasman Spirit*	Karachi, Pakistan	30,000
41.	April 27, 2003	Bouchard No. 120	Buzzards Bay, Massachusetts, USA	320
42.	November 13, 2002	*Prestige* oil spill	Galicia, Spain	63,000
43.	October 6, 2002	*Limburg* (bombing)	Gulf of Aden, Yemen	12,200
44.	November 23, 2001	Manguinhos refinery	Guanabara Bay, Rio de Janeiro, Brazil	97
45.	October 4, 2001	Trans-Alaska Pipeline gunshot spill	Alaska, USA	932
46	June 25, 2001	2001 Shell Ogbodo oil spill	Niger Delta, Nigeria	Unknown
47.	May 2001	2001 Shell Ogoniland oil spill	Niger Delta, Nigeria	Unknown
48.	March 15, 2001	*Petrobras 36*	, Campos Basin, Brazil,	274
49.	January 14, 2001	Amorgos oil spill	Southern coast, Taiwan	1,150
50.	January 2001	*Jessica*	Galapagos Islands, Ecuador,	568
51.	August 1, 2000	Pine River	Chetwynd, British Columbia, Canada,	850
52.	June 2000	Project Deep Spill	Helland Hansen ridge, Norway,	100
53.	June 2000	*Treasure*	Cape Town, South Africa,	1,400
54.	January 2000	Petrobras pipeline	Guanabara Bay, Rio de Janeiro, Brazil,	1,100
55.	December 12, 1999	*Erika*	Bay of Biscay, France,	25,000
56.	January 12, 1998	Mobil Nigeria oil spill	Niger Delta, Nigeria	5,500
57.	December 1997	*Nakhodka*	Sea of Japan, Japan	6,240
58.	September 27, 1996	*Julie N.*	Portland, Maine, USA	586
59.	February 15, 1996	*Sea Empress*	Pembrokeshire, UK	72,000
60.	January 19, 1996	North Cape	Rhode Island, USA	2,500
61.	March 31, 1994	*Seki* oil spill	United Arab Emirates	15,900
62.	January 7, 1994	*Morris J. Berman* oil spill	Puerto Rico	2,600
63.	January 5, 1993	MV *Braer*	,Shetland Islands, UK	85,000
64.	December 3, 1992	Aegean Sea	A Coruña, Spain	74,000
65.	April 26, 1992	*Katina P*	Maputo, Mozambique	72,000
66.	March 2, 1992	Fergana Valley	Uzbekistan	285,000
67.	July 21, 1991	*Kirki*	Coast of Western Australia, Australia,	17,280
68.	May 28, 1991	*ABT Summer*	Offshore, Angola	260,000
69.	April 11, 1991	MT *Haven*	Mediterranean Sea near Genoa, Italy	144,000
70.	January 23, 1991	Gulf War oil spill	Persian Gulf, Iraq	820,000
71.	June 8, 1990	*Mega Borg*	Gulf of Mexico, Texas, USA	16,501
72.	February 7, 1990	*American Trader*	Bolsa Chica State Beach, California, USA	981
73.	December 19, 1989	*Khark 5*	off Las Palmas de Gran Canaria, Spain,	80,000
74.	June 24, 1989	*Presidente Rivera*	Delaware River, Pennsylvania, USA	993

(continued)

Table 7.11 (continued)

S. No.	Dates	Vessel name or name of spill or cause of spill	Location	Spill size (t)
75.	March 24, 1989	*Exxon Valdez*	Prince William Sound, Alaska, USA	104,000
76.	November 10, 1988	*Odyssey*	off Nova Scotia, Canada,	132,000
77.	January 2, 1988	Ashland oil spill	Floreffe, Pennsylvania, USA	10,000
78.	December 6, 1985	*Nova*	Gulf of Iran, Kharg Island, Iran	70,000
79.	September 28, 1985	*Grand Eagle*	Delaware River, Pennsylvania, USA	1,400
80.	August 6, 1983	*Castillo de Bellver*	Saldanha Bay, South Africa	252,000
81.	February 4, 1983	Nowruz Field Platform	Persian Gulf, Iran	260,000
82.	March 7, 1980	*Tanio*	Brittany, France	13,500
83.	February 23, 1980	*Irenes Serenade*	Pylos, Greece	100,000
84.	November 15, 1979	MT *Independenţa*	Bosphorus, Turkey	95,000
85.	November 1, 1979	*Burmah Agate*	Galveston Bay, Texas, USA	8,440
86.	July 19, 1979	*Atlantic Empress*/*Aegean Captain*	Trinidad and Tobago	287,000
87.	June 3, 1979	Ixtoc I oil spill	Bay of Campeche, Mexico,	480,000
88.	January 8, 1979	*Betelgeuse*	Bantry Bay, Ireland	64,000
89.	March 16, 1978	*Amoco Cadiz*	Brittany, France	227,000
90.	February 15, 1978	Trans-Alaska Pipeline	Alaska, USA	2,162
91.	April 22, 1977	Ekofisk oil field	North Sea, Norway	27,600
92.	February 26, 1977	*Hawaiian Patriot*	Honolulu, Hawaii	95,000
93.	February 7, 1977	*Borag*	Northern coast, Taiwan	34,000
94.	December 15, 1976	*Argo Merchant*	Nantucket Island, Massachusetts, USA	28,000
95.	May 12, 1976	*Urquiola*	A Coruña, Spain	100,000
96.	January 31, 1975	*Corinthos*	Delaware River, Pennsylvania, USA	35,700
97.	January 29, 1975	*Jakob Maersk*	Oporto, Portugal	88,000
98.	August 9, 1974	VLCC *Metula*	Strait of Magellan, Chile	51,000
99.	December 19, 1972	*Sea Star*	Gulf of Oman, Iran	115,000
100.	January 17, 1971	*Arizona Standard*/*Oregon Standard* collision	San Francisco Bay, USA	2,700
101.	March 20, 1970	*Othello*	Trälhavet Bay, Sweden	60,000
102.	January 28, 1969	1969 Santa Barbara oil spill	Santa Barbara, California, USA	14,000
103.	March 18, 1967	*Torrey Canyon*	Isles of Scilly, UK	119,000
104.	December 30, 1958	*African Queen* oil spill	Ocean City, Maryland, USA	21,000
105.	1950s–1994	Guadalupe Oil Field	Guadalupe, California, USA	29,000
106.	1940s–1950s	Greenpoint, Brooklyn oil spill	Newtown Creek, New York, USA	97,400
107.	March 6, 1937	SS *Frank H. Buck*/ SS *President Coolidge* collision	San Francisco Bay, California, USA	8,870
108.	March 14, 1910–September 10, 1911	Lakeview Gusher	Kern County, California, USA	1,230,000

emulsions contain from 30% to 80% water. The emulsions usually occur after strong storms in the zones of spills of heavy oils with an increased content of nonvolatile fractions, especially asphaltenes. They can exist in the marine environment for over 100 days in the form of peculiar "chocolate mousses." The stability of these emulsions usually increases with decreasing temperature. The reverse emulsions, such as oil-in-water, droplets of oil suspended in water, are much less stable because

surface-tension forces quickly decrease the dispersion of oil. This process can be slowed with the help of emulsifiers – surface-active substances with strong hydrophilic properties used to eliminate oil spills. Emulsifiers help to stabilize oil emulsions and promote dispersing oil to form microscopic (invisible) droplets. This accelerates the decomposition of oil products in the water column.

4. *Oxidation of some oil components*
   Chemical transformations of oil on the water surface and in the water column start to reveal themselves no earlier than a day after the oil enters the marine environment. They mainly have an oxidative nature and often occur as a result of the ultraviolet light of the sun. These processes are catalyzed by some trace elements (e.g., vanadium) and inhibited (slowed) by compounds of sulfur. The final products of oxidation (hydroperoxides, phenols, carboxylic acids, ketones, aldehydes, and others) usually have increased water solubility. The reactions of photooxidation initiate the polymerization and decomposition of the most complex molecules in oil composition. This increases the oil's viscosity and promotes the formation of solid oil aggregates.
5. *Sedimentation*
   In the narrow coastal zone and shallow waters where particulates are abundant and water is subjected to intense mixing from 10% to 30% the oil in a spill is adsorbed on the suspended material and deposited at the bottom sediment. In deeper areas remote from the shore, sedimentation of oil, except for the heavy fractions, is an extremely slow process. Biological objects bring about "biosedimentation": plankton filtrators and other organisms absorb the emulsified oil and sediment it to the bottom with their metabolites and remainders. The suspended forms of oil and its components undergo intense chemical and microbial breakdown in the water column. When oil particles reach the bottom of seas and oceans, which are anaerobic their decomposition slows down considerably, if not totally stopped, the heavy oil fractions accumulated inside the sediments can be preserved for many months and even years.
6. *Aggregation*
   Oil aggregates in the form of petroleum lumps, tar balls, or pelagic tar can be found both in the open and coastal waters as well as on the beaches. They derive from crude oil after the evaporation and dissolution of its relatively light fractions, emulsification of oil residuals, and chemical and microbial transformation. The chemical composition of oil aggregates is changeable, but most often, its base includes asphaltenes (up to 50%) and high-molecular-weight compounds of the heavy fractions of the oil.

   Oil aggregates look like light gray, brown, dark brown, or black sticky lumps. They have an uneven shape and vary from 1 mm to 10 cm in size (sometimes reaching up to 50 cm). Their surface serves as a substrate for developing bacteria, unicellular algae, and other microorganisms. Many invertebrates (e.g., gastropods, polychaetes, and crustaceans), which are not affected by oil components often use them as shelter. Oil aggregates can exist from a month to a year in the enclosed seas and up to several years in the open ocean. They slowly degrade in the water column, on the shore (if they are washed there by currents), or on the sea bottom (if they lose their floating ability).
7. *Microbial degradation*
   The fate of most petroleum substances in the marine environment is ultimately defined by their transformation and degradation due to microbial activity. About a hundred known species of bacteria and fungi are able to use oil components to sustain their growth and metabolism. In pristine areas, their proportions usually do not exceed 0.1–1.0% of the total abundance of heterotrophic bacterial communities. In areas polluted by oil, however, this portion increases to 1–10%.

   Biochemical processes of oil degradation with microorganism participation include several types of enzyme reactions based on oxygenases, dehydrogenases, and hydrolases. These cause aromatic and aliphatic hydrooxidation, oxidative deamination, hydrolysis, and other biochemical transformations of the original oil substances and the intermediate products of their degradation.

   The degree and rates of hydrocarbon biodegradation depend on the following (Walker and Cowell 1974, 1977):

   (a) *The structure of the molecules*
   The paraffin compounds (alkanes) biodegrade faster than aromatic and naphthenic substances. With increasing complexity of molecular structure, that is, increasing the number of carbon atoms and degree of chain branching, as well as with increasing molecular weight, the rate of microbial decomposition decreases.

(b) *The physical state of the oil*
The degree of the dispersion of the oil is important in deciding the rate of microbial breakdown; the more dispersed the oil the faster the rate of microbial breakdown because of the greater contact with the microorganisms.

(c) *Environmental factors*
The environmental factors affecting microbial breakdown include the temperature, the availability of oxygen, and the nutrients available in the water.

(d) *The nature and numbers of the oil-degrading microorganisms in the water*
The species, composition and abundance of oil-degrading microorganisms in the water is important in deciding the rate of oil breakdown. Complex and interconnected factors influence biodegradation in the marine environment. These factors will be examined in a little more detail below.

(e) *Biodegradation of oil*
Of all the various factors, which affect the weathering or disappearance of oil, microbial degradation is the most important.

Microorganisms, especially bacteria, definitely play a part in the breakdown of oil. Many isolations have been made from oil and water of microorganisms capable of using crude oil as the only source of carbon. Table 7.12 shows the bacteria (including cyanobacteria), algae, and fungi, which can degrade oil and oil components (Raghukumar et al. 2001). Microbes degrading oil usually account for less than 1% of natural populations of microbes, but can account for more than 10% of the population in waters polluted with oil. Obligate hydrocarbon-degrading bacteria, designated as "hydrocarbonoclastic" and belonging to genera in the gamma-proteobacteria, are isolated regularly in marine water when an oil spill has occurred. Crude oil is a complex mixture of different hydrocarbons, and different bacteria have been shown to attack specific components of oil (Leahy and Colwell 1990). In one study, for instance, the degradation of PAHs (polycyclic aromatic hydrocarbons) was shown to be carried out mainly by *Cycloclasticus*. PAHs are chemical compounds that consist of fused aromatic rings and do not carry substituents. PAHs occur in oil, coal, and tar deposits, and are believed to be carcinogenic.

Table 7.12 Bacteria and fungi degrading oil (Modified from Gordon 1994; Raghukumar et al. 2001)

Bacteria	Fungi
Achromobbacter	*Allescheria*
Acinetobacter	*Aspergillus*
Actinomyces	*Aureobasidium*
Aeromonas	*Botrytis*
Alcaligenes	*Candida*
Arthrobacter	*Cephaiosporium*
Bacillus	*Cladosporium*
Beneckea	*Cunninghamella*
Brevebacterium	*Debaromyces*
Coryneforms	*Fusarium*
Erwinia	*Gonytrichum*
Flavobacterium	*Hansenula*
Klebsiella	*Helminthosporium*
Lactobacillus	*Mucor*
Leucothrix	*Oidiodendrum*
Moraxella	*Paecylomyces*
Nocardia	*Phialophora*
Peptococcus	*Penicillium*
Pseudomonas	*Rhodosporidium*
Sarcina	*Rhodotorula*
Spherotilus	*Saccharomyces*
Spirillum	*Saccharomycopisis*
Streptomyces	*Scopulariopsis*
Vibrio	*Sporobolomyces*
Xanthomyces	*Torulopsis*
Oscillatoria *Plectonema*	*Trichoderma*
Aphanocapsa	*Trichosporon*
Thalassolituus	
Alcanivorax	
Cycloclasticus	

Note: The last six in the column for bacteria are Cyanobacteria

On the other hand, the degradation of the branched alkane, pristane, was carried out almost exclusively by *Alcanivorax.* Bacteria related to *Thalassolituus oleivorans* were the dominant degraders in *n*-alkane (C12–C32). The marine bacterium, *Roseobacter*, was present among the various groups but was not dominant. Figure 7.7 shows the pathways for the breakdown of some oil components. Table 7.12 shows a list of microorganisms degrading oil.

7.3.2.2 Remediation of Oil Spills

Remediation and cleanup techniques for oil spills depend largely on the type of oil and the conditions

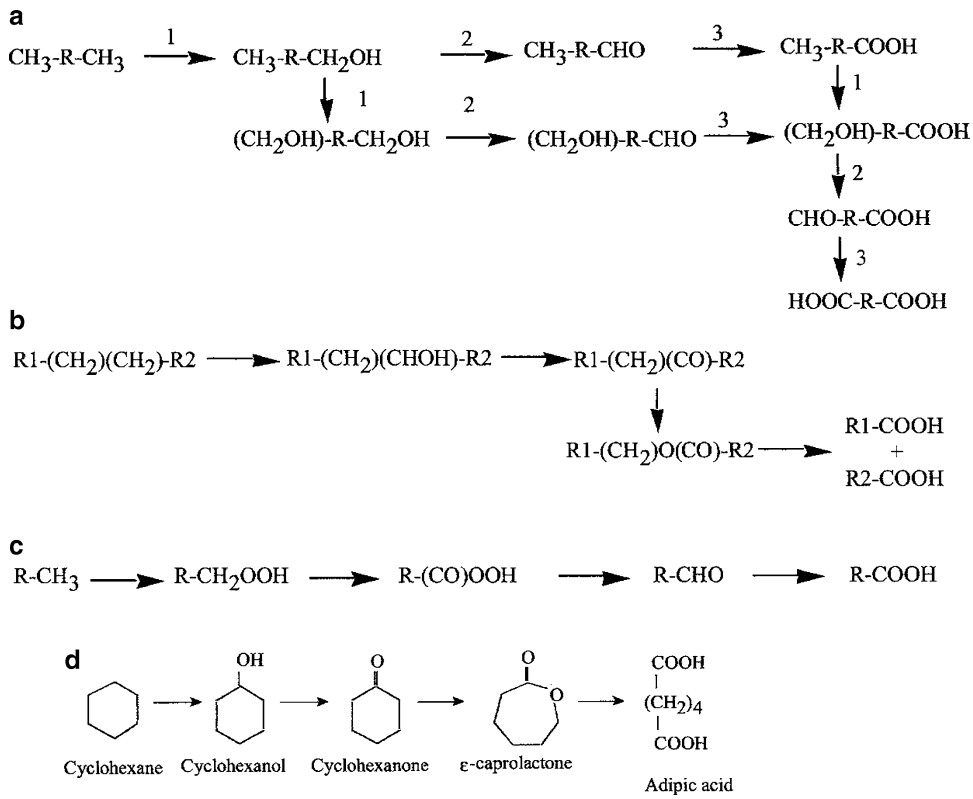


Fig. 7.7 Pathways for the breakdown of some oil components (From Harayama et al. 1999)

present at the location during the time of the spill. The method for the cleanup or remediation of oil spills may be physical or biological.

1. *Physical methods of remediation*
   (a) Containment and recovery using booms and skimmers
   Containment and recovery are the earliest methods used to remediate and clean up an oil spill. Long, floating plastic or rubber barriers or booms are placed around the floating oil slick. These act like fences, containing the oil and preventing it from further spreading. In addition, booms may be used to divert and channel oil slicks along desired paths, making them easier to remove from the surface of the water. Booms can be divided into several basic types. They are not very effective in rough waters, which flow over the barriers. Nonrigid or inflatable booms may be used; they are easy to clean and store, and they perform well in rough seas. They are, however, expensive, more complicated to use, and may puncture and deflate easily.

   After the oil is contained using booms, "skimmers" or boats that skim spilled oil from the water surface are used. In calm waters, vacuum skimmers are used to suck the oil and put it into storage tanks. In rough waters, floating disk and rope skimmers can be passed through the oil. The amount of oil recovered by booms and skimmers is small, and if used for long periods, may hamper the spread of oil, which helps its biodegradation.

   (b) Absorption
   Absorption is the technique employed in choppy or fast-moving waters, when methods like containment and removal fail. In this method, sorbent materials such as talc, straw, sawdust, and synthetic absorbents are added to the oil slick and they are removed when they have soaked up some of the oil. These sorbent materials act like a big sponge, removing oil but contaminated absorbent materials must be treated as toxic waste and present disposal problems. Furthermore, straw and sawdust can become waterlogged and difficult to remove.

(c) Dispersants

The adverse economic and environmental effects of offshore oil spills are greatest when the oil slick reaches the shoreline. On account of this, much effort is put into preventing offshore oil spills from reaching the shoreline. In calm seas, use of skimmers and booms to collect the oil at sea is the conventional method of cleanup and recovery. In situ burning (see below) is also used in such a situation. In rough seas, skimming or burning the oil is not effective, and the use of chemical dispersants appears to be the most suitable for cleaning up the oil spill.

Dispersants are chemicals that promote the formation of tiny oil droplets, and delay the reformation of slicks. They contain surfactants and/or solvent compounds that cause the oil sleek to break into small droplets in a process known in oil parlance as *dispersion*. After oil spills, oil droplets break down because of waves and currents during the dispersion process. Water and oil droplets then combined to form water-in-oil emulsions, which have high viscosity. Dispersants inhibit emulsion formation and promote oil dispersion. Thus, they can remove the spilled oil from the water surface and reduce the impact to environment, especially to the shoreline and sensitive habitats. The generated small oil droplets get transported or transferred into the water column due to wave action and sea turbulence. They subsequently move away from the contaminated area in accordance with the prevailing currents. They can then more easily adsorb onto suspended particulate matter and/or more easily biodegrade. After an oil spill, oil droplets break down because of waves and currents during the dispersion process.

Very light oils are, however, not easily dispersible because the formed droplets have to be very small to overcome buoyancy. For this reason, a high dosage of dispersant is required to cause the formation of such small droplets in very light oils. Very heavy oils on the other hand are much more resistant to dispersion because their high viscosity prevents the dispersant from penetrating them. Such penetration is necessary to produce dispersed oil droplets.

In very calm seas, the applied dispersant is not very effective as it tends to run off the oil and gathers in small pools within the slick. In very rough seas the use of large amounts of dispersants might not be needed because a high degree of dispersion occurs naturally due to the mixing effect of the waves.

The evaluation of dispersant effectiveness used for oil spills is commonly done using tests conducted in laboratory flasks, which attempt to mimic the conditions at sea using tests such as the swirling flask devised by the US EPA.

The advantages of dispersants over other types of remediation methods are that they can be used over a wide area through the use of aircraft and also in remote areas. Furthermore, in instances where other methods do not work well such as the use of booms in high seas, dispersants work excellently. By dispersing the oil it is brought in closer contact with microorganisms, which biodegrade it.

On the other hand, many dispersants are toxic and may have lethal or long-term effects on plankton, animals, or fish. They are ineffective on oils which are viscous because of their content of the heavier fractions of oil. Finally, they are not very effective in calm waters.

(d) In situ burning

In in situ burning of oil, the controlled burning of an oil spill takes place on the water's surface. It requires minimal equipment although some specialized equipment and training is required. In burning oil, no need exists for collecting and transporting recovered oil. The burning leads to the production of green house gases and may leave toxic residues in the water. Because the oil is gasified during combustion, the need for physical collection, storage, and transport of recovered product is reduced but can cause air pollution. At times, burning also leaves a toxic residue on the surface of water, thereby causing more pollution, rather than removing it from the natural environment.

2. *Biological methods of remediation (bioremediation)*

Bioremediation is the process of using biological means in remediating or ameliorating the effects of oil pollution. In general terms, it is the remediation of any pollution by the use of microorganisms in transforming harmful organic compounds, such as oil, into nontoxic and less dangerous compounds. Seawater contains a range of microorganisms that

can partially or completely degrade oil to water-soluble compounds and eventually to carbon dioxide and water. Two approaches are adopted in using microorganisms to remediate oil pollution: (i) biostimulation, which seeks to increase the activity of oil-degrading indigenous organisms and (ii) bioaugmentation, which is the introduction of microorganisms, which degrade oil into water (Smith and Osborn 2009).

(i) *Biostimulation*

Biostimulation of the indigenous microorganisms involves the provision of materials, which increase the activity of the oil-degrading microorganisms. This includes providing those nutrients known to be deficient in the sea environment, or providing conditions, which increase the efficiency of the microorganisms to utilize what is available.

- Addition of nutrients:
  Usually, the rate-limiting factor for oil degradation in the marine environment is inorganic nutrient concentration, and in particular, nitrogen (N) and phosphorous (P). Biostimulation through the addition of nutrients containing these two elements has been the most widely used bioremediation strategy. Oil degradation is significantly enhanced by this method.
- Addition of surfactancts:
  The hydrocarbons present in crude oil are mostly insoluble in water; furthermore, being hydrophobic the microorganisms, which degrade them need to be in contact with the oil for the membrane-bound enzymes, which breakdown the oil to act. Many of the bacteria produce surfactants, which reduce the surface tension of, and emulsify, the oil and enable contact between the microorganisms and oil. For this reason, the addition of surfactants to supplement those produced naturally is a widely used method to enhance degradation of oil. Surfactants reduce surface tension and increase the surface area of hydrophobic compounds such as oil, therefore, increasing its bioavailability. Biosurfactants produced by bacteria have been used for bioremediation. They have the advantage of nontoxicity when compared with chemical surfactants. In cases where the surfactants is membrane bound the microorganism is added directly to the oil spill (Maneerat 2005).

(ii) *Bioaugmentation*

The addition of oil degraders has been done in many cases, especially in the early stages of the spill (about 5 days). Several studies have shown that bioaugmentation with *Alcanivorax* leads to enhanced degradation of the branched alkanes (pristane and phytane). Similar observations have also been made that bioaugmentation with *Thalassolituus*, which causes increased degradation of *n*-alkanes. There have also been a few reports of failures.

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