LITERATURE REVIEW

2 LITERATURE REVIEW

The review is limited to the published work on classification of surfactants and detergents, metabolism of xenobiotic compounds with special reference to alkylbenzene sulphonates, acclimatization of microorganism to the surfactant, the problems associated with the presence of alkylbenzene sulphonate and the methods of treatment of detergent wastewater with special emphasis on the activated sludge process and its kinetics.

2.1 Surfactants

The hydrophobic groups in surfactants consist normally of hydrocarbon groups containing 10 to 20 carbon atoms (Cain 1977). These groups can be fatty acids, paraffins or alkylbenzenes. The hydrophilic portion of the surfactants comprises a solubilising group which can be carboxylates, sulphonates or sulphates. The surface active properties are then determined by the combination of the number and arrangement of the hydrocarbon groups together with the nature and position of the hydrophilic group. Hamirin (1985) stated that a carbon length of C_{12} to C_{20} is generally regarded as the range covering optimal detergency properties.

2.1.1 Classification of surfactants

McKinney (1957) and Swisher (1970) categorised surfactants according to the distribution of electrical charges on the molecule. The five categories are :

i. Anionic surfactants

Anionic surfactants are characterised by the hydrophobic portion of the molecule carrying a residual negative charge. Examples are alkylbenzene sulphonates or sodium salts of fatty acids.

ii. Cationic surfactants

These surfactants are characterised by the hydrophobic portion carrying a residual positive charge. An example is the quaternary ammonium ions.

iii. Non-ionic surfactants

Non-ionic surfactants are those in which a residual electrical charge on the molecule is not present. An example is the alcohol polyethoxylates.

iv. Amphoteric surfactants

Amphoteric surfactants are characterised by both negative and positive charges being present on the molecule. An example is the alkyldimethylbetaine.

v. Zwitterionic surfactants

Zwitterionic surfactants are similiar to amphoteric surfactants in that both have positive and negative charges on the molecule but differ in that zwitterionic surfactants do not dissociate in solution. An example is the

3 - (N,N - dimethyl - N - hexadecylammonio) propane - 1 - sulphonate

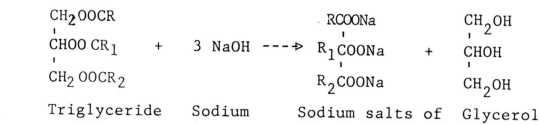
2.2 Detergents

Moore (1967) classified detergents into two main categories. The first category comprises soap or soapy detergents which were the earliest manufactured detergents. They include soap powders and flakes, household soaps and toilet soaps and are basically made by saponification of animal or vegetables oils and fats. The second category is that of soapless detergents, manufactured chiefly in the form of washing powders and liquids for clothes, dishes and other household articles.

Commercial detergent preparations usually contain only 10 to 30% of the active surfactant with the remaining consisting of polyphosphates and others. Higgins and Burns (1975) and Cain (1977) have reported that these preparations have a greater cleaning efficacy compared to an equal weight of pure surfactant.

2.2.1. Soaps or soapy detergents

As mentioned previously, the first type of commercial cleaning agents were simple soaps derived from reactions between caustic alkali and natural oils and fats. The most commonly used technique in soap making is the saponification process and the chemical reaction is as follows:



In this process, the fats and oils described chemically as triglycerides are hydrolysed with caustic alkali to form the sodium salts of fatty acids which is soap and glycerol. The resulting soap molecule has a carboxylate head, the hydrophilic group, and a long hydrocarbon tail, the hydrophobic part. The soap molecule in water ionises to form a negatively charged molecule making it an anionic surfactant (Swisher 1970).

2.2.2. Soapless detergents

Soapless detergents are essentially synthetic chemicals obtained by a sulphonation reaction between oleum or sulphur trioxide with a parent hydrocarbon chain. They can also be derived from reactions between alchols and ethylene oxide or by sulphating tallow alcohols.

Longman (1975) has further classified these detergents into anionic, cationic, non-ionic or amphoteric detergents depending on the distribution of electrical charges on the molecule.

2.3 A Comparison Of Cleaning Efficiency Between Soap And Soapless Detergents.

Soap, being the first commercial cleaning agent, is at present being successfully replaced by soapless detergents due to the following factors (Khanpara 1982):

i. Cleansing action of soap in hard or acid water is greatly reduced compared to soapless detergents. Soap reacts with calcium, magnesium and ferric salts found in hard water to form the respective insoluble salts. Further, soap in acid water tends to form non-ionised lipophilic fatty acids, thus making it insoluble in water. Soapless detergents do not react in the same manner.

ii. Soapless detergents can also be used effectively in cold and hot water and they prevent redeposition of dirt on fabrics. There is also a greater scope for formulation developments to suit newly developed fabrics and consumer needs.

iii. Soap is dependent on food and agricultural industries for its raw materials which can be natural oils or fats. Soapless detergents, however, were based on synthetic chemicals mainly from the petrochemicals industries where the supply is constant and the price is stable. It does not compete with the food industry and is not governed by the various supply and demand factors that affect food industries. Recently, Hamirin (1985) stated that the cost of producing synthetic detergents from the oil industries could be more expensive than previously anticipated. The rising costs could be attributed to the spiraling cost of oil. However, the high cost is compensated by its efficiency in cleaning processes.

2.4 Alkylbenzene Sulphonates

This is by far the largest group in general use, since dodecylbenzene sulphonate, the leading member of the group accounts for some 40 % of all detergents used in the world. These compounds are based on aromatic compounds combined with an aliphatic chain and a sulphonate group attached to the aromatic compound.

Swisher (1970) reported that alkylbenzenes were initially produced by the chlorination of kerosine and Friedel - Crafts alkylation of benzene. The chemical equation for this synthesis is as shown:

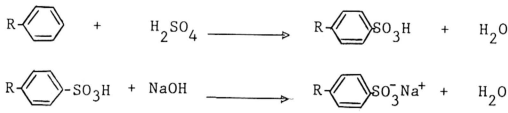
$$C_{12}H_{26} + Cl_2 \dashrightarrow C_{12}H_{25}Cl + HCl$$

$$C_{12}H_{25}Cl + \bigcirc \dashrightarrow C_{12}H_{25}\bigcirc + HCl$$

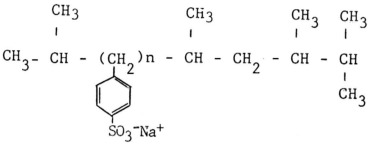
However, the stimulus for an enormous expansion in the production of alkylbenzene sulphonate surfactants was caused by the availability of cheap tetrapropylene based alkylbenzenes in the early 1950s (Cain 1981, Berg and Vora 1982). Berg and Vora (1982) also reported that alkylbenzene was produced in a cheap one-step process involving a Friedel - Crafts alkylation of benzene with a propylene tetramer which mainly contained a mixture of C_{12} olefin isomers.

Alkylbenzene sulphonates were obtained by sulphonation of the parent alkylbenzene with oleum or sulphur trioxide to give the sulfonic acid, which is then neutralised with sodium hydroxide to give the sodium salt.

The chemical reactions are as shown:-



where R-  based on a propylene tetramer can be shown as:



Tetrapropylene alkylbenzene sulphonates were generally replaced by the corresponding linear alkylbenzenes which were prepared either from linear paraffins via the intermediate chloro-paraffins or from linear olefins. This occured around 1964 (Swisher 1970) where practical techniques were developed for the production of linear paraffins. For example, linear paraffin was produced from hydrotreated kerosine and/or gas oil feedstocks by continuous liquid phase solid - bed adsorption. This allowed for the

successful replacement of the branched chain alkylbenzene sulphonates which were relatively non-biodegradable to the highly biodegradable linear alkylbenzene sulphonates.

2.5 Biodegradation Of Xenobiotic Compounds.

Alkylbenzene sulphonates are now generally accepted as being a threat to the environment due to the problems of biodegradation. King (1981) defined biodegradation as the conversion of chemical compounds to simpler substances through the action of microorganisms. In surfactant breakdown, primary biodegradation is said to occur when a characteristic property of the surfactant is removed while ultimate biodegradation involves the complete breakdown to carbon dioxide, water, oxides or salts of other elements and the synthesis of new cells (Painter 1974, Swisher 1970).

Surfactants derived from petrochemicals and other sources are considered xenobiotics compounds. Many authors (Paris et al 1982, Huttzinger and Veerkamp 1981, Slater and Somerville 1979) described xenobiotic compounds as organics which are not

natural to the system. They are normally absent from the ecosphere and are introduced by man, often by industrial processes involving synthetic chemicals. These compounds are also substances which biological organisms in the biosphere have not been exposed to during its evolutionary history.

Many xenobiotic compounds are known to be highly recalcitrant and tend to persist in our environment contributing to environmental pollution and are potential hazards to man (Alexander 1965). The list of chemical compounds considered as xenobiotics include chlorinated hydrocarbons (Omori and Alexander 1978, Stirling and Dalton 1979), aromatic compounds which included catechols, benzene and polyclic aromatic hydrocarbons (Haller and Finn 1979, Lippmann and Schlesinger 1979 and Neff 1979), sulphonated hydrocarbons which include alkybenzene sulphonates (Cain 1981, Brilon et al 1981), chlorinated dibenzo-dioxins in pesticides (Nash and Beall 1980, Klecka and Gibson 1980) and synthetic organic colourants (Evans 1977).

2.5.1 Factors influencing biodegradation of xenobiotic compounds

According to Alexander (1965) and Huttzinger and Veerkamp (1981) the difficulties encountered in the biodegradation of xenobiotic compounds are attributed to the following factors:

i. The structure of the xenobiotic compound

The resistance to biodegradation of the xenobiotic compounds to the action of the microorganisms can be attributed directly to the structure of the compound itself. These compounds have never been exposed to microorganisms in its evolutionary history and therefore the micro-organisms have not evolved appropriate enzyme systems for metabolism of these compounds. The continuous development of new chemicals within a short period of time is unfavourable in terms of the evolutionary adaptability of the micro-organisms to the new chemicals. However, Gibson (1982) has pointed out that microorganisms have been exposed to an array of chemicals produced by diagenesis. Many of these chemicals

produced by diagenesis bear little structural relationship to the biological products from which they are derived (Brooks 1977). The chemicals include many substituted polycylic aromatic hydrocarbons (Blummer and Youngblood 1975) formed by the thermal alteration of cellular materials and the existence of micro-organisms that metabolize aromatic hydrocarbons ranging in size from benzene to benzo (a)pyrene have been described (Gibson 1977).

Some of the examples of chemicals with structural resistance to biodegradation include terminal quaternary groups,non-alkyl substituents and extensive branching on an alkyl chain of an alkylbenzene sulphonate (Swisher 1963, Huddleston and Allred 1963, Straus 1963, Ryckman and Sawyer 1957). Alexander and Lustigmann (1966) had also observed that minor changes to the molecular structure of the compounds which are metabolisable could produce an analogue which is recalcitrant. This has been shown by substitutions of aromatic compounds with halogens, nitro, amino and sulphonate groups which sufficiently alter the structure, hence reducing the rate of trans-formation and may even completely prevent metabolism

(Alexander 1965, Cripps 1975, Chapman 1976, Furukawa et al 1978).

The molecular structure of the compound may also determine its physical state (adsorbed, gas-phase) where microbial degradation occurs slowly. This is shown by lypophilic compounds which have low solubilities in water (Huttzinger and Veerkamp 1981).

ii. Availability of microorganisms and uptake deficiencies

The biodegradation of compounds also depend on the availability of microorganisms which may not be found in environments with extremes of temperature, salinity, pH or the deficiency of some critical nutrient element (Alexander 1974). Biodegradation may also not occur when the compound is unable to enter the cell (Lara and Stokes 1952, Dagley 1954, Ornston 1971).

2.6 Metabolism Of Xenobiotic Compounds

Xenobiotic compounds, however, are known to be degraded in the environment since it has generally been shown that they do not accumulate in the environment although their rate of biodegradation may take a long time. This is in accordance to the principle of microbial infallibility (Alexander 1965) where microorganisms are said to have a remarkable capacity for adapting themselves to a host of ecosystems, environmental conditions and substrates due to their ready susceptibility to genotypic modication and frequent enzyme reorientation. It enables them to degrade recalcitrant xenobiotic compounds provided that the environmental conditions are conducive to microbial life and degradation of the specific compounds.

Hardy (1983) has recently described that degradative enzymes other than those derived or specified by chromosomal genes are involved in degradation of xenobiotic compounds. These are the plasmid coded enzymes. Plasmids are extrachromosomal elements found in many bacterial genera that enables them to code for important

properties that are not coded for by the main bacterial chromosome. These important properties include resistance to antiobiotics, production of toxins and degradation of complex organic molecules including components of mineral oils, the herbicide 2,4-D and detergents. These plasmid coded enzymes convert the growth substrates like alkylbenzene sulphonates, octane, toulene, camphor and salicylate to metabolites like acetaldehyde, pyruvate and isobutyrate which then enter the various metabolic pathways catalysed by chromosomal enzymes. Most degradative plasmids code for at least ten enzymes which are involved in the catabolism of a particular substrate. Degradative plasmids enable bacteria to grow on xenobiotic compounds and are important in the removal of organic pollutants from the environment.

However, normal enzymes are also important in the degradation of xenobiotic compounds. A xenobiotic compound can be metabolized by an enzyme which normally serves a physiological function in an organism, provided there is a similiarity in the structure of the xenobiotic compound to the compound which the enzyme normally acts upon. This is

exemplified by the conversion of a broad spectrum of hydrocarbons and halogenated alkanes by methane monooxygenases of methane oxidizing organisms (Higgins et al 1980, Stirling and Dalton 1979, 1980) and in dioxygenation of substituted benzoic acids (Reineke and Knackmuss 1978).

Xenobiotic compounds can also be degraded by co-metabolism. This is a process in which microorganisms, growing at the expense of one substrate, also have the capacity to transform other compounds without deriving any direct benefit from its metabolism (Horvath and Alexander 1970, Jacobson et al 1980). Co-metabolism was originally thought to be the result of a simultaneous attack on the growth promoting metabolites and the non-utilized co-metabolite by the activity of the enzymes of broad specificity. Ooyama and Foster (1965), Beam and Perry (1973, 1974) and de Klerk and van der Linden (1974) have shown that mirco-organisms growing at the expense of an alkane, co-metabolize cycloalkanes to the corresponding cycloalcohol or cycloalkanone. Co-metabolism has also been indicated in the breakdown of

complex branched and cyclic hydrocarbons (Perry 1979, Raymond and Jamison 1971) and in a secondary recalcitrant alkylbenzene sulphonate (Horvath and Koft 1972). Jamison et al (1969), Jensen (1963) and Beynon et al (1963) pointed out that co-metabolism may also occur through the activity of enzymes not directly associated with the catabolism of the growth substrate. Slater et al (1976) has shown that organisms growing on succinate will synthesize the enzyme dehalogenase which is not involved in succinate metabolism, but will metabolise chlorinated acetic acids without being able to utilize the products for growth.

2.7 Problems In Biodegradation Of Alkylbenzene Sulphonates.

Alkylbenzene sulphonates being xenobiotic compounds of complex chemical structures afford some problems in biodegradation. The breakdown of alkylbenzene sulphonates is easily explained if the structure of the alkylbenzene sulphonate is considered as consisting of three components.

These are:-

i. A long hydrocarbon side chain which is linear or branched.

ii. A benzene ring attached to the hydrocarbon chain.

iii. A sulphonate group attached to the benzene ring.

The degradation of the alkylbenzene sulphonates is generally believed to be initiated with the breakdown of the alkyl side chain followed by the sulphonate group and the benzene ring (Ryckman and Sawyer 1957, Cain et al 1972, Huddleston and Allred 1963). Berg and Vora(1982) reported that the ease of biodegradation of the alkyl side chain depends on the actual nature of the alkyl side chain which could be branched or linear.

2.7.1 Alkyl chain metabolism

In simple linear alkyl chains, biodegradation is initiated by ω -oxidation of the terminal methyl group in both hydrocarbons (van der Linden and Thijsse 1965, McKenna and Kallio 1965)

and surfactants (Huddleston and Allred 1963). Cain (1981) suggested that ω -oxidation is the oxidation of the terminal carbon at the ω -position which results in the successive formation of the C-terminal alcohol, aldehyde and carboxylic acid. The enzymes involved are an alkane monooxygenase and two dehydrogenases, and the reaction is :

$$R\text{-}CH_2\text{-}CH_3 \dashrightarrow R\text{-}CH_2\text{-}CH_2OH \dashrightarrow R\text{-}CH_2\text{-}CHO \dashrightarrow R\text{-}CH_2\text{-}COOH$$

The alkyl side chain is then degraded by β-oxidation (Lynen, 1955). β-oxidation involves oxidation of the carbon atom, with the splitting off of a two carbon fragment successively as acetyl Co-A (Overath et al 1969). Acetyl Co-A then enters the tricarboxylic acid cycle for the production of energy (Gottschalk, 1979). However, isomers with a B-methyl substituted side chain or with a gem-dimethyl-branched side chain cannot be degraded by this mechanism alone. In this case, the existence of an α and β oxidation mechanism has been shown (Martin and Stumpf 1959, Cain et al 1972). α -oxidation involves the loss of one carbon atom, that is the carboxyl carbon, and the carbon atom two, that is the α - carbon becomes oxidized to being the new carboxyl

group. Cain (1981) reported that this combination of an α and β-oxidation is known to be rare in microorganisms and could explain why alkylbenzene sulphonates may persist in the environment. However, with prolonged acclimatization, they are still degraded (Huddleston and Allred, 1963).

The problems associated with the alkyl chain metabolism arose mainly due to the branched forms of the alkyl chain. Ludzack and Ettinger (1960) reported that tert-alkylbenzene sulphonates do not degrade as readily as n-alkylbenzene sulphonates. Numerous authors (Fall et al 1979, McKenna and Kallio 1964, Pirnik 1977 and Schaeffer et al 1979) also reported that methyl branching increases resistance of hydrocarbons to microbial attack. Terminal quaternary groupings and compounds in which the benzene moiety is on a tertiary carbon also offer resistance to biodegradation (Swisher 1963, Ryckman and Sawyer 1957). In addition, Schaeffer et al (1979) has also reported that terminal branching inhibits biodegradation of hydrocarbons.

Gledhill (1975) suggested that the recalcitrance of branched alkyl moieties could probably be due to the inability of the microorganisms to initiate β-oxidation. This has been attributed to the hindrance to β-oxidation at the branched point. Methyl branching at the β position of the alkyl chain is also known to block β-oxidation, requiring ω -oxidation (Martin and Stumpf 1959, Beam and Perry 1973), α-oxidation (Pirnik 1977) or β alkyl removal (Cantwell et al 1978).

2.7.2 Metabolism of sulphonate groups

The sulphonate by itself is known to be biologically stable (Cain 1981) due to the C-S bond of an attached sulphonate group which is thermodynamically stable. This is reflected in the rarity of naturally occuring sulphonates. The removal of the sulphonate group from the alkylbenzene is an important step in the biodegradation of alkylbenzene sulphonates. Higgins and Burns (1975) reported that the breakdown stage of the alkylbenzene sulphonate is still unclear although the breakdown of the alkyl chain

is generally thought to occur before that of the aromatic benzene ring. However, Swisher (1972) reported that alkyl chain degradation preceeded the breakdown of the benzene ring and sulphonate. Willets and Cain (1972), on the other hand showed that desulphonation of the benzene ring occured before β-oxidation of the alkyl side chain. Willets (1973a,1973b) later reported that desulphonation is in fact the prerequisite to benzene ring biodegradation in alkylbenzene sulphonates.

The mechanism of desulphonation is thought to occur by either of the three mechanism of reactions below:

i. Hydroxylative desulphonation of alkylbenzene sulphonates (Willets and Cain, 1972)

$$R\text{-}SO_3H + H_2O \text{ ---- } R\text{-}OH + 2H^+ + SO_3$$

ii. A monooxygenase mechanism of desulphonation (Cain et al 1972)

$$R\text{-}SO_3H + O_2 + NADH + H^+ \text{ --- } R\text{-}OH + H_2O + SO_3^- + NAD$$

iii. A reductive mechanism of desulphonation (Huddleston and Allred, 1963)

$$R\text{-}SO_3H + NADH + H^+ \text{ --- } R\text{-}H + NAD^+ + H_2SO_3$$

In all the three mechanisms, a sulphite group is the first product of desulphonation (Willets and Cain 1972, Johnston et al 1975). Sulphite is than oxidised to sulphate which is the final desulphonation product (Focht and Williams 1970, Thysse and Wanders 1974, Ripin et al 1971).

2.7.3 The benzene ring metabolism

The aromatic compound, benzene, in alkylbenzene sulphonates is generally regarded to be degraded after alkyl chain breakdown and desulphonation. The products formed after degradation of the side chain and desulphonation are benzoate or phenylacetate. This is as shown below:

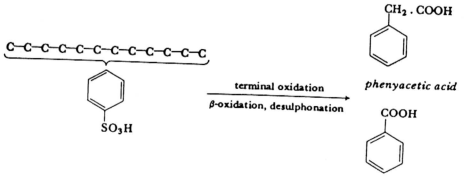


Figure 2.1 and 2.2 illustrate the pathways of phenylacetic acid degradation and microbial oxidation of benzoic acid (Willets and Cain 1972, Willets 1974, Bird and Cain 1974). In the case of benzoate, the initial step in its subsequent breakdown involves the addition of molecular oxygen by a monooxygenase resulting in the formation of a catechol. Catechol is further degraded by one or two routes, depending upon whether the ring is cleaved between the hydroxyl groups (ortho cleavage) or adjacent to them (meta cleavage). Both reactions are catalysed by dioxygenase enzymes, the former by catechol-1,2-oxygenase, the latter by catechol-2,3-oxygenase. The ensuing catabolic routes of the cleavage products results in the formation of acetyl-CoA and succinate for the ortho cleavage and acetaldehyde and pyruvate for the meta cleavage. These products are then subsequently chanelled into the energy deriving pathways of the bacteria.

The degradation of phenylacetate is through the ortho or para-hydroxyl derivatives resulting in the formation of fumaric and acetoacetic acid or δ-carboxymethyl-α- hydroxymuconic semialdehyde (Pirnik 1977).

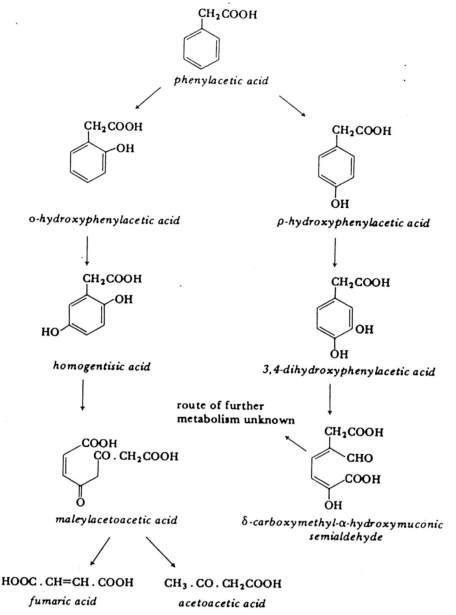


Fig 2.1: Pathways of phenylacetic acid degradation

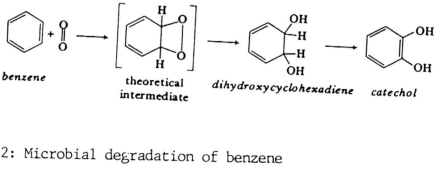


Fig 2.2: Microbial degradation of benzene

2.8 Acclimatization During Surfactant Biodegradation

As mentioned previously, the biodegradation of xenobiotic compounds is made possible by the metabolic versatility of the microorganisms. This includes the capacity of the microorganism to acclimatize to the organic compounds which it could not metabolize earlier. Slater and Somerville (1979) refer to acclimatization as the adaptation of the bacteria's existing enzyme systems for the breakdown of the novel chemical structures encountered or through the acquisition of novel metabolic pathways in the bacteria.

The enzymes present in microorganisms can generally be classified into two main types. They can either be constitutive or inductive enzymes. Constitutive enzymes are enzymes essential to the very existence of the microorganisms and are present in significant amounts in the cells irrespective of the environmental conditions. These enzymes are especially involved in the conversion of readily utilizable compounds to energy in the various energy deriving pathways (Sanwal 1970, Gibson 1968).

The other group of enzymes are the inductive or inducible enzymes which are normally present in low amounts in the cells. The presence or activity of these enzymes are generally initiated or triggered by the presence of certain substrates under conditions when the cells are normally under stress (Swisher 1970). These substrates are generally not readily assimilable and need these enzymes for their breakdown (Richmond 1968). The inducer triggers the de novo synthesis of these inducible enzymes which are matched to the chemical structure of the compound. This enables the enzymes to modify or breakdown the compounds to products suitable for entering the cell's fundamental metabolic reactions catalysed by constitutive enzymes.

There is a lag period involved for the inducible enzymes to be formed. According to Swisher (1970), this is the period of acclimatization. Inducible enzymes are generally subjected to catabolite repression, especially by the addition of readily utilizable substrates. Glucose and succinate have been indicated as good catabolite repressors of inducible enzymes for the biodegradation of aromatic compounds(Ornston 1966, Hegeman and Rosenberg 1970). Cain et al (1972) reported

that sulphite represses desulphonation of alkylbenzene sulphonates.

The development of the new metabolic potential of microorganisms to metabolise xenobiotic compounds has been termed as one of acquisitive evolution. The studies of this new metabolic potential of microorganisms has indicated that four primary ways are generally involved:

i. Constitutive production of a previously inducible enzyme

The presence of a new constitutive enzyme activity could be explained by a mutation either in the regulatory gene associated with an inducible enzyme activity or a mutation in the gene specifying a normal constitutive enzyme (Hegeman and Rosenberg 1970). Lin et al (1976) later suggested that the first step in acquiring a novel catabolic activity was a mutation in the regulator gene to constitutive synthesis of an enzyme that was normally inducible. Numerous reports such as those by Kim (1963) and Lederberg(1951) described this mechanism to be the most commonly observed mechanism by which bacteria acquire the ability to grow on novel substrates.

ii. A change in the specificity of induction of a pre-existing enzyme possesing limited activity towards the novel compound.

This could occur by a mutational modification of the relevant repressor gene which may lead to recognition of the novel substrate and the formation of a 'new' inducible enzyme (Lester and Bonner 1956, Mortlock and Wood 1964, Mortlock 1976).

iii. The acquisition of decreased sensitivity to the normally generated toxic or growth inhibiting metabolites from a novel compound.

Mayo and Anderson (1969) have reported an example of this acquisition of decreased sensitivity in mutants of Aerobacter aerogenes growing on L-mannose. It was shown that although the wild type organisms could induce the formation of enzymes for its breakdown, only the mutants could grown on the L-mannose. This was explained by the fact that L-glyceraldehyde, a metabolite of L-mannose, inhibited bacterial growth and glycolysis in the wild strains while the mutants could overcome this inhibition.

iv. Acquisition of permeability to a metabolisable compound.

Acquisition of permeability to a metabolisable compound has been shown in microorganisms which are unable to metabolise compounds of biological importance. Gutnick et al (1969) reported that these compounds are often intermediates of the tricarboxylic acid cycle and pentose phosphate cycle. The loss of permeability barrier in the mutants seems to be the explanation for the mutants ability to metabolise these intermediates

Two general theories have been proposed to explain the origin of these metabolic pathways. On one hand, Horowitz (1965) has suggested that retrograde stepwise construction of catabolic pathways occur by tandem gene duplication. This can also be termed as vertical evolution (Clarke 1981). It is the process by which evolution occurs by duplication of genes of consecutive steps of the pathway, beginning with the last steps of the pathway. Wu et al (1968) proposed an alternative theory which described the possibility of duplication of an entire pre-existing pathway

of enzymes. This could occur by duplication of each of the genes for the enzymes responsible for the successive steps of the pathway. Numerous reports (Hartley 1974, 1976, Wu 1976, Clark 1978) have supported this view. The alternative theory may be termed as horizontal evolution whereby an archetypal enzyme of broad specificity coded by a gene duplicated many times and, by divergent mutation, could give rise to a group of enzymes with high specificities and high rates of reaction.

More recently, numerous reports (Reineke and Knackmuss 1978, Knackmuss 1981, Chakrabarty 1980) have described that naturally occuring plasmids play a central role in the evolution of new strains of bacteria with novel catabolic capabilities. This is primarily due to the possibility of alternative convergent and divergent pathways specified by plasmid and chromosomal genes giving a great flexibility in the evolution of new catabolic pathways. Plasmids are also important in that the transmissible catabolic plasmids enable genes for catabolic enzymes to be spread rapidly through a bacterial

population (Jones and Sneath 1970).

Acclimatization must be considered as a key factor in surfactant biodegradation. Huyser (1960) found that 5 to 6 days were generally needed for the breakdown of linear alkylbenzene sulphonates in unacclimatized river water whereas approximately 1 to 2 days are required with acclimatized river water. Cain (1977) has also described that acclimatization to benzene ring degradation takes longer than that for alkyl chain length. A change in substrate that is from 3-phenyl to 6-phenyldodecane sulphonate, can also delay benzene ring degradation of the 6-phenyl isomer for several days. Over this period the culture re-acclimatizes to the newer substrate. The difference in acclimatization to linear alkylbenzene sulphonates and to branched alkylbenzene sulphonates has also been mentioned. WPRL (1968) reported that the trickling filter microorganisms took 2 to 4 weeks to acclimatize to linear alkylbenzene sulphonates while it took 12 to 13 weeks to acclimatize to branched alkylbenzene sulphonates. The percentage of biodegradation in the two cases were 90-95% and 65-75% respectively.

2.9 Microbiology Of Surfactant Biodegradation

The principal agents of surfactant breakdown are bacteria although some algae and fungi are also known to degrade surfactants (Wurtz-Arlet 1964, Davis and Gloyna 1967, Cain 1977). Most of the bacteria that are known to degrade surfactants have been isolated using an enrichment or selective culture technique. Some of the microorganisms that have been isolated are listed in Table 2.1.

Table2.1 Biodegradation of surfactant molecules by pure cultures of fully identified microorganisms.

Organism	Surfactants degraded	Reference
Alcaligenes faecalis	branched ABS*	Marion, 1966
Escherichia coli	branched ABS	Huddleston & Allred, 1963
Proteus vulgaris	branched ABS	Huddleston & Allred, 1963
Pseudomonas aeruginosa	branched ABS	Swisher, 1970
Pseudomonas fluorescens	branched ABS	Huddleston & Allred, 1963
Serratia marcescens	branched ABS	Huddleston & Allred, 1963
Sphaerotilus species	branched ABS	Pipes & Jones, 1963
Arthrobacter Bacillus	general	Cain, 1977
Citrobacter, Nocardia	general	Cain, 1977

* ABS Alkylbenzene sulphonates

2.10. Environmental Effects Of Detergents Containing Alkylbenzene Sulphonates.

The environmental problems associated with the use of detergents arose mainly due to the use of branched alkylbenzene sulphonates (Hammerton 1955, Pattison 1967, Truesdale and Eden (1974). This was proven when the introduction of linear alkylbenzene sulphonates eliminated many of the problems caused by branched alkylbenzene sulphonates (Huddleston and Allred 1963, Allred et al 1964). Huddleston and Nielsen (1979) and Nielsen and Huddleston (1983) later showed that the linear alkylbenzene sulphonate carbon is completely biodegradable. However, branched alkylbenzene sulphonates are also known to be biodegradable, but, over a longer period of time and therefore contribute to many environmental problems. The environmental effects of the detergents containing branched alkylbenzene sulphonates are as follows:

i. Toxicity of the surfactant itself

Manganelli (1952) and WPRL (1968) have reported the toxicity of branched alkylbenzene sulphonates to

bacteria and protozoa in sewage treatment processes. According to Janicke (1973) alkylbenzene sulphonates at concentrations of 62.5 to 125 mg/l and 12.5 to 125 mg/l inhibited the growth of Escherichia coli and Pseudomonas species, respectively. It was also reported that alkylbenzene sulphonates at concentrations of 10 to 25 mg/l was toxic to protozoa in sewage treatment processes (ERL 1978). In the anaerobic digestion of sewage sludge, it has been shown that concentrations of alkylbenzene sulphonates in excess of 1.5% (dry-solid basis) inhibits methane production (Degens et al 1952, Pitter 1974 , Bruce et al 1966).

Acute toxicity of branched alkylbenzene sulphonates to various freshwater fish species have also been documented (Henderson et al 1959, Surber and Thatcher 1963, Arthur 1970). Abel (1974) reported that a concentration as low as 7.4 mg/l of branched alkylbenzene sulphonate can be toxic to some species of freshwater fish.

ii. Toxicity of partially degraded surfactants

Numerous reports (Patterson et al 1967, Alexander 1974, Cook 1979) have shown that for certain organic

compounds the degradation products are more toxic and pose a greater environmental hazard than the original compounds themselves. Borstlap(1964), however, working on alkylbenzene sulphonates has showed that degradation products of alkylbenzene sulphonates are less toxic than the original compound itself.

iii. Effects on oxygen transfer

Microorganisms suspended in an aqueous medium require dissolved oxygen for oxidation. The oxygen is usually supplied by diffusion from the atmosphere into the aqueous medium. In aerobic treatment systems, the oxygen is supplied by forced aeration whereby oxygen transfer to the microorganisms occurs in a series of steps (Bennet 1980).

Surface active agents in water, however, have been shown to lower the overall mass transfer coefficient k_La or oxygen transfer in bubble aeration systems (Mancy and Okun 1960, Koide et al 1976, Eckenfelder and Barnhart 1961) since the surfactants adsorb on the gas-liquid interface. The adsorption of the large surfactant molecules reduces the surface tension of water (O'Connor 1960 ,

Meijboom and Vogtlander 1974), reduces the bubble size (Zieminski et al 1967), lowers the terminal velocity of the bubbles (Griffith 1962, Kawase 1982) and increases the drag coefficient (Raymond and Zieminski 1971). Accordingly, surfactants are believed to reduce the liquid phase mass transfer coefficient k_L by depressing the hydrodynamic activity and by offering resistance for the entrance of gas molecules into the liquid (Stenstrom and Gilbert 1981). However, according to Mancy and Okun (1960) and Eckenfelder and Barnhart (1961) higher surfactant concentrations results in an increase in the interfacial gas-liquid area per unit volume caused by the formation of smaller bubbles and this results in an increased overall mass transfer coefficient. The reduction of oxygen transfer rates is especially felt in rivers and other natural water systems where self purification processes are severely hindered. Consequently also in aerobic sewage treatment processes, the reduction in oxygen transfer rates will result in higher costs in power and equipment needed to supply the necessary amount of oxygen to the microorganisms.

iv. Foaming

Numerous authors (Gowdy 1953, Martin 1954, Hansen and Derderian 1975), described foams essentially as gas dispersions in liquids resulting from mechanical agitation or forced aeration of liquids containing surface active agents. Foaming is generally observed in sewage treatment plants and in rivers which receive effluents containing surface active agents. According to (Rudolfs et al 1949, Degens 1954, McKinney 1957, ERL 1978) the problems created by excessive foaming include:

a) Microbial flocs tend to be carried over with the foam from aeration tanks resulting in the reduction of active biomass in the mixed liquor of aerated treatment systems.

b) The sight of foams does not blend with the surroundings and are therefore aesthetically undesirable.

c) Reduction in visibility due to foam build-up in rivers. This could be hazardous to boats.

d) Transmission of pathogenic organisms from sewage treatment system by foam to surrounding areas.

v. Eutrophication

Eutrophication generally means the enrichment of water by organic and inorganic materials, in particular by phosphates (Owens and Wood 1968). The general symptom of eutrophication is the increase in the nutrient content of the water resulting in algal blooms . The death of the algal blooms will exert a great oxygen demand and this will cause the death of other aquatic life resulting in foul odours.

Polyphosphate builders which may constitute 40 to 60 percent of the detergent formulation have been described to be responsible for eutrophication (Devey and Harkness 1972, Jenkins et al 1972). However, no direct confirmation of the contribution of polyphosphate builders to eutrophication have been reported (Higgins and Burns 1975).

2.11 Methods Of Treatment Of Detergent Wastewater

Biological methods such as the activated sludge process, oxidation ditch, oxidation pond, aerated lagoon, trickling filters and anaerobic digesters are widely adopted for wastewater treatment. The principle and practice of these biological methods involve the oxidation of organic wastes in water by bacterial action. However, biological methods have their drawbacks. Nitrate and phosphates are not efficiently reduced and are known to cause eutrophication in receiving waters (Higgins and Burns 1975, Weaver 1969). Nitrate which is known to increase in trickling filter and activated sludge systems (Ademoroti 1985) can cause the disease methemoglobinemea in infants (APHA 1980). Ademoroti (1985) also reported that the reduction of metals is also limited and, according to Gledhill (1975), recalcitrant alkylbenzene sulphonates are not removed.

However, chemical and physical treatment methods have been widely used as pre-treatment steps (Weber 1972) to make the wastewater more amenable to biological treatment. These physical and chemical treatment systems employed in the

treatment of detergent wastewater include foaming (Flynn and Andres 1963, Klein and McGauhey 1965, Rubin 1963, Grieves and Bewley 1973), coagulation and flocculation with acid, alum and a tallow flocculation aid (Flynn and Andres 1963), flocculation with sulphuric acid, lime and alum (Hashim et al 1985), flocculation and clarification with activated carbon (Hassan et al 1985), flocculation with lime, ferrous sulphate and chlorine water (Vaughn et al 1956), oxidation with Fenton's reagent (Eisenhauer 1965), precipitation of alkylbenzene sulphonates with a cationic polymer (Samples 1967) and vaccum diatomite filtration with and without ion exchange (Flynn and Andres 1963).

2.11.1 Foaming treatment of detergent wastewater

Lemlich (1972) and Clarke and Wilson (1983) have described the foam separation process which has been used successfully in the treatment of several wastes. In certain instances surface active agents have been added to promote foaming and separation of a non-surface active component in wastewater. This includes flotation of dichromate (Grieves and Schwartz 1966), phosphate (Grieves 1972),

chromic hydroxide (Bhattacharyya et al 1971), iron-cyanide complexes (Busch et al 1980) and lead (Thackston et al 1980, Slapik et al 1982).

The theory underlying the foam separation process is simple. Since, surface active agents such as alkylbenzene sulphonates tend to collect at gas-liquid interfaces, foaming as a result of forced aeration provides an efficient means of generating and collecting the gas-liquid interfaces (Rubin 1963, Viesturs et al 1980). The foam is therefore encriched with the surface active agent and their content in the residual bulk liquid is hence depleted. Consequently, the foam can be collected and collapsed to produce a surface active agent rich foamate.

Many studies (Rubin 1963, Jenkins 1966) have shown the foam separation process to be an efficient and economical method of removing alkylbenzene sulphonates in sewage plants containing low levels of detergents in the region of approximately 5 to 20 mg/l. However, Hassan et al (1985) reported that in treated detergent

wastewater containing 164 mg/l of alkylbenzene sulphonates, only a 16% reduction was recorded after the foam separation process.

2.11.2 Activated carbon use in the treatment of detergent wastewater.

Suffet and McGuire (1980) claimed that activated carbon adsorption is one of the most effective and dependable technologies for the removal of the broad spectrum of dissolved organic impurities found in wastewaters. It has generally been used in combination with other treatment methods including fixed film systems, activated sludge processes and ozonation. In general activated carbon treatment has been widely employed in the removal of trace concentrations, usually less than 1.0 mg/l of various pollutants.

Activated carbon treatment of detergent wastewater has not been very successful (Flynn and Andres 1963, Hassan et al 1985). Hassan et al (1985) reported only a 33% reduction in alkylbenzene sulphonates with 20,000 mg/l of powdered activated carbon.

2.11.3 Chemical coagulation and flocculation of detergent wastewater

According to Grutsch and Mallat (1976), chemical treatment processes are generally a prerequisite before biological treatment. This is especially so for the removal of phosphates and metals which are not amenable to biological treatment (Ademoroti 1985). In detergent wastewater, phosphates can be easily removed with lime or alum treatment (Ferguson et al 1970, Ferguson and King 1977).

However, alkylbenzene sulphonates cannot be precipitated out due to its inability to precipitate out as insoluble magnesium, calcium or ferrous salts in hard water (Cain 1977). In the chemical treatment of detergent wastewater containing alkylbenzene sulphonates, chemical oxidation has been used in conjunction with ozone treatment (Degremont 1971) and a combination of hydrogen peroxide and ferrous salt (Eisenhauer 1965). Vaughn et al (1956) has also reported the use of lime, ferrous sulphate and chlorine

in which there was a 50% reduction in the anionic detergent of the treated wastewater. They also reported a 30% reduction in anionic detergent at pH 8.8 using aluminium sulphate. Hashim et al (1985) also described the use of sulphuric acid, lime and alum treatment of detergent wastewater where an efficiency of 80% removal of branched alkylbenzene sulphonate was achieved. It was believed that most of the alkylbenzene sulphonates was removed by adsorption onto the floc particles produced with lime and alum additon.

2.11.4 Anaerobic treatment of detergent wastewater

Meynell (1976) and Wolfe (1971) described anaerobic digestion as a naturally occurring process involving fermentation of organic wastes by specialised bacteria in the absence of oxygen. Anaerobic treatment is generally used in the treatment of strong organic wastes, containing high amount of suspended solids and sludge obtained from aerobic processes and sedimentation (Tebutt 1977, Nemerow 1978). However, according to (Klein and McGauhey 1965 and Cain 1981) anaerobic treatment of detergent wastewater is ineffective. This is primarily due to the fact that

the initial step in alkyl chain metabolism involves ω-oxidation which requires molecular oxygen. The requirement for molecular oxygen severely retards alkylbenzene sulphonates degradation in anaerobic digesters and explains why alkyl oxidation cannot be effected when alternative electron acceptors such as sulphate and nitrate replace molecular oxygen (Little 1977). This is also true for the benzene ring which can only be partially degraded by reactions involving hydroxylations in anaerobic systems (Allred et al 1964, Taylor et al 1970).

2.11.5 Aerobic treatment of detergent wastewater

Aerobic treatment involves the microbial oxidation of organic matter in which the oxygen requirements of the treatment is supplied by deliberate aeration or by other means (Forster 1977). The two systems commonly used in aerobic treatment are the fixed film biological system and the system in which microorganisms are in suspension. A commonly used system for the former is exemplified by the trickling filter and for the latter by the activated sludge system.

2.11.5.1 Trickling filter

Jenkins et al (1967) carried out studies and described the use of the trickling filter system in studies on surfactant biodegradation. WPRL(1968) had reported that 90 to 95% degradation of the linear alkylbenzene sulphonates and 65 to 75% degradation of the branched tetrapropylene based alkylbenzene sulphonates could be achieved by trickling filter systems. In the fixed film process there are two zones of microbial growth. These are an aerobic zone determined by the depth to which oxygen penetrates and an anaerobic zone extending from the aerobic zone to the support surface. The aerobic zone which is actively involved in alkylbenzene sulphonate degradation only occupies a film thickness of 50 to 100um compared to a total film thickness of 0.1 to 2.0mm whereas the anaerobic zone will only be able to partially degrade the alkylbenzene sulphonate molecule (Little 1977). The operation of the trickling filter system is also known to be severely hampered when the

COD value is greater than 500 mg/l. This is due to oxygen transfer limitation into the biofilm or microorganisms actively oxidizing the organic compounds (Harris and Hansford 1976).
This would severely hamper the trickling filter process treating detergent wastewater which have high chemical oxygen demand values as well as surfactants which lower oxygen transfer into liquids containing them. This would further limit the thickness of the active, aerobic biofilm zone degrading the alkylbenzene sulphonates.

2.11.5.2 Activated sludge treatment

The success of the activated sludge process is dependent on a high concentration of a mixed culture of microorganisms maintained in a well flocculated and suspended state by agitation (Forster 1977,Tebutt 1977). Carbon dioxide and water are the final products of the microbial oxidation process. The oxygen requirements are for:

i. Conversion of organic matter

$$\text{Organic matter} + O_2 \xrightarrow[\text{enzyme}]{\text{bacteria}} CO_2 + H_2O + \text{Energy}$$

ii. Reproduction of new cells

$$\text{Organic matter} + P + NH_3 + O_2 \xrightarrow[\text{energy}]{\text{bacteria}} \text{New cells} + CO_2 + H_2O$$

iii. Degradation of other cells

$$\text{Cells} + O_2 \xrightarrow[\text{enzyme}]{\text{bacteria}} CO_2 + H_2O + NH_3 \text{ or } NO_3 + \text{Energy}$$

The activated sludge process itself comprise 3 steps. The first is the transfer step in which the soluble organics are absorbed through the cell wall and there being broken down. The second step of conversion involves the synthesis and oxidation of the organics. The acclimatization to the organic compounds also occurs in this step. The third step is the flocculation step where the organisms which include bacteria, protozoa and fungi (Pipes 1966), form flocs by various mechanisms (Coackley 1969, Esser and Kues 1983).

As indicated above, the formation of flocs and its settlelability is a major criterion in the success of the activated sludge process. This is primarily due to the fact that 50 to 90% of the settled flocs or activated sludge is recycled to the aeration zone to recommence stabilization.

2.11.5.3 Activated sludge process and its modifications

In general, the commonly used conventional activated sludge system essentially consists of an aeration tank, a secondary settler and a sludge recycle system. Mixing in the reactor is ideally taken to be of plug flow type. The conventional activated sludge process operates with the sludge in the stationary growth phase (Winkler 1981).

Many modifications have been made to the basic design of the conventional activated sludge process. These modifications were aimed at achieving:

i. more efficient aeration
ii. better contact between sludge and pollutants
iii. shorter retention time
iv. lower quantities of waste sludge

Lawrence and McCarty (1970) have reviewed these modifications in detail, and the most commonly known ones are step aeration, pure oxygen activated sludge system, contact stabilization, high rate treatment and extended aeration.

The F/M is defined as

$$F/M = \frac{S_o - S}{X\theta} \qquad 2.1$$

where:

S_o influent substrate concentration, mg/l

S effluent substrate concentration, mg/l

X microorganism concentration or volatile fraction of the mixed liquor suspended solids, mg/l

θ hydraulic retention time, days

For most conventional activated sludge reactors, the optimum value of F/M lies between 0.2 to 6 kg BOD_5/kg MLSS-day.

ii. Nutrition

The problems with industrial wastes are the presence of high concentrations of organic matter, the question of degradability of the major components of the organic matter and the ratio of nitrogen and phosphorous to the organic matter. The concentration of nutrients in wastewaters is generally very low and

nutrients have to be supplemented. The nutrient balance is usually quoted in relation to the content of readily degradable carbonaceous material expressed as BOD and, for conventional operation, the nutrient balance is 0.03 to 0.06 kg nitrogen per kg BOD and 0.007 to 0.01 kg phosphorous (as phosphate) per kg BOD. Jones (1975) and Pirt (1975) stated that the nutrients are mainly utilized to provide maintenance energy whereby nitrogen and phosphorous are utilized by bacteria during the organic removal process for the synthesis of new cells. The deficiency of either of these nutrients may cause dispersed or filamentous growth and/or limit BOD removal. The requirements may be more exactly determined by these relationships:

$$N \text{ (lb/day)} = 0.123 \left(\frac{x}{0.80}\right) (X) + 0.07 \left(\frac{0.8-x}{0.8}\right) (X)$$

$$P \text{ (lb/day)} = 0.026 \left(\frac{x}{0.80}\right) (X) + 0.01 \left(\frac{0.8-x}{0.80}\right) (X)$$

x = degradable fraction of biomass

X = volatile suspended solids, mg/l

iii. Temperature

The wastes received by activated sludge plants usually have a temperature in the range of 0°C to 40°C. Keefer (1962) reported that at 25°C a lower

BOD and smaller amounts of suspended solids were produced than at 12°C . Hurwitz et al (1961) found that cellulose could be degraded at 25°C but not at 12°C. He also reported that at higher temperatures, acclimatization took longer and the sludge never regained good characteristics above 44°C. The activity of the protozoa was severely inhibited above 36°C, and protozoa were not present above 43°C. An increase in effluent total suspended solids has also been reported and the settlelability of the sludge worsens above 35°C (Powell and Lessard 1976).

Most enzymatic reactions involving micro-organisms can be described by an Arrhenius type of temperature relationship where:

$$k' = Ae^{-E/RT} \qquad 2.2$$

k' kinetic constant at temperature T K

E activation energy

R gas constant in cal/g-mol- K

The modified Arrhenius equation is widely used to describe the temperature effects on the substrate removal rate (Eckenfelder 1967)
that is

$$k_{T2} = k_{T1}\ \theta_t^{(T2 - T1)} \qquad 2.3$$

where

$k_{T1,\ T2}$ are substrate utilization rate coefficients at temperature T1 or T2^{o}C and
θ_t temperature coefficient

iv. pH

Keefer and Meisel (1951) found that activated sludge microorganisms could acclimatize to any pH value in the range of 6.0 to 9.0 but if the pH was above 10 or below 5, the microbial population could not be called activated sludge. They also observed that the sludge had much better settling characteristics at a pH of 6.5 to 7.5. The major effect of low pH was attributed to the development of copious growths of fungi which replace the flocculant sludge.

In many cases, bacterial oxidation will modify the pH. For instance, caustic alkalinity will be

neutralized by reaction with carbon dioxide evolved during bacterial respiration. In fact, the removal of 1 lb of BOD_5 means 0.5 lb of alkalinity as calcium carbonate will be neutralized. If high concentrations of salts of organic acids are present, they will react to produce sodium carbonate and raise the pH whereas sulphonates will yield sulphuric acid as a byproduct and will require neutralization (Eckenfelder et al 1985).

v. Oxygen supply

The three primary considerations in the study of oxygen supply to the activated sludge microorganisms are:

a. the dissolved oxygen concentration maintained in the mixed liquor
b. the length of the anaerobic period during sludge return
c. the length of the time the sludge is aerated during each pass through the aeration tank.

A high concentration of dissolved oxygen in the mixed liquor does not necessarily produce good BOD reduction. In fact, Winkler (1981) had reported that 2 mg/l of dissolved oxygen was

satisfactory for this process. The effect of dissolved oxygen in the mixed liquor is related to the length of the anaerobic period during sedimentation and the sludge return. According to Gaudy and Turner (1964), long anaerobic periods are known to destroy the effectiveness of sludge. The sludge has a maximum capacity to take up organic matter, and aeration of the sludge restores this capacity.

The variation of dissolved oxygen in the reactor can be described mathematically by:

$$\frac{dC}{dt} = k_L a \cdot (C_{sw} - C) - r \qquad 2.4$$

where:

$k_L a$	overall oxygen transfer coefficient, h^{-1}
C_{sw}	saturation concentration of oxygen in pure water, mg/l
C	concentration of dissolved oxygen in sample, mg/l
t	time, h
r	respiration rate of activated sludge, mg O_2/l/h

$k_L a$ is a function of air-flow rate, temperature etc.

2.11.6 Aeration in the presence of surfactants

In the activated sludge process, metabolism sets the rate of oxygen demand. The function of forced aeration is then to transfer oxygen to the liquid at such a rate that oxygen never becomes the limiting factor in process operation.

The rate of mass transfer dM/dt in mg/l can be expressed as

$$\frac{dM}{dt} = k_L A(Cs - C) \qquad 2.5$$

where

k_L liquid film coefficient, m/h

A cross sectional area through which diffusion occurs, m^2

Cs saturation concentration of dissolved oxygen, mg/l

C dissolved oxygen concentration, mg/l

By introducing the volume of the liquid V in m^3 the above equation can be written as

$$\frac{1}{V}\frac{dM}{dt} = \frac{dC}{dt} = k_L \frac{A}{V} (Cs - C) \qquad 2.6$$

$$= k_L a \ (Cs - C)$$

where

$k_L a$ overall mass transfer coefficient, h^{-1}

The determination of $k_L a$ and values can be carried out by a steady or non-steady state test (Stukenberg et al 1977, Benefield 1980).

The α factor varies with many process conditions including wastewater quality, intensity of mixing or turbulence, suspended solids concentration and method of aeration. Holroyd and Parker (1952) reported that the alpha factor for a fine bubble diffuser that generated 0.28cm mean bubble diameter could be as low as 0.5 in the presence of 20 to 100 mg/l of surfactants. The same surfactant concentrations reduced the alpha factor of a 30 cm diameter disc surface

aerator to only 0.8. Baars (1955) reported alpha factors ranging from 0.4 to 0.9 for fine bubble diffusers that generated 0.25 cm mean bubble diameter in water containing 4 to 10 mg/l of anionic surfactants. He also tested the Kessener brush aerator under similar conditions and reported that alpha factors ranged from 1.0 to 2.0 in water containing high surfactant concentrations. Downing et al (1960) also reported an alpha factor of 2.0 for the Searle aerator (modified brush aerator) in the presence of 10 mg/l of alkylbenzene sulphonates.

The saturation concentration of oxygen in water depends upon salinity, temperature and the partial pressure of the oxygen in contact with water. The presence of dissolved salts can be corrected for by introducing a β factor, defined as:

$$\beta = \frac{\text{saturation concentration in wastewater}}{\text{saturation concentration in tap water}}$$

Under process conditions, the value of $k_L a$ is usually less for wasterwater than for tap water because of the presence of soluble organic compounds,

particularly surface active materials. To compensate for the effects of surface active agents on oxygen transfer, an α factor is introduced, where

$$\alpha = \frac{k_L a \text{ of wastewater}}{k_L a \text{ of tap water}}$$

2.11.7 Effect of branched alkylbenzene sulphonates on the activated sludge process.

The effect branched alkylbenzene sulphonates has on the activated sludge process are numerous but they can be categorised into three main areas. Firstly, the toxicity of branched alkylbenzene sulphonates to bacteria and protozoa which has been discussed in Section 2.10. Surfactants also decrease the rate of oxygen transfer between the air bubbles and the wastewater. In addition the foam generated results in a decrease in suspended solids concentration in the aeration tank.

The effect of surface active agents on the rate of oxygen transfer have been discussed by Mancy and Okun (1960), McKeown and Okun (1963) and O'Connor (1960). Their studies have shown that with addition of small amounts of aerosol O.T. and alkylbenzene sulphonates, there was a

rapid decrease in the absolute rate of oxygen transfer to a minimum value. The minimum occurred at the critical micelle concentration of the surfactant and further addition did not affect the absolute coefficient. However, according to Mancy and Okun (1960), at surfactant concentrations above the critical micelle concentration the overall rate of oxygen transfer increased.

In their studies, Downing and Boon (1963) reported that the value of $k_L a$ was reduced considerably by the presence of surfactants in the liquid phase of the mixed liquor to an extent depending upon its concentration. The introduction of a detergent containing sewage will tend to cause a deterioration of an activated sludge plant with a low dissolved oxygen concentration whereas the performance of a plant in which the dissolved oxygen concentration is high may be substantially unaffected.

The substantial amount of foam caused by branched alkylbenzene sulphonate compounds also causes the active biomass in the activated sludge process to be removed with the foam layer. This reduce the biomass concentration in the mixed liquor aeration tank. Jenkins (1966) reported that in foam fractionation treatment,

43% removal of total suspended solids and a 50% removal of volatile suspended solids into the foam layer was possible.

2.12 Biological Reaction Kinetics

The biological stabilization of organic matter should be carried out as quickly and completely as possible in the activated sludge process. Therefore, the knowledge of the kinetics of aerobic treatment is important in identifying key process design parameters. These key process parameters include solids retention time θ_c (Chiang 1977, Lawrence and McCarty 1970, Fujimoto et al 1983, Sherrard 1977, Thērien and Perdrieux 1981), dissolved oxygen and oxygen uptake rate (Chen et al 1980, Olsson and Andrews 1978, Huang et al 1985, Andrews 1971) and hydraulic retention time (Vandevenne and Eckenfelder 1980). The kinetic parameters are also important in the prediction of plant performances and indicating which aspects of the process would benefit from further investigation.

2.12.1 Kinetics of waste utilization

Two approaches to the mathematical formulation of the relationship between waste utilization or growth rate and waste concentration in the reactor have received wide support. One approach describes this relationship by a continuous function, the other approach describes this relationship by a discontinuous function (Garret and Sawyer 1952).

The continuous function approach typified by the Monod (1949) equation expressed the relationship between bacterial growth rate and concentration of the growth limiting nutrient. This equation has been used in pure bacterial culture growth studies. The corresponding substrate utilization rate equation can be written as follows:

$$\frac{dS}{dt} = \frac{kXS}{K_s + S} \qquad 2.7$$

where

$\frac{dS}{dt}$ rate of substrate utilization per unit volume of reactor

S substrate concentration in reactor, mg/l

k maximum rate of substrate utilization per unit weight of microorganisms occuring at high waste concentrations, day^{-1}

K_s half velocity constant equal to the waste concentration when dF/dt is equal to 1/2 of the maximum rate of substrate utiliation, mg/l

X microorganism concentration, mg/l

The above equation is of the same hyperbolic form as the Langmuir adsorption isotherm and the Michaelis-Menten enzymatic susbstrate utilization equation. The equation when combined with the appropriate material balance equations and taking into account endogenous metabolism can be applied to form a widely used mathematical model for describing the activated sludge process with sludge recycle (Lawrence and McCarty 1970, Goodman and Englande 1974, Pearson 1966).

The discontinuous function approach is to explain the observed substrate concentration and microbial growth relationship in the assimilation of wastes by

activated sludge. The waste or substrate utilization rate equations are as follows:

High substrate concentration zero order	$\frac{dS}{dt}$	$= k_1 X$
Low substrate concentration first order	$\frac{dS}{dt}$	$= k_2 XS$

where dS/dt, X and S are as previously defined and k_1 and k_2 are proportionality constants.

The approach represented by these equations or slight modifications of the equations has been applied to the description of activated sludge kinetics (Garret and Sawyer 1952, Eckenfelder 1963, McKinney 1962, McKinney and Ooten 1969).

It was later shown that the models developed for the design of the activated sludge process with sludge recycle by McKinney (1962) and Eckenfelder (1971) were similar (Goodman and Englande 1974). Vandevenne and Eckenfelder (1980) later reported that when the influent substrate concentration is constant, the Lawrence and McCarty (1970) model yields the same results as the Eckenfelder's model.

2.12.2 Monod kinetics and a steady state mathematical model to describe a completely mixed activated sludge process with sludge recycle.

In this study the Monod (1949) kinetic equation adopted by Lawrence and McCarty (1970) in their steady state kinetic model for completely mixed activated sludge reactors with sludge recycle and soluble substrate was used in the evaluation of the kinetic parameters. The equations in this model can be derived as follows:

The rate of substrate utilization dS/dt is given by

$$\frac{dS}{dt} = \frac{kXS}{K_s+S} \qquad 2.8$$

where

k maximum rate of substrate utilization, day^{-1}

X microorganisms concentration, mg/l

S substrate concentration, mg/l

K_s substrate concentration at which rate of substrate utilization is 1/2 the maximum rate, mg/l

In the activated sludge system with recycle, the operation has always been described to be in the declining phase of growth and stationary growth phase (Goodman and Englande 1972). Therefore any expression for the rate of microbial growth should take into account endogenous metabolism (Gram 1956, Stewart 1958).

The rate of microbial growth can be related to the rate of substrate utilization (Moser 1958 , McKinney 1962, Herbert 1959) by the following expression :

$$\frac{dX}{dt} = -Y\frac{dS}{dt} \qquad 2.9$$

where

$$Y = \frac{\text{weight of microorganisms newly formed}}{\text{weight of essential substrate utilized}}$$

and is known as the growth yield coefficient. As for the microbial growth rate in which endogenous metabolism is taken into account, the rate expression can be written as follows :

$$-\left(\frac{dX}{dt}\right)_{\text{endogenous}} = -k_d X \qquad 2.10$$

where

k_d is the microbial decay coefficient, day^{-1}

From Eqn 2.8, 2.9 and 2.10, the net rate of microbial growth accounting for endogenous metabolism can be written as follows :

$$\left(\frac{dX}{dt}\right)_{net} = -\frac{YdS}{dt} - k_d X \qquad 2.11$$

By substituting Eqn 2.8 into Eqn 2.11 yields

$$\frac{dX}{dt} = \frac{YkXS}{K_s + S} - k_d X \qquad 2.12$$

when Eqn 2.12 is divided by X, it yields

$$\frac{1}{X}\frac{dX}{dt} = \frac{YkS}{K_s + S} - k_d \qquad 2.13$$

where

$\frac{1}{X}\frac{dX}{dt}$ = k_o which is the specific growth rate,

The specific growth rate can be related to the solids retention time θ_c by $k_o = 1/\theta$. This yields

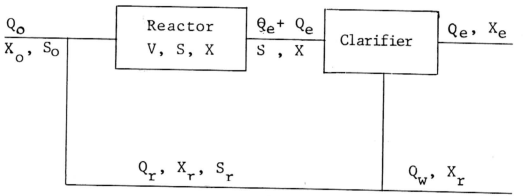
$$\frac{1}{\theta_c} = \frac{YkS}{K_s + S} - k_d \qquad 2.14$$

Solving the above equation for S results in

$$S = \frac{K_s\ (1+k_d\theta_c)}{\theta_c\ (Yk-k_d)-1} \qquad 2.15$$

The application of Eqns 2.8 to 2.15 to completely mixed activated sludge process with sludge recycle can than result in a system which can be represented by Fig 2.3.

Fig. 2.3 Schematic representation of a completely mixed with sludge recycle process.



A material balance for the net change of microbial mass and substrate in an activated sludge process with sludge recycle is:

i. Mass balance for the substrate around the biological reactor

$$\frac{VdS}{dt} = Q_o S_o + Q_r S - (Q_o + Q_r)S - \frac{kSXV}{Y(K_s+S)} \qquad 2.16$$

ii. Mass balance for the mircoorganisms around the biological reactor

$$\frac{VdX}{dt} = Q_o X_o + Q_r X_r - (Q_o + Q_r)X - \frac{kSXV}{K_s+S} - k_d XV \qquad 2.17$$

where

V	volume of aeration tank, l
Q_o	influent flow rate, l/day
Q_r	sludge recycle flow rate, l/day
Q_w	waste sludge flow rate, l/day
X_o	microorganism concentration in influent, mg/l
X_r	microorganism concentration in recycle, mg/l

Other parameters are as previously defined.

Taking the recycle ratio as $R = Q_r / Q_o$ and a hydraulic retention time based on fresh feed as $\theta = \frac{V}{Q_o}$
Eqn 2.16 and 2.17 become

$$\frac{dS}{dt} = \frac{1}{\theta}(S_o - S) - \frac{kSX}{Y(K_s + S)} \qquad 2.18$$

and

$$\frac{dX}{dt} = \frac{1}{\theta}(X_o + RX_r - (1+R)X) + \frac{kSX}{K_s+S} - k_d X \qquad 2.19$$

For a steady state reactor $\frac{dS}{dt} = \frac{dX}{dt} = 0$ 2.20

and the material balance for the Eqns 2.18 and 2.19 become

$$So - S - \frac{kSX\theta}{Y(K_s+S)} = 0 \qquad 2.21$$

$$X_o + RX_r - (1+R)X + \frac{kSX\theta}{K_s+S} - k_d X \qquad 2.22$$

If biomass entering with fresh feed is negligible, the material balance for the microbial mass is

$$RX_r - (1+R)X + \frac{kX\theta S}{K_s+S} - k_d X\theta = 0 \qquad 2.23$$

Eqn 2.21 can be rewritten as

$$(S_o - S)Y = \frac{kXS\theta}{K_s+S} \qquad 2.24$$

and the hydraulic retention time can be related to the solids retention time θ_c by

$$\theta = \theta_c(1 + R - RX_r/X) \qquad 2.25$$

Substituting the Eqn 2.21 and Eqn 2.25 into Eqn 2.24 and rearranging yields

$$\frac{Y(S_o - S)}{X} - k_d\theta = \frac{\theta}{\theta_c} \qquad 2.26$$

which can also be written as

$$\frac{S_o - S}{X\theta} = \frac{(1)}{Y}\frac{(1)}{\theta_c} + k_d Y \qquad 2.27$$

Eqn 2.15 and Eqn 2.27 are widely used in determining the kinetic coefficients k_d , Y, K_s in the completely mixed activated sludge process with sludge recycle.

The minimum solids retention time θ_c^m at which process failure occurs can be written down as:

$$(\theta_c^m)^{-1} = \frac{YkS_o}{K_s + S_o} - k_d \qquad 2.28$$

where

θ_c^m minimum solids retention time, day