Anaerobic Bioflocculation as a Mechanism for the Removal of Grease from Wool Scouring Effluent

by

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I hereby declare that this thesis is my own account of
my research and contains as its main content work
which has not previously been submitted for a degree at
any university.

Wipa Charles

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To my husband

Stephen

Abstract

Effluent produced by the wool scouring process is highly polluted with emulsified grease, dirt particles, salts, and detergent. The major problem in treating this waste stream is the wool grease which is resistance to biodegradation. The removal of grease from the effluent would lead to a more readily degradable waste stream, and therefore suitable for further biological treatment processes. This study aimed to investigate anaerobic destabilisation (flocculation), rather than degradation, of wool grease emulsion from wool scouring effluent (WSE). The process therefore can serve as a pretreatment step, prior to a conventional biological process.

The results from this study show that emulsified wool grease in WSE could be removed by bioflocculation under anaerobic conditions. After 110 days of continuous operation, a two-stage anaerobic process treating a high grease (>10 g/L) effluent removed 70 to 90% grease and approximately 60 to 86% COD at a combined hydraulic residence time (HRT) of 4 to 10 days. With low grease (<10 g/L) effluent grease removal was reduced. At a HRT of 3 days a single stage anaerobic process removed 40 and 44% grease (37 and 43% COD) at 20 °C and 37 °C respectively. Since the supernatant of the treated effluent still contained residual grease of over 1.5 g/L, further purification was necessary. The supernatant was readily treated by an aerobic activated sludge process, reducing the grease concentration from about 1.5 g/L to less than 0.1 g/L, in the final effluent, with an HRT of 3 days.

Methane production and volatile fatty acids consumption of both the above anaerobic systems were negligible. The majority of the grease was removed by flocculation as a result of anaerobic bacterial activity. The mechanisms of this process were investigated by a series of batch experiments. It was found that: (1) appropriate gentle mixing between wool scouring effluent (WSE) and anaerobic sludge resulted in the absorption

of wool grease from the liquid phase to the sludge phase, (2) further destabilisation of the wool grease emulsion was obtained when the mixed liquor is left undisturbed. The process thus required a short gentle mixing period of approximately 15 minutes to enable complete contact between the sludge and WSE, and a longer settling period of 2 to 4 days to provide appropriate time for the microbes to destabilise wool grease emulsion and transfer it to the sludge phase.

The process of destabilising the wool grease from wool scouring liquor was found to result from the activities of suspended microbes in the anaerobic sludge, which could successfully grow in WSE, rather than the bulk biomass as required in a conventional anaerobic digestion process. General microscopic observation indicated that during the process of bioflocculation a large number of mixed bacterial cells (>10^8 cells/ml) were present in the supernatant and only a small number appeared within the flocculated grease. No evidence of bacterial cell aggregation was observed in the process. It was hypothesised that the mechanism involved the partial degradation of detergent.

Detergent analysis revealed that anaerobic microbes (taken from the sludge of a municipal wastewater treatment plant) had an ability to partially degrade non-ionic surfactants (nonylphenol polyethoxylates - NPEO) by shortening the hydrophilic ethoxylate chain, resulting in the reduction of surfactant properties. This is likely to be one factor causing coagulation and subsequent flocculation of wool grease in the liquor. Other factors such as production of biopolymers and enzymes by microbes may also play a role, and should be further investigated as they beyond the scope of this thesis.

Ten different bacteria strains were isolated from the supernatant of successfully flocculated WSE samples. Six strains were found to grow in raw WSE as a pure culture. Only three strains caused some flocculation of wool grease, although the reduction of grease from the supernatant was not as effective (20-30%) as that using the mixed culture (60-80%). However, the results were not reproducible when different

WSE samples were used, thus no definite conclusions could be obtained from this experiment.

The efficiency of anaerobic bioflocculation was found to vary greatly (30% to 80% grease removal) depending on the source of wool scouring effluent. The concentration of bacterial substrate, grease and free detergent (rather than total detergent) were all found to effect the efficiency of the process. At a constant loading rate, the efficiency of the process was found to increase with increased grease concentration in WSE. A rationalisation of the scouring process to minimise detergent use and produce higher concentration grease and suint WSE is a likely benefit of bioflocculation process. These findings lead to the recommendation of a proposed treatment scheme.

The main conclusion drawn from this study is that the anaerobic biological removal of wool grease in WSE is due to the destabilisation of the wool grease emulsion resulting in grease flocculation. Since the process does not require further additives, such as chemical flocculant or oxygen, the removal of the bulk of the grease by simple anaerobic bioflocculation appears to be a useful part of an economic treatment system.

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List of Abbreviations

AF	anaerobic filter
BOD	biochemical oxygen demand
cm	centimetre
COD	chemical oxygen demand
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSTR	continuously stirred-tank reactor
d	day
EO	ethylene oxide
g	gram
G	gravity
HPLC	high performance liquid chromatography
hr	hour
HRT	hydraulic residence time
HST	heavy solids tank
i.d.	internal diameter
kg	kilogram
L	litre
m	metre
mg	milligram
min	minute
MJ	megajoule
μg	microgram
μl	microlitre
μm	micrometre
ml	millilitre

mm	millimetre
NP	nonylphenol
NPEO	nonylphenol polyethoxylates
PC	proteinaceous contaminant
rpm	revolutions per minute
SS	suspended solids
TS	total solids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids
v/v	volume per volume
WRONZ	Wool Research Organisation of New Zealand
WSE	wool scouring effluent

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Chapter 1 Introduction

1.1 Introduction

Australia is the world's largest wool producer supplying 70% of the world's traded apparel wool (Anon., 1989). In 1992 alone, Australia produced 875 million kilograms of greasy wool with a value of over three billion dollars (Castles, 1994). The income earned from the export of this wool plays a major role in the Australian economy, with the bulk of raw wool exported without any value-added processing. To be able to increase the value of this wool, processes such as scouring are required. Wool scouring, however, consumes vast quantities of water and produces an effluent which is highly contaminated.

Wool as shorn from the sheep ('greasy wool') contains an appreciable amount of grease, dirt, vegetable matter and other extraneous material. The removal of these impurities from wool fibre during the scouring process involves the counter-current washing of raw wool with detergent in hot water. Typically 8-10 litres of water is used for each kilogram of greasy wool. For a wool with a scouring yield of 56 percent, the average grease content would be 12 percent, the suint content 6 percent and the dirt content 23 percent (Anderson & Wood, 1974). With the addition of detergent at 7 kg per 1 000 kg of (merino) wool (Stewart, 1985), the effluent from this process is considered as one of the most polluted industrial wastewaters. There are more than 40 scouring mills in Australia which produce not only 240 000 tons of clean wool a year, but also a huge amount of highly polluted liquid waste. (Wright, 1993).

Since wool grease can be recovered as a valuable by-product (lanolin) the majority of scouring plants around the world use disc centrifuges to recover this product (Christoe & Bateup, 1987). However, only the best quality grease (approximately 25

to 45% of the total) is removed. The concentration of grease as well as other pollutants in the final discharge is still too high for their disposal to be considered environmentally acceptable, and too expensive for sewer disposal. For this reason, further treatment is required.

The scouring effluent is a highly stable water-emulsion of grease, dirt particles, salts, and detergent. It typically contains 3 000 to 20 000 mg/L wool grease, 7 000 to 15 000 mg/L suint salts (salts produced by natural secretions) and 10 000 to 30 000 mg/L dirt (sand, vegetable matter and fibre). The biological oxygen demand (BOD_5) and chemical oxygen demand (COD) of the effluent can be as high as 40 000 mg/L and 120 000 mg/L respectively, in the scouring wastewater before it is combined with the less polluted rinse water (Christoe & Bateup, 1987; Townsend *et al.*, 1989).

Wool grease causes major problems in the treatment of this waste stream because of its resistance to biodegradation (Cail *et al.*, 1986; Chao & Yang, 1981; Isaac & Cord-Ruwisch, 1991; Good, 1987). The separation of grease from the effluent will lead to a more easily degradable waste stream while concentrated wool grease may be recovered as a by-product.

A number of physico-chemical and biological pretreatment processes have been investigated to remove this bulk grease. These include acid-cracking, chemical flocculation, solvent extraction, aerobic and anaerobic digestion (Christoe, 1977; McLachlan *et al.*, 1978 a, b; Stewart, 1985). Chemical processes usually suffer from high operating costs due to the large amounts of chemicals required, and their application has therefore been determined by economic factors (Gibson *et al.*, 1982). Aerobic degradation can be economically acceptable if a large land area is available, but can also be expensive when mechanical aeration is required (Robinson & Gibson, 1985). Anaerobic digestion processes are low in operating cost, however the treatment of highly polluted effluent requires relatively long retention times of 20 to

30 days (Rodmell & Wilkie. 1983; Whitaker & Stewart, 1985; Genon *et al.*, 1984). Thus the required plant is usually fairly large with correspondingly high capital costs. Apart from the above problems, the use of a biological process to treat wool scouring effluent (WSE) encounters serious problems due to the extremely high grease load. In addition to blocking some parts of the system, oily materials, which are more difficult to degrade than other organic matters, tend to coat biological flocs, thereby interfering with the mass transfer in the system (Cheremisinoff *et al.*, 1989). In all cases, pretreatment processes to remove some grease are necessary.

A recent study by Cord-Ruwisch *et al.* (1990) demonstrated the destabilisation of grease emulsion using aerobic bacteria which caused partial degradation (oxidation) and partial flocculation of the wool grease (settling of biomass and undegraded lanolin). The process, however was confronted by several problems, namely the large amount of air required (high operating cost), foam formation and the large amount of sludge produced.

1.2 Objectives of the study

A study was undertaken which aimed to investigate whether bioflocculation (destabilisation of grease emulsion), rather than degradation, of wool grease from WSE could also occur under anaerobic conditions. Based on the findings in the literature (Cord-Ruwisch *et al.*, 1990; Roth *et al.*, 1989), it is known that the wool grease emulsion in scouring effluent may be destabilised (coagulated) upon aerobic bacterial action. This effect would be utilised within an anaerobic treatment process in order to achieve separation of the grease and water phases in the shortest possible residence time. The study therefore involved;

(1) the investigation of the principal mechanisms by which microbes destabilise the emulsion;

(2) the optimisation of this process to achieve the highest possible grease flocculation in the shortest residence time;

(3) an investigation into the feasibility of running this process as a continuous system; and

(4) examination of the possibility of employing aerobic post-treatment to purify the effluent from the anaerobic bioflocculation system.

1.3 Structure of the thesis

Having defined the purpose of this investigation, a relevant literature review of wool scouring effluent characteristics and treatment is presented in Chapter 2. Chapter 3 contains the relevant materials and methods used in the laboratory experiments. A survey of liquor streams at a local wool scouring plant, characterising its wastewater for use in the experiments is described in Chapter 4.

Chapters 5 and 6 contain results and the discussion in relation to the mechanisms of the anaerobic bioflocculation process, while the investigation of operating the process in a semi-continuous system is described in Chapter 7. The conclusions of the thesis are presented in Chapter 8.

Chapter 2 Literature Review

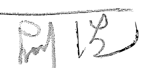
2.1 Raw wool and its contaminants

Raw wool is the natural coat of sheep, removed by shearing. In addition to wool fibre, it contains appreciable amounts of wool grease, suint (the skin excretion of the sheep), inorganic dirt (clay, sand), organic dirt (urine, faeces, organic soil components), vegetable matter, and moisture (Wood, 1983). The exact quantities of these impurities in the fleece vary between differing climatic and pastoral conditions, with seasonal fluctuations and with the breed and condition of sheep (Castles, 1994).

In Australian merino fleece wools, these contaminants can make up 40 % by weight of raw wool, comprising (Stewart, 1985; Taylor, 1986);

- Grease	15-16	%
- Dirt	15-20	%
- Suint	5-6	%
- Protein contaminants	4	%
- Vegetable matter	2	%

2.2 Scouring techniques and their relationship to effluent problems



Wool scouring may be regarded as an extraction process in which the contaminants are extracted from wool by a detergent solution. When the wool is scoured, the grease is emulsified, the suint is dissolved and the dirt is suspended in the scouring liquor. A number of scouring processes have been developed and fully reviewed in the literature (Stewart, 1985; Wood, 1983), from semi-continuous or batchwise operations to steady-state countercurrent systems, as are used in most scourers.

There has been an increase in research into scouring and the treatment of the final effluent in recent years. It is generally agreed that the first step in any process is to rationalise the scouring operation so that the final effluent is preferably of low volume and of low grease content, since it is the residual grease which affects the pollution load dramatically (Gibson *et al.*, 1982).

In the conventional scour (Figure 2.2.1) the wool passes sequentially from bowl 1 through to bowl 5. Detergent is added to one or more of the first three bowls. Water is added to the last bowl and flows countercurrently to bowl 1 and is then discharged. The effluent from this process contains all of the contaminants removed from the wool as well as the detergent.

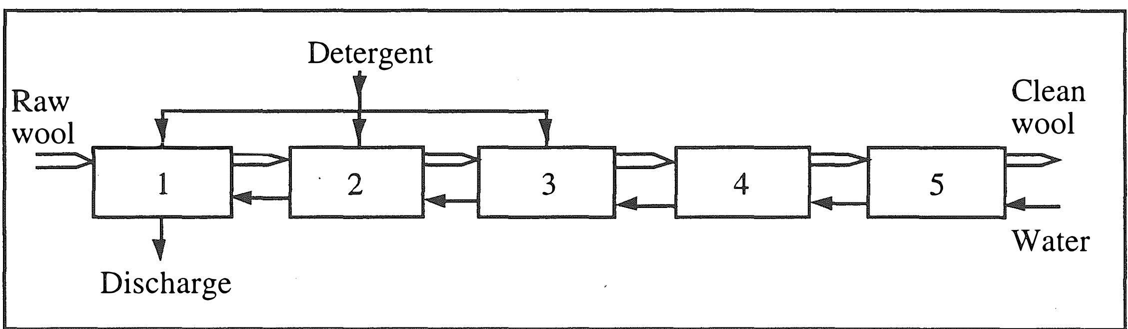


Figure 2.2.1 Simple countercurrent scouring system (Warner, 1986; Wood, 1983)

By adding contaminant recovery devices, such as settling tanks, centrifuges, dissolved-air flotation, (Figure 2.2.2), dirt is removed, grease can be recovered, and treated water can be recycled to the first bowl. This recycling of water reduces water consumption and hence the quantity of wastewater effluent requiring treatment (Christoe & Bateup, 1987).

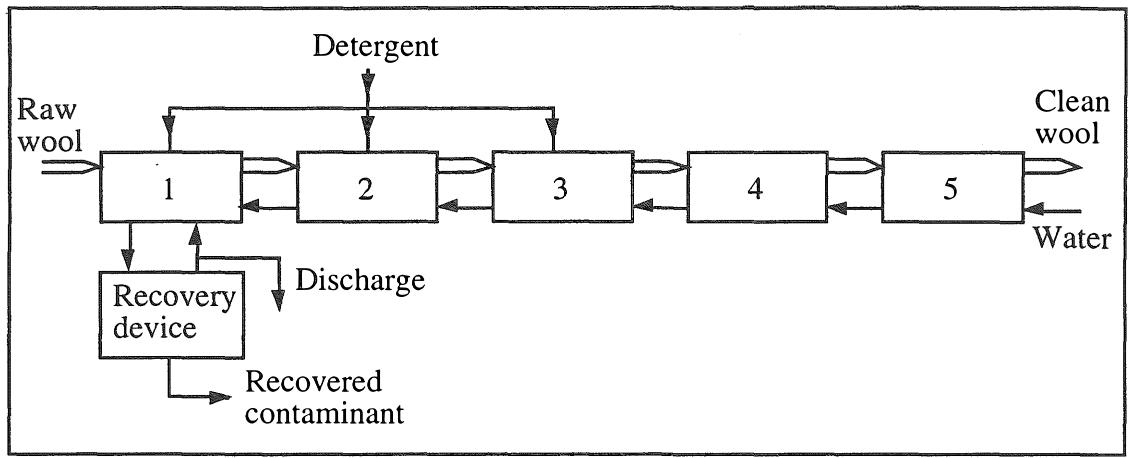


Figure 2.2.2 Countercurrent scouring with a recovery loop (Warner, 1986; Wood, 1983)

The WRONZ rationalised scour system (Stewart *et al.,* 1974), introduced by the Wool Research Organisation of New Zealand (WRONZ) in 1974, was the first system to incorporate both dirt and grease recovery devices (Figure 2.2.3). The system employed a heavy solids tank (HST) for removal of dirt, a disc centrifuge for grease recovery, and recycling of the majority of the liquor back to the scour. This system improves grease recovery and the water consumption is cut to a third (3.5 L/kg wool) (Gibson *et al.*, 1982). As a result, the amount of wastewater from the system is also much decreased.

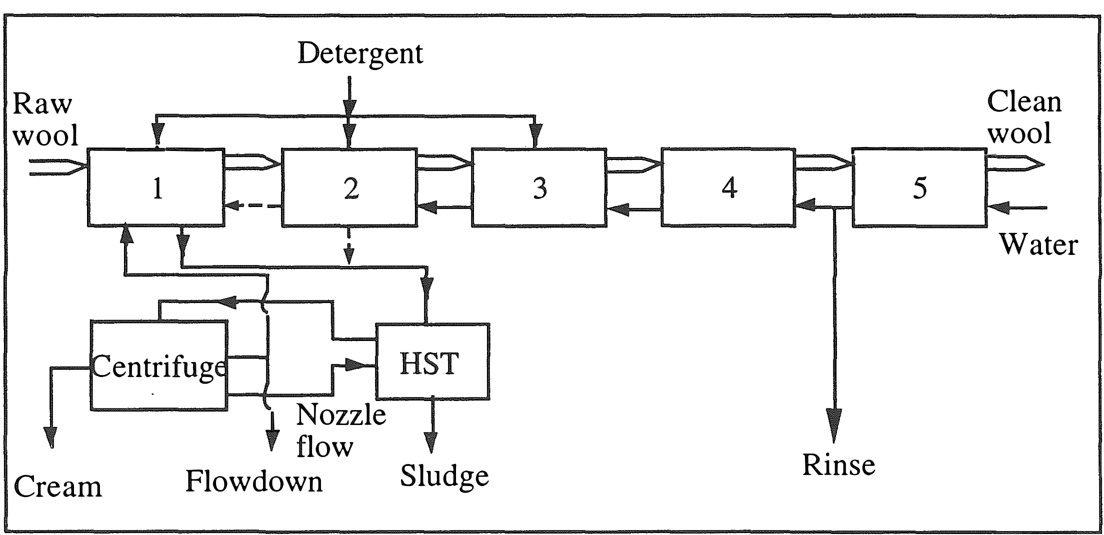


Figure 2.2.3 The WRONZ system (Wood, 1983)

In 1979, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) introduced the CSIRO Lo-flo Process (Figure 2.2.4). It was originally designed for effluent treatment rather than scouring improvement. The principle behind this process involves destabilisation of stable particle suspension by increasing the concentration of substances, namely, suint, detergent and sodium carbonate or other salts, already in the system (Brooks, 1980). In ordinary scouring liquors, the concentrations of these substances are too low to cause destabilisation. In the Lo-flo process, their concentrations are increased several times simply by reducing water input to the bowls.

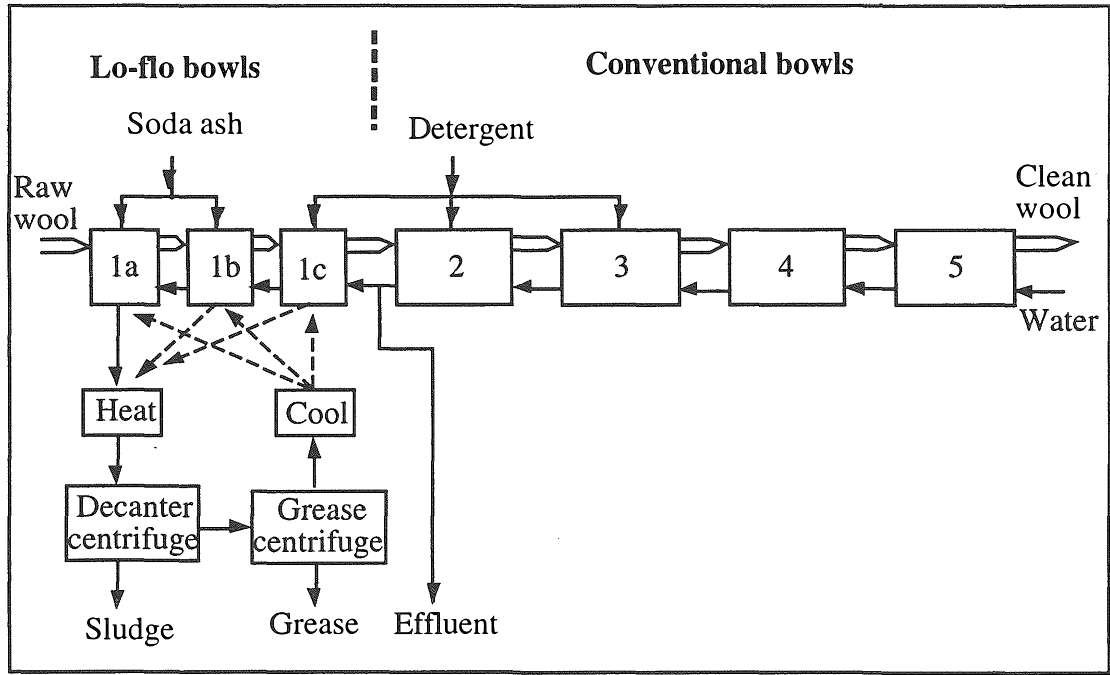


Figure 2.2.4 The CSIRO Lo-flo process (Wood *et al.*, 1979)

An arrangement has been adopted in which three bowls are installed in place of an existing first bowl, and water input is restricted in these bowls to less than 1 L/kg raw wool, thereby increasing the dissolved solids concentration to 10% or more

(Wood, 1983). The liquor is pumped and heated before removing dirt and grease by using a decanter centrifuge and a grease centrifuge, respectively. The water phase is then cooled and returned to the bowls.

A sludge content of about 50% solids is obtained which can be disposed of via landfill. Grease recovery is approximately doubled compared with conventional scouring. However, its quality is reduced because of a high content of free acid, alcohol and detergent (Warner & Wood, 1980). There is no liquid effluent when running with some wool types; with others, a small volume (0.5-1 L/kg) needs to be treated and/or disposed of (Wood, 1983).

The WRONZ mini-bowl system had adopted the concept of small scouring bowls to the WRONZ Rationalised Scour System to obtain maximum scouring efficiency. Because of the design of the bowls, the minimum flowdown is about 1.5 L/kg, which can be evaporated or otherwise treated before discharge. At these water input rates, the liquors are at least partially destabilised and grease recovery is significantly increased (Wood, 1983).

Gibson *et al.* (1982) compared the different scouring systems in terms of grease and dirt removal efficiency (Table 2.2.1). By rationalising the scouring process, the pollution load could be reduced dramatically.

Table 2.2.1 Comparison of pollution load in the final effluent using different scouring systems.

Scouring process	% Grease recovery	COD (kg/hr) Grease recovery	Sludge	Final effluent	Reduction of pollution load
Conventional	-	-	-	474	-
Conventional + HST + centrifuge	30	107	45	322	32%
WRONZ	43	155	67	252	47%
WRONZ mini-bowl	65	240	70	164	65%

Based on a scouring train capable of scouring 5 000 tonnes greasy wool per annum of a relatively clean Merino wool at a liquor ratio of the order of 10 L/kg greasy wool (summarised from Gibson *et al.*, 1982).

2.3 Characteristics of wool scouring effluent

Aside from the detergent, the main components of a scouring liquor are natural products originating either from the sheep (grease, suint, protein, etc.) or from accumulation in the field (dirt). Table 2.3.1 shows the relative contributions of the major components of scouring liquor to its pollution load. The contributions are based on both the biological load (BOD_5) and the concentrations in the liquor (Christoe, 1986).

Table 2.3.1 Relative contributions of components of a scouring effluent to the pollution load (Christoe, 1986).

Component	Contribution to pollution load	
	Organic load	Suspended matter
Suint	Medium	None
Proteinaceous contaminants	Medium	Medium
Inorganic dirt	None	High
Wool grease	High	Medium
Detergent	Low	Low

2.3.1 Suint (Dissolved solids)

Suint has long been defined as the water soluble part of the raw fleece, which was secreted from the sweat or sudoriferous glands. It is composed largely of potassium salts of organic acids, the potassium content being fairly constant between breeds at about 25-27%. Potassium accounts for 90% of the cations present, the balance being sodium, iron, aluminium, calcium and magnesium (Stewart, 1985). The organic acids fraction in suint contains amino acids, urea, volatile acids (primarily acetate and propionate), and dicarboxylic acids (predominantly succinate) (Truter, 1956).

Although the known organic components are biodegradable, only 55% of the suint COD was found to be biodegradable in aerobic treatment (Cord-Ruwisch *et al.*, 1990) and only 29% of the suint could be converted to methane in an anaerobic process in 30 days (Isaac, 1991).

2.3.2 Proteinaceous contaminants

Proteinaceous contaminants may be classified as being water soluble or particulate proteins. The water soluble protein is commonly included as part of suint (Stewart, 1985). Particulate proteins are mainly skin flakes from the sheep. The biodegradation of this protein should be easier than that of the wool fibre itself because they contain very few cross-linking amino acids (Christoe, 1986). The proteinaceous contaminants became important since they were found to have a major effect on wool scouring process (Anderson, 1983), the recovery of wool grease, and effluent treatment (described in section 2.3.4.3).

2.3.3 Dirt

The inorganic dirt in raw wool, mainly comprising sand and clay, varies in particle size and type depending on the environment and behaviour of the sheep. It might vary from large grains to colloid size particles. Organic dirt originates in faecal matter and possibly in organic soil components. The large particles are normally removed by in-plant recovery devices, such as settling tanks and centrifuges. The fine particles may freely suspend in the liquor or sometimes become entrapped in the oxidised grease droplets (Figure 2.3.4.3.2)

2.3.4 Wool grease

2.3.4.1 Composition of wool grease

Wool grease comprises oxidised grease and unoxidised grease. The oxidised grease is located on the top half of the growing fibre and has had a longer exposure to air, sunlight, and water. The unoxidised grease is located on the root half of the fibre

and has had only a relatively shorter exposure after excretion. Both fractions are generally present in similar proportions (Anderson & Wood, 1962).

Both oxidised and unoxidised wool grease have a melting point of around 40 °C. Since the removal of grease from wool by detergent solution is slow and difficult, 40 °C is the lowest temperature at which scouring processes are effective (Stewart, 1985). Normally, scouring temperature is in the range of 55-65 °C.

Wool grease can be recovered during the scouring process. After refining, it is called lanolin, a valuable by-product widely used in the cosmetic and pharmaceutical industry. Lanolin is a highly complex mixture of lipids which are esters and polyesters of high molecular weight alcohols and fatty acids (Fawaz *et al.,* 1973; Truter, 1956). By using ion-exchange chromatography on resins and silica column, Fawaz *et al.* (1973) found that lanolin consisted of 9 fractions as follows;

- free acids	0.5 %
- hydrocarbons	0.6 %
- esters of sterols and triterpenealcohols	35.4 %
- mono and diesters of aliphatic alcohols	23.7 %
- monohydroxyesters of sterols and triterpene alcohols	14.7 %
- monohydroxyesters of aliphatic alcohols	5.3 %
- free aliphatic alcohols	6.5 %
- free sterols	4.1.%
- free dihydroxyesters and diols	7.9 %

2.3.4.2 Biodegradation of wool grease

Although wool grease contains alcohol esters of fatty acids which are among the most highly reduced substrates available to microorganisms (Rose, 1976), they are present in the solid form. Since bacteria can only readily oxidise dissolved organic

matter, wool grease degradation requires an additional step to transform the solid grease into dissolved matter. The initial step in catabolism of these fatty-acid esters involves hydrolysis, by intracellular or extracellular lipases (Rose, 1976), to yield monohydric alcohol and free fatty acids. These products can then be further degraded to yield ATP (Rose, 1976). However, due to the chemical complexity of wool grease, the first step of solubilising (hydrolysis) is likely to be rate limiting (Cail *et al.*, 1986), as is the case for other organic solids like cellulose (Figure 2.3.4.2.1). Because of the high reduction state 1 g of wool grease is likely to yield at least 0.5 g of biomass when degraded aerobically.

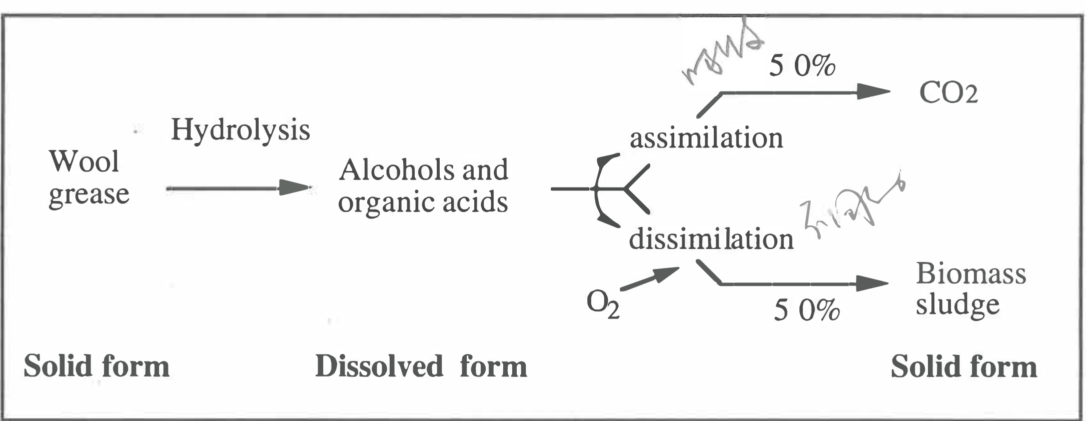


Figure 2.3.4.2.1 Schematic diagram of aerobic wool grease biodegradation (Ralf Cord-Ruwisch, personal communication)

In an activated sludge treatment process, wool grease in WSE was found to be degradable (Cord-Ruwisch *et al.*, 1990; Mickelson *et al.*, 1989). Mickelson *et al.* (1989) determined bacterial strains which had the ability to synthesise esterase enzymes capable of cleaving p-nitrophenyl palmitate. This study also found that the presence of ciliated protozoa enhanced the rate and extent of wool grease degradation. With the absence of ciliates, bacteria alone were able to generate the free fatty acids and sterols from the sterol esters. Degradation of the free fatty acids

occurred but the cholesterol, lanosterol and dihydrolanosterol accumulated with extremely slow breakdown. In the culture with ciliates similar early steps occurred and the sterols were rapidly degraded during culture growth.

Under anaerobic conditions, carbon dioxide, the major electron acceptor in anaerobic digestion leading to methane production, cannot be used by the lipid degrading bacteria. These bacteria use hydrogen as an electron carrier to allow lipid oxidation via interspecies hydrogen transfer. Due to thermodynamic limitations these obligate hydrogen producing bacteria are known to be very slow growing and easily inhibited by hydrogen accumulation (Isaac & Cord-Ruwisch, 1991). Moreover, the hydrolysis products are still lipophilic and largely insoluble in water, which raises the possibility of mass transfer limitations decreasing the rate of wool grease degradation. This was confirmed by Isaac (1991) finding that emulsified wool grease was very difficult to degrade anaerobically with less than 10% COD conversion to methane in 30 days and only 30-40% COD conversion to methane after 100 days. On the contrary, a number of studies have shown a successful degradation of wool grease under anaerobic condition as described in detail in section 2.4.3.2.

2.3.4.3 Stability of wool grease emulsion

The first step in the process of flocculating the wool grease emulsion in WSE is the destabilisation or breaking down of the grease emulsion. The stability of this emulsion in wool scouring liquor therefore has a direct impact on the degree of grease flocculation. During the scouring process, wool grease is liquefied and then emulsified in the liquor by a non-ionic surfactant, generally of the nonylphenol polyethoxylate type (see section 2.3.5). The mechanisms for the removal of grease from the fibre involves adsorption of surfactant molecules into grease droplets followed by detachment of the grease droplets (Tokiwa & Immamura, 1984). The

long hydrophobic hydrocarbon tails (nonyl group) penetrate the grease droplet but the hydrophilic ethoxylate groups surround the grease surface and form hydrogen bonds with the water (Atkins, 1982). By this process the hydrophobic properties of grease droplets are converted to hydrophilic (Figure 2.3.4.3.1). The formation of this film around the dispersed droplets, so called micelles, promotes emulsion stability and hence helps to prevent flocculation and coalescence (Shaw, 1980). Two factors influence the stabilisation of these hydrophobic sols: (1) the concentration of surfactant in the solution, and (2) the number of ethoxylate groups in the surfactant molecules. Thus the stability of emulsions increases with an increase in the number of ethoxylate groups (the length of hydrophilic tail) (Glazman, 1966).

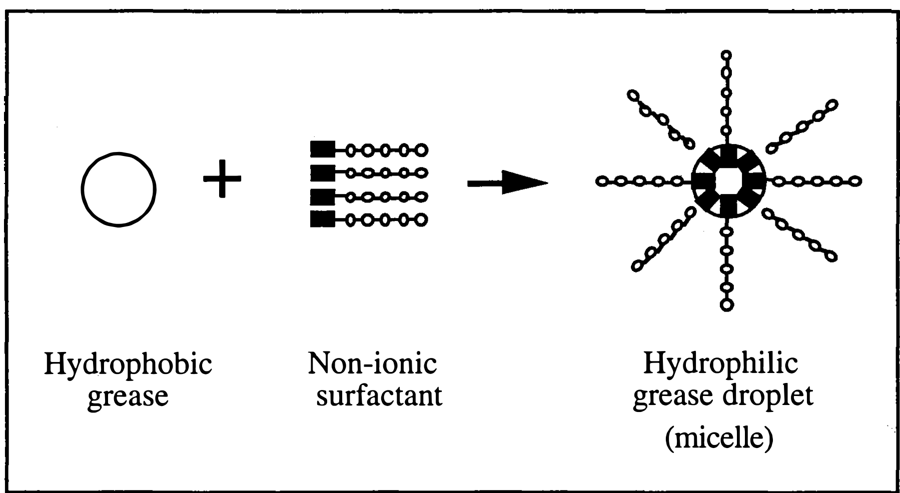


Figure 2.3.4.3.1 Schematic drawing of emulsification of grease by non-ionic surfactant (modified from Denneis *et al.*, 1979)

The basic theory of emulsion stability concerns the balance between the attractive and repulsive forces of the particles (Moss & Dymond, 1978; St. Angelo, 1989). Attractive forces (London-Van der Waal's forces) tend toward destabilising an emulsion, whereas the repulsive force (electrical double layers of like sign) would stabilise the emulsion by keeping the droplets separated. For dispersion to occur the

emulsion' s repulsive interactions must be increased to the point where they overcome the attractive interactions; to aggregate particles, the reverse applies (Rosen, 1978).

In wool scouring liquor, due to the variation and complexity of wool grease and other contaminants present on the wool fibre, the formation of emulsions in the scouring is far more complex than in the case of a classical emulsion as described above. Anderson & Wood (1962) suggested that the ability of wool grease to form oil-in-water emulsion depended on the surface activity of the grease component, which in turn depends on the degree of oxidation of the grease. Wood (1983) classified emulsion in WSE into three types (Figure 2.3.4.3.2):

(A) unoxidised grease forming classic emulsion droplets consisting of a sphere of melted grease with a surface film of detergent molecules;

(B) oxidised grease forming complex emulsion droplets that contain dirt particles and liquor drops; and

(C) mineral dirt particles with adhering oxidised grease.

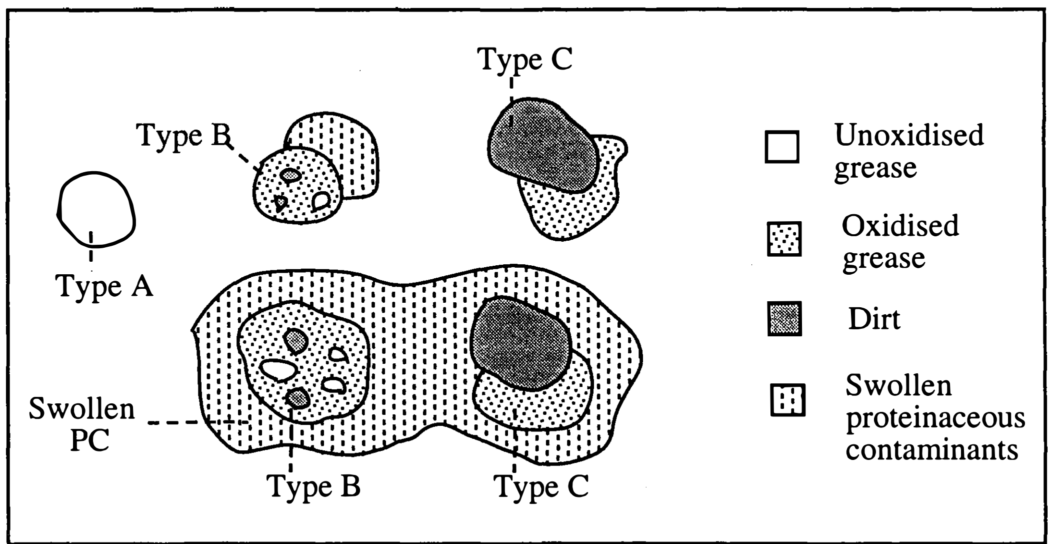


Figure 2.3.4.3.2 Schematic representation of a scour liquor (Warner, 1986)

Subsequent studies by Anderson (1983) have shown that proteinaceous contaminants (PC) play an important role in emulsion stability. Particles of types B and C (Figure 2.3.4.3.2) are often entrapped within masses of swollen PC matrix. As a result they are difficult to remove by settling because of their low specific gravity (similarity in densities to the liquor), and they may form bulky sludges because of the mass of highly swollen PC.

The quality of grease itself was also found to influence the stability of the resulting emulsion. Anderson & Christoe (1981) found that the stability of an oxidised grease emulsion is pH-dependent while unoxidised grease (lanolin) emulsion was not affected by pH. This is consistent with the view that the ionisation of fatty acids and other ionisable groups in the grease increases with pH. Thus, a high quality (unoxidised) grease having a low degree of oxidation and free acid content produces a less stable emulsion than one made from a more oxidised grease having a higher free acid content. In addition, oxidised grease has a high surface-activity and interacts strongly with dirt, proteinaceous and amphiphilic matter, and water. Emulsified droplets of this grease have a density close to WSE liquor and are therefore difficult to remove from the liquor by gravity based separation processes such as settling (Anderson & Wood, 1962).

2.3.4.4 Breaking of wool grease emulsions (Demulsification)

Demulsification or the breaking of an emulsion refers to three different phenomena (Fukushima, 1984; St. Angelo, 1989): (1) Flocculation, the first stage in demulsification, is the sticking together of droplets to form clusters. Normally this is associated with electrical phenomena at the droplet surface, reducing the repulsive force to such an extent that the Van der Waal's forces of attraction are dominant (Glazman, 1966). (2) Coalescence, which is irreversible, in which the droplets in a

cluster join together to form larger ones, leading ultimately to two liquid layers. (3) Creaming, the rising or falling of the droplets under the action of gravity, which normally occurs to the droplets which are larger than 1 μm and of a density different from that of the dispersion medium, regardless of the surface charge (Davies & Rideal, 1963; Fukushima, 1984).

A number of techniques are used in industry to accelerate emulsion breakdown. These include centrifuging, filtration, heating, freezing, application of electrical fields, and chemical additives (Davies & Rideal, 1963; Shaw, 1980). Due to the complexity of the contaminated grease emulsions formed in wool scouring liquor, these emulsions may be demulsified by different techniques according to their stability. Unoxidised grease, which is less stable, normally forms droplets with diameters between 1 and 10 μm (Stewart, 1985) and specific density of 0.89-0.95 g/cm^3 (Warner, 1986) and can be simply removed by gravity. Since this product is valuable most wool scouring plants employ centrifuges to accelerate the separation of grease. From the centrifuge, three distinct phases are formed (Warner, 1986):

(1) an upper phase, containing all of the unoxidised grease droplets which float to the surface, forming a cream (but do not coalesce into a grease layer at this stage), requiring a further purification process (described in Warner, 1986; Wood, 1983) to obtain anhydrous wool grease ;

(2) a sludge phase, containing most of the dirt particles and a proportion of the oxidised grease droplets. It may have a solid content as high as 35%. Of the solids in the sludge, about 20% may be grease (Wood, 1983);

(3) an intermediate phase, with a density of approximately 1.0 g/cm^3 (Warner, 1986), containing an emulsion of oxidised grease droplets and associated swollen PC (containing many other contaminants) in a solution of detergent, suint, and other water soluble substances from the fleece. This intermediate phase is normally discharged into the environment and may still contain up to 50% of the total grease (Wood, 1983).

The build-up of dissolved solids in the liquor was found to result in the destabilisation of grease emulsion (Wood *et al.,* 1979). This lead to the development of the CSIRO Lo-flo process as described earlier in section 2.2. The mechanism of the destabilisation of grease emulsion within the concentrated liquor is still unclear. Anderson & Christoe (1981) suggested that the coagulation was due to electrical double-layer compression. The amount of electrolyte required for destabilisation depends upon the quality of the grease, the type and concentration of other solutes, e.g. suint, and the pH of the scouring liquor.

Besides the maximisation of in-plant grease recovery, oxidised grease emulsion discharge from a scouring plant can be broken (flocculated, destabilised) in a number of ways, including reduction in pH (acid cracking), addition of polyvalent cations, and extraction by solvent (as detailed in section 2.4.1 and 2.4.2).

In a biological process, aerobic microbes were found to be able to destabilise wool grease emulsions (Christoe *et al.*, 1976; Cord-Ruwisch *et al.*, 1990). The mechanism of the process is not yet clear. Poole (1991) suggested that the destabilisation of wool grease emulsions was due to bacterial cell adherence to the emulsified grease particles, as a result of biopolymer bridging. Another possibility is that the surface of emulsions presented by the surfactant (NPEO) may be altered through microbial degradation. The degradation of NPEO is generally reported as a reduction in the length of the ethoxylated hydrophilic tail (detailed in section 2.3.5.1) resulting in the reduction of surfactant properties, causing demulsification (Glazman, 1966).

2.3.5 Non-ionic surfactant

Surfactants (or surface-active agents) are the major active constituents in many consumer cleaning products. With the presence of a strongly hydrophilic group and

a strongly hydrophobic group linked in the same molecule, a surfactant has the ability to interface between hydrophilic and hydrophobic phases. The hydrophilic group in the surfactant is more or less readily soluble in water. However, the hydrophobic group is repelled by water, so that there is a tendency for that portion of the molecule to leave the aqueous phase. This leads to a higher concentration of surfactant at the surfaces or boundaries than in the main body of the solution. In the presence of an immiscible liquid, the surfactant molecules orient themselves with the hydrophilic groups in the water phase, the hydrophobic groups toward the other liquid. This promotes dispersion and emulsification as grease droplets (Schick, 1967).

The term non-ionic surfactants refers chiefly to polyoxyethylene and polyoxypropylene derivatives, but other surfactants are also included in this category, such as anhydrohexitol derivatives, sugar esters, fatty alkanolamides, and fatty amine oxides (Schick, 1967). However, two principal types of non-ionic surfactants are the alcohol ethoxylates and the alkylphenol ethoxylates. Both contain a long hydrophobic alkyl chain linked by an ether bridge or a phenolic group respectively to a long hydrophilic ethoxylate chain (Figure 2.3.5.1); the latter may contain 5-25 (or even more) ethylene oxide units (Cain, 1977).

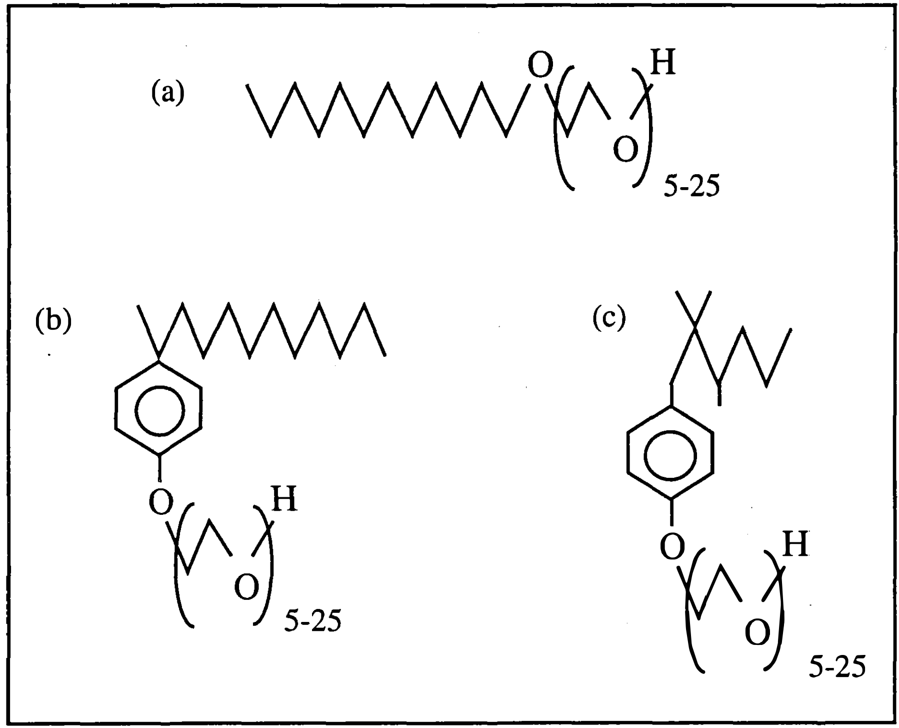


Figure 2.3.5.1 Non-ionic surfactants (a) alcohol polyethoxylates; (b) linear alkylphenol polyethoxylates; (c) branched alkylphenol polyethoxylates

Non-ionic detergents have been used for scouring wool for over forty years (Christoe, 1986). The most popular non-ionic detergent used in the scouring industry is the alkyl (mainly octyl- or nonyl-) phenol polyethoxylate type with a longer oxyethylene side chain containing approximately five to twenty oxyethylene groups shown in the formula below.

$$CH_3 - [CH_2]_{7-8} - C_6H_4 - O - [CH_2CH_2O]_{5-20} - H$$

In Australia, the most common detergent used, nonyl phenol polyethoxylate, NPnEO (ICI product Lissapol TN450), is based on a condensate of nonylphenol with 8.5 moles of ethylene oxide (Christoe, 1986). It is a clear liquid at 20 °C with a melting point of approximately 0 °C and a specific gravity of 1.056 g/ml at 20 °C . As TN

450 is miscible with water, a uniform concentration is readily achieved in the aqueous phase (Illingworth, 1988).

The level of detergent used depends upon the type of wool being scoured. Coarse carpet wools require about 0.3-0.4% detergent by weight of greasy wool whereas merino fleece would require double the amount. In general, most Australian scourers would use 0.6-0.8% (Bateup, 1986). The minimum concentration of detergent in any particular bowl varies depending on the function of the bowl, e.g., the main scouring bowls must contain as much as 0.2-0.3% w/v of detergent with the later bowls reduced to 0.05-0.1% w/v (Bateup, 1986). Some of this detergent is removed with the wool grease by centrifugation, but most of it is discharged with the effluent (Stewart, 1985).

2.3.5.1 Biodegradation of non-ionic surfactants

The biodegradation of a non-ionic surfactant can occur when given suitable acclimatisation (Cain, 1977). The process can be sub-divided into two phases, primary biodegradation and ultimate biodegradation (Swisher, 1987). Primary biodegradation occurs when the surfactant undergoes change to the extent that its characteristic property (surface activity) is lost. Completion of primary biodegradation may still leave appreciable amounts of organic residues undegraded. Ultimate biodegradation is the conversion of all the components in the surfactant molecule (i.e. parent surfactant and all its intermediate metabolites) into mineral salts, carbon dioxide, water and/or normal components of the microbial cells.

In principle, three mechanisms of primary biodegradation are available by which bacteria may gain access to utilisable carbon in ethoxylated surfactants (White, 1993): (1) ω-/β- direct oxidation of the alkyl chain, (2) central ether scission resulting in a hydrophile/hydrophobe separation, and (3) ethoxylate shortening.

However, the steric bulk of the aryl nucleus was found to restrict central scission, and biodegradation of ethoxylated surfactant therefore rely mainly on alkyl and ethoxylate shortening (Cain, 1977; White, 1993). Extensive branching of the alkyl chain further precludes ω-/β-oxidation, therefore leaving ethoxylate-shortening as the dominant pathway. As a result, these surfactants are observed to undergo extensive primary biodegradation (removal of surfactant properties) but relatively restricted ultimate degradation to carbon dioxide and normal cell components (White, 1993).

A number of authors have demonstrated that nonylphenol polyethoxylate can be at least partially degraded in the natural environment (Ahel *et al.,* 1994b; Zoller, 1994) and sewage treatment plants (Ahel & Giger, 1985b; Brunner *et al.*, 1988; Giger *et al.*, 1981; Holt *et al.*, 1986).

In sewage treatment where activated sludge and anaerobic sludge treatment are employed, the parent nonylphenol polyethoxylate was found to transform to a shorter derivative NPnEO (mainly NP1EO and NP2EO) and nonyl phenoxy carbolic acid (Figure 2.3.5.1.1) with adequate supplies of oxygen in an activated sludge process (Brunner *et al.,* 1988; Giger *et al.*, 1987). During anaerobic sludge treatment nonylphenol is formed by the degradation of NP1EO and further educts. Nonylphenol which is not significantly degraded under anaerobic conditions (Ahel *et al.*, 1994a) then accumulates in the digested sewage sludge (Figure 2.3.5.1.1). The abundance of the different metabolites varies depending on treatment conditions. Approximately 50% (molar basis) of NPnEO in the sewage was transformed to NP and accumulated in the digested sludge (Brunner *et al.*, 1988).

$C_9H_{19}-C_6H_4-O-[CH_2CH_2O]_n-CH_2CH_2OH$

n = 0-20 (NPnEO)

aerobic wastewater treatment

anaerobic

$C_9H_{19}-C_6H_4-O-CH_2CH_2O-CH_2COOH$

(NP2EC)

$C_9H_{19}-C_6H_4-O-CH_2CH_2O-CH_2CH_2OH$

(NP2EO)

anaerobic sludge treatment

$C_9H_{19}-C_6H_4-OH$

(nonylphenol)

Figure 2.3.5.1.1 Aerobic and anaerobic biotransformation pathways of nonylphenol polyethoxylates in sewage treatment plant (modified from Ahel *et al.*, 1994a)

In the wool scouring process, the effluent contains up to 1-2% v/v of nonylphenol polyethoxylate surfactant. Under a laboratory simulated natural (aerobic) ecosystem, Roth *et al.* (1989) found that NPnEO in WSE was degraded during the first 4 weeks, from approximately 1.2% to 0.24% v/v, with no further detectable breakdown up to 16 weeks. In their experiment, however, surfactant was analysed by the ammonium cobaltothiocyanate method (Longman, 1978). This method relies on the reaction of non-ionic detergent (cobalt thiocyanate active substances) with aqueous cobalt thiocyanate solution to give a cobalt-containing product extractable into an organic liquid in which it can be measured (APHA, 1989). The determination of surfactant

by this method did not allow for the measurement of partial degradation, which may have taken place after the fourth week of the experiment.

When intensive oxygen was supplied t the activated sludge process treating WSE, Mickelson & Smith (1989) reported that for a 2 day residence time 70% of the NPnEO was completely degraded with no accumulation of nonylphenol.

2.3.6 Pollution load of wool scouring effluent

The quality and quantity of effluent from wool scouring vary greatly depending upon the type of wool being scoured, the process of scouring (which will determine the water and detergent consumption), and the degree of in-plant grease and dirt recovery (see section 2.1 and 2.2).

Even though most scouring plants employ in-plant sedimentation tanks to remove settleable solids and centrifuges to recover grease, the effluent from the process still contains high concentrations of pollutants. As can be seen from Table 2.3.6.1 the typical effluent from wool scouring contains 3 000 to 20 000 mg/L wool grease, 7 000 to 15 000 mg/L suint salts, and 10 000 to 80 000 mg/L dirt. The biological oxygen demand (BOD_5) and chemical oxygen demand (COD) of the effluent can be as high as 40 000 mg/L and 120 000 mg/L respectively. How to measure.

It must be noted that scouring wastes are difficult to degrade biologically due to a high level of residual grease which is resistant to biodegradation (see section 2.3.4.2), and hence the BOD_5 value does not provide a true indication of the organic pollution load. McCracken & Chaikin (1980) suggested the use of a 35 day BOD figure which was considered to be a better estimate for realistic biological oxygen demand. However, the measurement of COD is a more reliable method for estimating the pollution load. The ratio of COD: BOD_5 varies depending on the

effluent; for untreated scouring effluent the ratio is about three to four (Christoe, 1986, Mickelson *et al.,* 1989).

Table 2.3.6.1 Characteristics of wool scouring effluent reported in the literature

Author Parameter	Buckley *et al.*, 1989	Townsend *et al.*, 1989	Wilson & King, 1983	Genon *et al.*, 1984	Cail *et al.*, 1986	Christoe *et al.*, 1976	Anderson & Wood, 1974	Jones, 1973
Total solid (mg/L)	33 000	10 000-30 000	-	-	-	10 000-80 000	-	1 129-64 448
Suspended solid(mg/L)	-	-	-	15 000	9 000	3 000-8 000	6 000	-
Grease (mg/L)	21 000	10 000-20 000	8 000-10 000	5 000	7 000	3 000-10 000	4 000	-
Suint salt (mg/L)	7 000	7 000-15 000	-	-	-	-	-	-
COD (mg/L)	30 000	50 000	50 000-100 000	45 000	25 000	15 000-120 000	-	-
BOD_5 (mg/L)	-	-	20 000-40 000	22 000	8 000	3 000-25 000	6 700	30 000-40 000
pH	-	-	-	7.8	-	7-10	8	9.0-10.4

2.4 Treatment of wool scouring effluent

Various wastewater treatment processes have been investigated to treat wool scouring effluent, some of which have been used commercially. They may be

classified into 3 main types of treatment processes; (1) chemical; (2) physical; and (3) biological.

2.4.1 Chemical methods

To treat the stable emulsion in wool scouring effluent, chemicals can be added to break down the extremely stable grease-dirt particles that remain suspended in the solution after centrifuging. Chemical destabilisation can be achieved via:

2.4.1.1 Acid cracking

In this process, the effluent is acidified to a pH of less than 4 with sulphuric acid, and the liquor is allowed to settle. A clear middle layer is discharged. A top greasy layer and bottom sludge layer are combined, heated to boiling, and then filtered under pressure. The filtrate passes to a decanter where molten grease is separated from the water.

When scour liquors contain non-ionic surfactants, acid cracking processes are less effective. These problems have been solved by hot-acid cracking. The non-ionic scour liquors are also destabilised at pH 2.5-3.0, and the process is carried out at medium to high temperature (>60 °C), with even better effluent clarity achieved if the process is taken to near boiling. A COD reduction of 80-85 % can be attained but the plant does require regular supervision and maintenance so that cracking is efficient (Gibson *et al.*, 1982).

A serious problem arises from the use of this process, in the form of environmental air pollution problems associated with odour from the acid-cracking tanks and the filter process. However, by using technology developed by McCracken & Chaikin

(1978b) the hot acid process is carried out in a closed system which can dramatically reduce these problems.

The drawbacks of acid cracking are their expensive running costs due to the large amounts of chemicals, H_2SO_4 and NaOH, required. Moreover, the effluent from the process contains high SO_4^{2-} concentrations which can cause subsequent problems in the receiving environment.

2.4.1.2 Chemical flocculation

A number of chemical coagulating agents have been examined to destabilise the emulsion and produce a clear supernatant layer after settling. The most common are di- or trivalent electrolytes of iron salts (ferrous sulfate and ferric chloride), aluminium salts (alum and aluminium chlorohydrate) and calcium chloride. At room temperature, maintaining a pH of 5-6, an effluent of great clarity and high purity can be obtained (Christoe, 1977). However, these produce a large quantity of sludge or result in the production of low-grade grease.

Synthetic polymeric flocculants have been investigated that are capable of flocculating colloidal dirt in wool scouring effluent (McLachlan *et al.*, 1978b). It has been found that up to 80% of the dirt in the liquor may be removed as a 20% w/v solids sludge, which is readily de-waterable using a solid bowl centrifuge, concentrating to 45-50 % solids. Although the use of these flocculants in a recycling process has no effect on grease recovery or wool quality, they become very expensive at the concentration required for strong wool scouring effluent. The cost could be reduced if a less expensive coagulant such as bitterns (magnesium-rich waste from the recovery of salt from sea water by solar evaporation) is available (Stewart, 1985). This process involves adding 6% v/v bitterns to WSE and storing it in a holding pond for several days before centrifuging to recover wool grease.

The advantages of flocculation processes are that they can easily be controlled by a feedback system and their space requirement is small except when bitterns is used. The disadvantages are high chemical expense due to large dosage rates of flocculants (up to 1.5% liquor mass) required to achieve satisfactory levels of treatment. The sludge produced is both voluminous and difficult to dewater. Besides, this process does not remove dissolved impurities. Therefore, a secondary treatment, such as a biological process and sludge treatment are required.

2.4.1.3 Incineration

Wool grease has a calorific value (40 MJ/kg) which is comparable to fuel oil (43 MJ/kg) (Christoe, 1986). However, injecting a normal scour liquor into a furnace would be uneconomical because of the large quantity of water that is present. As a result, the usual method involves firstly increasing the solid content of the effluent to about 60-70 % by passing it through a series of evaporators. The concentrate is then incinerated to produce heat. The only waste is the ash from the furnace. This process has high capital equipment and running costs. However, it is the only compact treatment mill capable of reaching virtually zero discharge standards applied by some countries.

2.4.2 Physical methods

2.4.2.1 Ultrafiltration

When scouring liquor is pumped under pressure through a semi-permeable membrane, only water and low molecular-weight contaminants, mainly suint salts, pass through the membrane barrier. The high molecular weight particles, grease and dirt, remain behind and thus are concentrated. The particular pore size of the

membrane can be chosen to reject chemical species greater than a certain molecular-weight (Buckley *et al.*, 1989; Townsend *et al.*, 1989).

There are a number of problems associated with using ultrafiltration for scouring effluents, namely:

- severe erosion of the membrane surface can be caused by mineral particles in the scouring liquor.
- the flux of liquor across the membrane gradually drops due to the formation of contaminants on the surface of the ultrafilter. To maintain the flux rate, the membrane requires cleaning. Over time, cleaning loses its effectiveness and the membrane needs replacement. With scouring liquors, this occurs frequently, thus it is an expensive operation.
- sludge of 20-25% solid content is usually obtained from the process. It therefore needs further dewatering before disposal.
- the ultrafiltrate is not suitable for reuse in the scouring process since it contains a high suint load.

Ultrafiltration can be used more efficiently with a modified desuint scouring system by using it to treat separated desuint liquor. As a result of a low suint content in the liquor, the membrane erosion problems are reduced. Moreover, the introduction of dynamic (formed on site) membranes of hydrous zirconium(4)oxide on tubular sintered stainless steel supports has been shown to have potential for treating strong wool scouring effluent (Buckley *et al.*, 1989). The process is, however, still considered to be high in capital and operating costs.

2.4.2.2 Evaporation

In this process, the impurities, both soluble and insoluble, are completely separated from the water, which can be recycled. Technical difficulties as well as a high

energy requirement tend to make the process expensive, but it has been widely used in Japan and Germany for wool scouring liquors (Wood, 1983) and its use is spreading, especially where stringent regulations of pollution discharge standards apply. In one application, additional wool grease is recovered by centrifuging the concentrated liquor after the evaporation process (Wood, 1983).

2.4.2.3 Solvent extraction

When sufficient solvent is added to a scouring liquor, the detergent can no longer stabilise the organic phase. This causes the emulsion to break up giving a grease-rich solvent phase.

The addition of 4-5 % pentanol together with a synthetic polyelectrolyte has been found to remove 85-95 % grease and 50-75 % BOD_5 (McLachlan *et al.*, 1978b). This process uses the principle of solvent destabilisation with n-pentanol and a polyelectrolyte to give a grease rich top solvent phase, a middle phase containing essentially suint liquor and a bottom dirt phase. These three streams are then distilled on packed columns to separate the constituents.

Recently, a three stage cross-current extraction process (Suiyuan *et al.*, 1989) was investigated at the laboratory scale (Figure 2.4.2.3.1). Extractant N_{235} and kerosene were added to the liquor at each step. After sufficient agitation, three separate phases were produced. The top extractant phase from step 1 was washed with a dilute solution of sodium hydroxide, then reused while the top phase from step 2 and 3 could be reused directly. The bottom phases, enriched wool grease mixed with dirt and extractants, from each step were combined and then treated with hot methanol to recover wool grease from the mixture. The bottom raffinate phase passed to the next extraction step.

The effluent from the process was precipitated by poly-ferric flocculant before discharge. Overall COD removal is greater than 93% and most of the wool grease could be recovered.

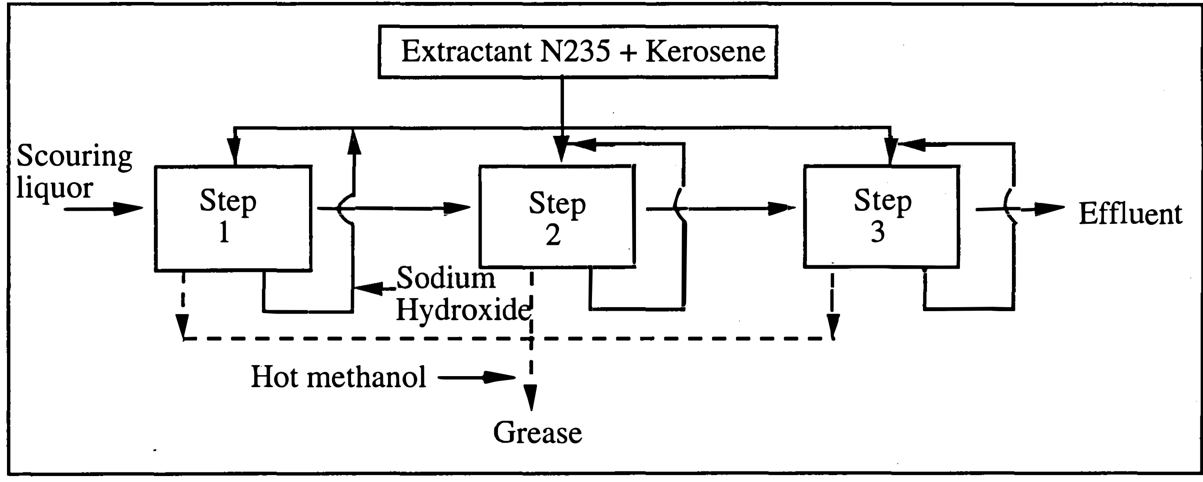


Figure 2.4.2.3.1 Three stage cross-current extraction (Suiyuan *et al.*, 1989)

There are a number of problems associated with solvent extraction, such as poor phase separation, the quality of recovered wool grease is poor with respect to colour and other characteristics, and high operating costs due to the expensive solvent used, (i.e. solvent recovery costs and solvent losses). Solvent extraction is therefore not considered commercially viable.

2.4.3 Biological methods

In biological treatment, bacteria and other microorganisms break down and metabolise the soluble and colloidal organic materials in the wastewater, thereby reducing BOD and COD. These may be classified into aerobic (in the presence of oxygen) and anaerobic (in the absence of oxygen). In aerobic processes the microorganisms convert the organic carbon to carbon dioxide and new cells, while

anaerobic microorganisms produce methane as well as CO_2. In either process, hydrogen acceptors must be present (oxygen for aerobic microbes; sulphates, nitrates, carbon dioxide for anaerobic microbes).

In effluents with high oil or grease concentration such as WSE, grease is adsorbed by the microorganisms faster than it can be metabolised. In trickling filters, oil tends to coat the microbial surfaces and reduce the transfer of more readily oxidisable organics. In activated sludge systems, the adsorbed oil tends to impair sludge settling characteristics. Resulting sludge losses may be so high as to reduce the microbial level in the system enough to cause reduced efficiency and possible system failure (Cheremisinoff *et al.,* 1989).

2.4.3.1 Aerobic processes

With sufficient oxygen supply, the microorganisms in an aerobic treatment system consume the organic matter and transform it by means of aerobic metabolism, partly into new microbial biomass and partly into the end products (CO_2, H_2O) (Eckenfelder *et al.*, 1985; Verstraete & Vaerenbergh, 1986).

In aerobic growth, considerable energy is available for cell synthesis, resulting in a relatively high yield coefficient (a = g dry cells formed/g of substrate used). The yield coefficients may range from 0.2 - 0.8 depending on the substrate and time of aeration (Tebbutt, 1983). The expected yield coefficient is related to the oxidation level of the organic substrate. Highly reduced compounds such as lipids result in high yield coefficients. In WSE, where the main contaminant is grease, the yield coefficient is expected to be high. The aerobic treatment of this wastewater would therefore produce a considerably high amount of biomass.

During the aerobic treatment process, adequate oxygen must be provided. The amount of oxygen needed for endogenous respiration is about 1.4 mg of oxygen per mg biomass (VSS) per day (Eckenfelder *et al.*, 1985). The overall oxygen requirement (mg/L processed) may be calculated as (Eckenfelder *et al.*, 1985):

$$\text{Oxygen} = a'Sr + 1.4\, byx_v$$

a' = amount of oxygen (mg O_2/mg organics) needed to oxidise organic matter

b = fraction of degradable biomass oxidised per day

Sr = amount (mg/L) of BOD or COD removed

x_v = biomass (VSS) concentration

y = degradable fraction of biomass

This equation shows the direct correlation between the amount of oxygen required and BOD (or COD) in the liquor being treated. To be able to maintain aerobic conditions in the system at all times, the loading rate of the process is generally limited by the amount of air supplied by aerators. In highly polluted wastewaters such as WSE, where BOD and COD are extremely high (see section 2.3.6), intensive aeration, or long HRT, is required. Together with the large amount of sludge produced, which requires treatment prior to disposal, make aerobic treatment of WSE very expensive.

Three aerobic processes, namely activated sludge, biofilter (trickling filter) and aerated lagoons, have been investigated to treat WSE for a number of years. Apart from the high running cost due to aeration, Rodmell & Wilkie (1983) found that an aerobic process treating WSE was sensitive to the variation in ambient temperature, especially in cool climates. In addition, many problems are encountered due to wool grease, since it is not readily biodegradable (Chao & Yang, 1981), and detergents,

which produce relatively stable emulsions (Christoe, 1977; McLachan *et al.*, 1978a). Moreover, in the process, grease tends to coat biological flocs, thereby interfering with the operation of the system by reducing the biological activity and oxygen transfer rate (Hatch, 1974), resulting in a high concentration of sludge organic matter in the treated effluent. Another problem is sludge disposal. The typical biological sludge from both activated sludge and trickling filter processes only contains 2-3% solids. Sludge conditioning (thickening) using either chemicals or anaerobic digestion followed by dewatering (using centrifuges, filter process) is needed to concentrate the solids content to 25%. Therefore, about 50% of the effluent treatment costs are in sludge treatment and disposal (Christoe, 1986).

McCracken & Chaikin (1980) reported the use of UNISAS or Superactive Sludge Process to treat WSE. This process was developed by the University of New South Wales for treatment of high strength wastes (Figure 2.4.3.1.1).

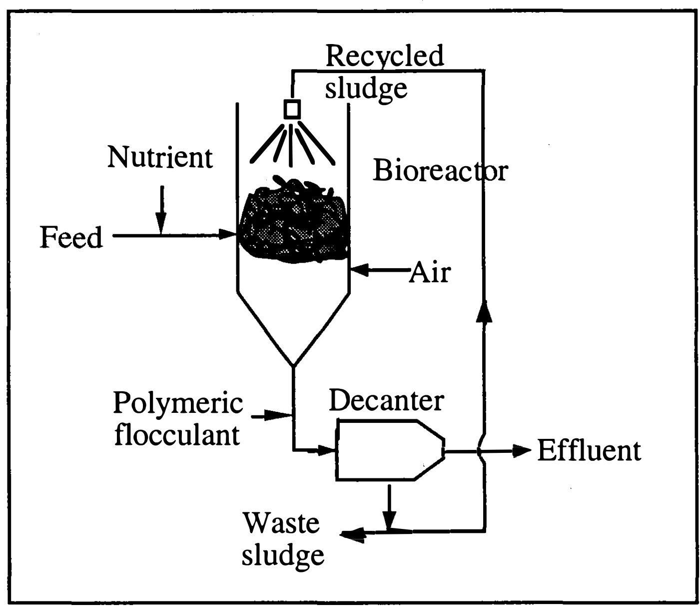


Figure 2.4.3.1.1 UNISAS Process (MaCracken & Chaikin, 1980)

It is a high-rate version of the conventional activated sludge process operating at a temperature of 35 °C. A high rate of air is injected to give sufficient oxygen. Extra nutrient is added on the basis of regular C, N, and P analysis. A high biomass concentration is obtained by recycling the sludge after concentrating by a decanter centrifuge. As a result, rapid biological breakdown can be obtained. It is claimed that the high biomass concentration and the control over the dissolved oxygen concentration afford the process great stability to shock loadings and periods of mill shutdown (MaCracken & Chaikin, 1980).

Although the efficiency of the process was high (84 % BOD_5 reduction at HRT of 1 day), it is noticeable that the concentration of pollutants in the wastewater used in the experiment was relatively low, averaging 2 000-4 000 mg/L of wool grease and 3 000-5 000 mg/L of BOD_5. It is doubtful whether the process would be still suitable for WSE with higher pollutant concentrations, which may be up to 20 000 mg/L of wool grease (Table 2.3.6.1). Moreover, an examination of the grease content of the mixed liquor and the centrifuged sludge indicated that a considerable amount of grease was not degraded but rather removed by the centrifuge. The UNISAS process is probably more suited to the treatment of the rinse stream, mixed mill effluent, or used as a purification step. However this application may be prohibited by the fact that the process is very high in capital and running costs.

Rodmell & Wilkie (1983) investigated the use of activated sludge and oxidation ponds to treat the combined flows of WSE and municipal wastewater. The results indicated that the combined wastes could be successfully treated by an activated sludge process, when the food to microorganism ratio (F:M) is great enough (approximately 0.3), and a long HRT (24 hrs) is provided. The process was, however, considered uneconomic. A more economical option, an oxidation pond of 1 m depth (without aeration) was investigated. However, apart from problems with the pond turning anaerobic in the warmer months (New Zealand summer December

to February), the results showed unsatisfactory reduction of pollutant, mainly due to the variation in composition of WSE.

Chao & Yang (1981) investigated the in-plant separation of concentrated wash streams from rinse water. The rinse water was treated by an activated sludge process, while wastewater from the wash stream was discharged to an anaerobic digester treating aerobic sludge. This modification resulted in a large reduction of the strength and waste loads to the aerobic process, and improved final effluent quality. Their study also investigated the disposal of digested sludge by injection into the soil surface. After six months of system operation, no effect on the ground water had been observed. The process, however, required large areas of land which not every wool scouring plant would have access to.

Christoe *et al.* (1976) demonstrated the use of aerobic pretreatment to partially destabilise wool scouring liquor. Their study showed that a low level aerobic treatment of 1-2 hrs gave less than 10% COD removal. However, this pretreatment greatly increased the efficiency of subsequent chemical flocculation. At the maximum, the flocculation efficiency was 15% greater for grease, 15% greater for COD, and 35% greater for SS than the efficiency without biological pretreatment. They also investigated the effect of sequencing the operation, and found that a combined aerobic followed by chemical flocculation treatment gave higher quality effluents than straight aerobic treatment, or straight flocculation treatment, or chemical followed by biological, or chemical-biological-chemical treatment. The sludges from the processes were rather bulky, about 40-80% of the liquor volume, which required further treatment before disposal. It was suggested to reduce the sludge volume to less than 15% by heating to 60 °C before dewatering by filtration. The additional sludge handling costs made this process uneconomic.

A more recent study has also shown the aerobic biological destabilisation of the liquor by an aerobic process (Cord-Ruwisch *et al.*, 1990). In this study, a short residence time (2-10 hrs HRT) activated sludge process was employed to treat the extremely high COD effluent. A reduction of 60-80% COD was obtained due to partial grease degradation, but mainly precipitation of wool grease into the sludge phase occurring during the growth of the microorganisms. However the process suffered from scum and foam formation

Roth *et al.* (1989) investigated the microbial ecology of aerobic biodegradation of WSE in a laboratory simulated natural ecosystem. It was found that partial clarification occurred during the first 8 weeks as indicated by the reduction of COD, surfactant breakdown and lighter colour. Flocculation was observed during a period of 8-12 weeks and steady stage developed after 14 weeks. Bacteria were isolated at fortnightly intervals and fully described. In the presence of sunlight, algae were found to contribute to the clarification of this wastewater. After 14 weeks, COD reduction was 72%. In the aerobic treatment process, a naturally occurring population of bacteria and a single ciliate species have been found to be able to degrade wool grease and detergent in WSE (Mickelson *et al.*, 1989). The experiment showed that the ciliates were essential to the successful performance of the mixed culture by enhancement of the rate and extent of wool grease breakdown.

2.4.3.2 Anaerobic processes.

With high-strength wastewaters containing 10 000 mg/L COD or over (Eckenfelder *et al.*, 1985), it becomes difficult to maintain aerobic conditions. The physical limitations of oxygen transfer equipment may prevent satisfaction of the oxygen demand with consequent onset of anaerobic conditions. In such circumstances it is possible to achieve COD reduction by anaerobic processes (Tebbutt, 1983).

In an anaerobic reactor, three groups of bacteria participate in the anaerobic conversion (Lemke, 1988): the hydrolytic and acid-forming (fermenting) bacteria transform organic matter to soluble organics by an enzymic hydrolysis reaction. The soluble organics are converted to volatile acids by acetogenic bacteria, then to methane and CO_2 by methanogenic bacteria (Pfeffer, 1979). The gas generated is typically 65-75% methane, and can be combusted to heat the reactor. In anaerobic systems, less energy is obtained from the organic conversion, and, hence, the growth yield is much less than for aerobic systems. Hence sludge produced is only about one-fifth as much as in aerobic treatment (Eckenfelder *et al.*, 1985).

In the past anaerobic digestion was commonly used to condition the waste sludges from aerobic treatment. In recent years there has been renewed interest in its use for strong effluents, such as piggery waste. Wool scouring effluent is well suited to treatment by anaerobic processes. This is supported by the characteristics of the waste itself, namely, the high organic load, the lack of heavy metals and toxic substances, the BOD_5/COD ratio, and the mean discharge temperature (Genon *et al.*, 1984). A number of investigations have shown that the wool scouring waste can be treated sufficiently by means of anaerobic digestion (Genon *et al.*, 1984; Isaac & Cord-Ruwisch, 1991; Rodmell & Wilkie, 1983; Whitaker & Stewart, 1985).

Due to the resistance to biodegradation of wool grease, WSE was found to be one of the most difficult substrates to be degraded anaerobically. It required digestion for 2 to 3 times longer than most of the other wastes to achieve a COD reduction of 50% (Pipyn & Verstraete, 1979) or a long HRT of 15-20 days is required to achieve a 75-80% reduction in BOD_5 (Rodmell & Wilkie, 1983; Whitaker & Stewart, 1985). Isaac & Cord-Ruwisch (1991) suggested that to successfully convert most of the organics in WSE into biogas, a very long residence time of about 30 days was required. Consequently, the large digester size required for the process questions the economics of such a treatment. However, in contrast to waste streams containing

easily hydrolysed compounds (such as carbohydrates, or sugars, which acidify rapidly and cause operational problems, with pH change and inhibition of the methanogens by high concentrations of VFAs) WSE is unlikely to cause digester failure due to acidification. This would make its anaerobic digestion more stable and readily controlled (Cail *et al.*, 1986; Isaac & Cord-Ruwisch, 1991) .

Recent developments in design and operation of anaerobic digestion processes have resulted in significant improvements in the rate, process control and cost effectiveness. A new development, the so called High Rate Anaerobic Treatment, which is based on two basic principles (Lettinga *et al.*, 1987) ; (1) a high retention of viable anaerobic sludge in the reactor and (2) also a sufficient contact between incoming wastewater and the retained sludge. The details of its design and operational conditions have been fully described in the literature (Callander & Barford, 1983; Idris, 1990; Lettinga *et al.* , 1983; Lettinga *et al.* , 1984; Sobkowicz, 1988).

The efficiency of high rate anaerobic processes, however, relies on the reduction of soluble solids. The presence of high concentrations of suspended solids in wastewater may reduce the process efficiency and also cause operating problems. Pretreatment process to remove suspended solids is recommended (Souza, 1986). Specific problems may be encountered in treating WSE due to a relatively high grease content in the liquor. Grease which is difficult to be degraded has a potential to form a scum layer and cause blockage (Lettinga *et al.*, 1983). The removal of the grease prior to the effluent entering the treatment system is therefore required.

Two of the high rate anaerobic processes, Upflow Anaerobic Sludge Blanket (UASB) and Anaerobic Filter Process were investigated for treatment of WSE, at a pilot-plant scale by Whitaker & Stewart (1985). Neither of the processes were found to perform as well as a Continuously Stirred-Tank Reactor (CSTR). At a HRT of 30

and 12 days, only 30% and 48% of BOD_5 reduction obtained from UASB and anaerobic filter process, respectively. This compares to an 80% BOD_5 reduction from the CSTR (HRT = 22 days). In addition, both processes suffered from the flotation of grease or scum causing blockage in the system and resulting biomass loss. It is noticeable that the processes used in the experiment did not respond favourably when the concentration of the feed was increased. This suggested that certain substances or conditions (possibly high grease concentration) might be inhibiting digestion.

Cail *et al.* (1986) investigated the feasibility of a semi-continuous digester system to treat WSE. The process is similar to a sludge-blanket reactor by virtue of the mode of operation. High cell densities are maintained in the reactor, thus achieving much higher treatment rates than those obtained using a conventional mixed digester. With the assistance of chemical flocculant (9-10 g/m^3 reactor/day of Zetag 88N) and polyelectrolytes (3-10 g/m^3 reactor/day) to maintain the biomass concentration in the reactor, an efficiency of 56% COD and 47% grease removal was obtained, at a HRT of 2.8 days (space loading rate of 9 kg COD/m^3reactor/day). In comparison to the conventional stirred anaerobic system investigated by Rodmell & Wilkie (1983), a similar COD removal of 55% (40% grease reduction) was achieved at a significantly longer HRT of 20 days (space load of 3.3 kg COD/m^3reactor/day). The concentration of WSE (7 g/L for grease and 25 g/L for COD) used in the study by Cail *et al.*, (1986) was however considerably lower than that used by Rodmell & Wilkie (1983) (14 g/L for grease and 66 g/L for COD).

Cail *et al.* (1986) also found that anaerobic digestion of WSE was rate-limited by wool grease which was resistant to biodegradation. Thus the further increase in the space load would only result in a lowered COD removal efficiency. To solve this problem, an enzyme (Actizyme) of 1% w/v was used for pretreating raw WSE before feeding to the system. This showed a significantly improved efficiency, 70%

removal was obtained for both COD and grease at a HRT of 2.8 days. However, with the addition of flocculant, polymer, and enzyme, the process became expensive and impractical.

Resent studies by Oellermann (1991) and Oellermann *et al.* (1992) employing an anaerobic reactor, with intermittent recirculation mixing mode, achieved a removal of up to 86% COD after a relatively short HRT of 2.4 days (organic loading of 18 310 mg COD/L/day). They also achieved successful post treatment using aerobic digestion which further reduced COD from 5 400 mg/L (average COD from the anaerobic process) to 1 200 mg/L. This gave an over all process efficiency of 97.2%. These studies, however, did not report the amount of methane production. It is therefore not possible to conclude whether wool grease had undergone full biodegradation, part adsorption and/or flocculation.

2.4.3.3 Bioflocculation process

The term bioflocculation generally refers to a dynamic process of floc formation resulting from synthesis of extracellular polymers by living cells (Dugan, 1987). This is essential to the functioning of biological waste treatment systems (Manahan, 1990). In wastewater treatment technology, bioflocculation is mainly discussed in relation to cell aggregation and the flocculation of bacterial flocs in aerobic biooxidation systems (particularly activated sludge) (Easson *et al.*, 1987). Since the separation and flocculation of those microorganisms and colloidal material which comprise the activated floc is an essential prerequisite for the efficient and economical operation of an activated-sludge wastewater treatment plant (Horan & Eccles, 1986), the understanding of bioflocculation mechanisms is crucial. It is well documented that this process involves polymeric substances of bacterial origin, particularly extracellular polysaccharides (Dugan, 1987; Easson *et al.*, 1987; Horan & Eccles, 1986; Novak & Haugan, 1981; Unz, 1989). However, in many cases,

extracellular proteins (Logan & Hunt, 1988), carbohydrates (Sato & Ose, 1980), lipids (Crabtree *et al.,* 1966; Forster, 1985), and nucleic acids (Novak & Haugan, 1981; Sato & Ose, 1980) are also common.

A number of microorganisms, including bacteria and algae (Lavoie & Noue, 1987), were found to produce water soluble extracellular ionic polymers which are effective in flocculating and sedimentating not only the bacterial cells but also other suspended particles in a manner analogous to synthetic polyelectrolyte flocculants (Easson *et al.*, 1987; Koizumi *et al.*, 1991; Levy *et al.*, 1992). The microscale phenomena of attachment were believed to involve some combination of hydrogen bonding and ionic, dipolar and hydrophobic interactions (Logan & Hunt, 1988). However, a number of articles have shown that the excess or accumulation of these biopolymers, particularly soluble polysaccharides which have high water-holding capacity (Easson *et al.*, 1987), can act to stabilise suspensions and prevent flocculation, resulting in poor sludge settling characteristics and less compact flocs (Harris & Mitchell, 1975; Lavoie & Noue, 1987; Urbain *et al.*, 1993). The reason for this phenomenon is not yet clear, however Harris & Mitchell (1975) suggested that the excess biopolymer may cause particle surface saturation which prevents optimum bridging between such particles.

In activated sludge processes treating wool scouring effluent, Cord-Ruwisch *et al.* (1990) observed the destabilisation and flocculation of wool grease emulsions which resulted in grease accumulation in the sludge. The mechanism of this process is not yet clear although Poole (1991) suggested that cell adherence to the grease particles by biopolymer bridging is a possible mechanism. In this study the term **bioflocculation** refers to the phenomenon of destabilisation of wool grease emulsion upon microbial action resulting in the coagulation/flocculation of wool grease from the WSE liquor.

2.4.4 Other Methods Used to Treat Wool Scouring Effluent

Due to the complexity and inconsistency of the WSE, no single treatment process has been found to be successful and economic. A number of researchers have investigated alternative options by combining the above processes, rationalised the scouring process to benefit effluent treatment, and attempted to utilise cheap materials in the treatment process. The following are some of the processes available:

2.4.4.1 Anaerobic-aerobic lagooning

When land is available, anaerobic/aerobic systems followed by spray irrigation is relatively cheap to install and operate.

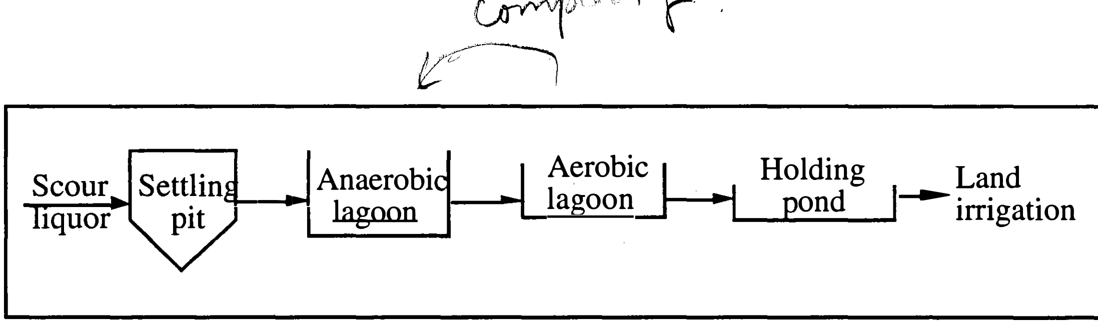


Figure 2.4.4.1.1 Anaerobic-aerobic lagooning process

The process (Figure 2.4.4.1.1) comprises the removal of heavy solids in a settling pit. The overflow passes to an anaerobic lagoon with a minimum 12 days retention followed by an aerobic lagoon with surface aerators for 5 days, and then finally to a holding pond of 1 m depth for at least 25 days. An efficiency of 93 % BOD_5 removal has been reported before spray irrigation (Gibson *et al.*, 1982).

2.4.4.2 The Traflo-W System (Harker & Bruce, 1974).

The effluent to be treated is monitored for alkalinity and sufficient acid is added to bring the pH value down to 6. A predetermined amount of ferrous sulfate solution (0.5-1.5%) is then added and this is followed by a suspension of lime sufficient to take the pH back to a value of 7.5-8.0. The voluminous sludge, about a third of the total volume of effluent, is produced, and is too huge to be directly disposed. Filtration is therefore essential to reduce this sludge volume.

Straight filtration is impossible because the filter cloth rapidly blinds, so it is necessary to add a filter aid. The cost of iron or aluminium salt and filter aid is very high if commercial grades are used. In the Traflo-W process, the ferrous sulphate solution used is waste liquor from a steel pickling process, and the filter aid is wood flour from saw milling. An alternative to filtration for dewatering the sludge is centrifuging, which is reported to work well (Harker & Bruce, 1974). Several variations of this process have been designed and commissioned in mills throughout the world. The variations have centred on the availability of cheap flocculants and filter aids (Stewart, 1985).

2.4.4.3 Centrifuging and evaporation (Alfa-Laval Process) (Beier, 1983).

The process removes as much grease and dirt as possible by using grease and decanter centrifuges. The degreased effluent is then evaporated to form a concentration of about 30% solids before it is passed to a sedimentation tank where it is finally combined with the sludge from the decanter. The water recovered from the evaporator is returned to the scour, thus there is no liquid effluent from the plant. The only waste is sludge which can be disposed to land.

2.4.5 Summary and cost comparison of wool scouring effluent treatment processes

A summary of treatment technologies for wool scouring effluent as well as relative costs are presented in Table 2.4.5.1. There are various methods which can be used effectively to treat the waste. However, the selection of the methods to be used must follow careful consideration since the processes have been developed for varying conditions. Christoe (1986) suggested some factors relevant to the selection of treatment methods for WSE:

- type of scouring technique,
- local discharge regulations,
- the location of the mill,
- the size of the mill,
- availability of land,
- access to treatment facilities,
- cost and availability of chemicals and energy,
- cost of sludge disposal.

Table 2.4.5.1 Summary of some effluent treatment processes for wool scouring effluent, at pilot scale or currently used (Christoe, 1986; Christoe & Bateup, 1982; Gibson et al., 1982; Stewart, 1985).

Type	Process	% COD removal	Comparative cost	
			Capital	Running
Aerobic	(a) Two stage activated sludge	85-90	High	Medium
	(b) UNSW 'Unisas' high rate activated sludge at elevated temperature	98-99	Medium	High
Anaerobic	Conventional mixed reactor	80	High	Medium
Anaerobic/Aerobic	Settling followed by anaerobic lagoon, aerobic lagoon and final dilution pond-land irrigation.	100	Low	Low
Chemical flocculation	(a) Flocculation with alum, ferric chloride or calcium chloride preferably with polyelectrolyte. Sludge dewatering via rotary vacuum filter or decanter.	85-90	Medium	Medium
	(b) Flocculation with 6% v/v bitterns followed by centrifugation.	80-85	Medium	Low
	(c) Traflo-W system. Ferrous sulphate/lime/ filter aid coagulation- rotary vacuum filter.	85-95	Medium	High

Type	Process	% COD removal	Comparative cost	
			Capital	Running
Acid cracking	(a) Traditional batch acid cracking with sulphuric acid , settling, filter pressing to give grease.	80-85	Low	Medium
	(b) UNSW continuous hot acid cracking at pH 2.5-3 in reactor followed by neutralisation, flocculation and sedimentation and continuous sludge dewatering via decanter centrifuge.	85	Medium	Medium
Solvent destabilisation	Alcohol destabilisation process uses pentanol to crack emulsion to give high grease phase, suint phase and dirt which are separated by steam distillation columns.	75	Medium	Low
Centrifuge/Evaporation	Alfa Laval process. Removing grease and dirt by centrifuging, then followed by evaporation.	99	High	High
Evaporation /Incineration	Evaporation of scour liquor to 60-70% solid content, sludge disposal by incineration.	100	High	High

2.5 Discussion

The high levels of pollution inherent in wool scouring effluent have long been acknowledged. The treatment processes available to the wool scouring industry are both many and varied as described in the literature review. Increasingly tougher legislation relating to acceptable effluent discharges and rising energy costs have forced industry generally to reassess their processing and waste disposal technologies. An essential initial step towards efficient pollution removal is the rationalisation of the scouring process (see section 2.2) to minimise pollution load to the environment. However, this also generally results in effluents of very high strength.

Since the effluent from wool scouring is generally a non-toxic wastewater, available physical, chemical and biological means can be and are used. The choice of process is usually determined by economic factors. As shown in Table 2.4.5.1 anaerobic or aerobic lagoons, followed by irrigation, offer the most cost effective solution. However not all scourers are fortunate enough to have adequate areas of land available in a favourable location to install such systems. Most physical and chemical processes, although easy to operate and control, are high in capital and running costs. Biological processes may still offer a cheaper alternative option.

Emulsified wool grease which represents the major part of organic load in WSE is not water soluble and therefore not directly accessible to microorganisms (see section 2.3.4.2). Consequently, a process that relies on biodegradation requires a long residence time resulting in high capital and running costs (e.g. aeration in an aerobic process). Thus biological treatment is not generally suitable unless the wool grease is removed first.

The use of chemical agents, such as acid or flocculant, to separate wool grease from WSE has been well investigated (see section 2.4.1.). Apart from expensive chemicals being used and large sludge volumes produced, the effluent is normally not suitable for biological post-treatment. Recently, aerobic microbes have been found to be able to destabilise (flocculate) wool grease from WSE (Cord-Ruwisch *et al.,* 1990, Roth *et al.*, 1989). However, the process requires expensive aeration, and moreover suffers from an excessive foaming problem.

An anaerobic process is more attractive than an aerobic process because it eliminates the need for aeration, thus having a much smaller energy requirement, minimises sludge production, and produces methane which is a source of energy to the process. Anaerobic microbes have not previously been investigated for their ability to destabilise wool grease emulsion. However, it has been observed that sludge from anaerobic lagoons, particularly the first lagoon, contains an appreciable amount of wool grease (John Sheehan, personal communication). Therefore the success of the lagooning system, which has been claimed to remove 97% of COD, can be hypothesised to be due not only to microbial degradation but also partial destabilisation of wool grease from WSE. The optimal conditions for anaerobic destabilisation of grease emulsion can be expected to be different from those for biomethanation. Anaerobic bioflocculation is likely to occur more readily than grease biodegradation observed in the aerobic process (Cord-Ruwisch *et al.*, 1990), and thus requires a shorter residence time and is more economical to operate. The success of such a process would therefore provide a cheaper alternative method for the treatment of WSE.

Chapter 3 Materials and Methods

3.1 Introduction

In this chapter, the general materials and methods used in the study are described. These include the preparation of sludge and WSE samples, the set up of experimental reactors, the isolation of bacteria from the process, and analytical procedure. The details of the specific methodology for each experiment can be found together with their results in the relevant chapters (chapter 4-7).

3.2 Preparation of sludge and feed (WSE)

3.2.1 Anaerobic sludge

3.2.1.1 Anaerobic digester sludge from municipal wastewater treatment plant

Anaerobic sludge used in all the experiments, unless otherwise stated, was taken from the primary anaerobic digester at a municipal wastewater treatment plant (Subiaco, Western Australia) and used without any preparation.

3.2.1.2 Lagoon sludge

Anaerobic lagoon sludge, used in section 5.8, was taken from the first and second anaerobic lagoons at a local wool scourer (Jandakot, Western Australia). The sludge was collected by throwing a metal bucket into the centre of the lagoon, then allowing the bucket to slowly sink to the bottom. Just after the bucket touched the bottom of the lagoon, it was slowly pulled to the side and quickly lifted before reaching the side of the lagoon.

3.2.1.3 Sludge supernatant

Sludge supernatant was obtained by centrifuging the anaerobic sludge at 105 G for 5 min, just enough to remove the bulk of the sludge from the liquor but still leaving suspended microbes in the supernatant.

3.2.2 Aerobic sludge

Aerobic sludge used for start up of the aerobic process was collected from the same wastewater treatment plant as the anaerobic sludge (Subiaco, Western Australia). The collection point was the bottom of the secondary sedimentation tank where activated sludge was returned to the aeration tank. The sludge was then used without any preparation.

3.2.3 Wool scouring effluent

WSE was collected from the main wash liquor (Stream 5 in Figure 4.1.1) at the local wool scouring plant. The effluent was from scouring Australian merino fleece wool using a NPnEO (TN 450) non-ionic surfactant (see section 2.3.5).

For batch experiments, a bulk supply of WSE was collected and preserved for all experiments requiring raw WSE. It was also used as a growth substrate (agar and broth, see section 3.4.1). For the semi-continuous experiments WSE samples were obtained from the plant on a fortnightly basis. Grease content in the WSE was analysed and samples with grease concentration of higher than 10 g/L were used in the two-stage bioflocculation system (see section 7.3), while samples with less than 10 g of grease /L were used in the single-stage system (see section 7.2). During the experimental period, preserved samples were supplied to the system daily.

The preservation of WSE samples was achieved by autoclaving the samples at 132 °C for 15 min, and then storing at 4 °C so as to maintain the same composition in feed material during the experimental period. Although autoclaving was found to affect the anaerobic digestion process, from the preliminary experiment (Appendix 1), it did not measurably affect the anaerobic bioflocculation process.

3.3 Experimental reactors and set up

3.3.1 Grease flocculation in mixed batch reactors

In batch experiments, two different sizes (70 ml and 170 ml) of clear glass test-tubes with rubber stoppers (Figure 3.3.1.1) were used as reactors. Three glass tubes passing through the stopper were used to provide an (1) effluent withdrawal tube, (2) nitrogen displacement tube, and (3) gas sampling tube. After the reactors were filled with the required content, air in the head space was displaced by nitrogen gas. Then the reactors were completely sealed.

The mixing mode for these reactors was achieved by placing the reactors on a Coulter Mixer® as shown in Figure 3.3.1.2 (a mixer that mixes samples in vials laid on the rollers, by imparting a continuous rotating and tilting motion to the vials).

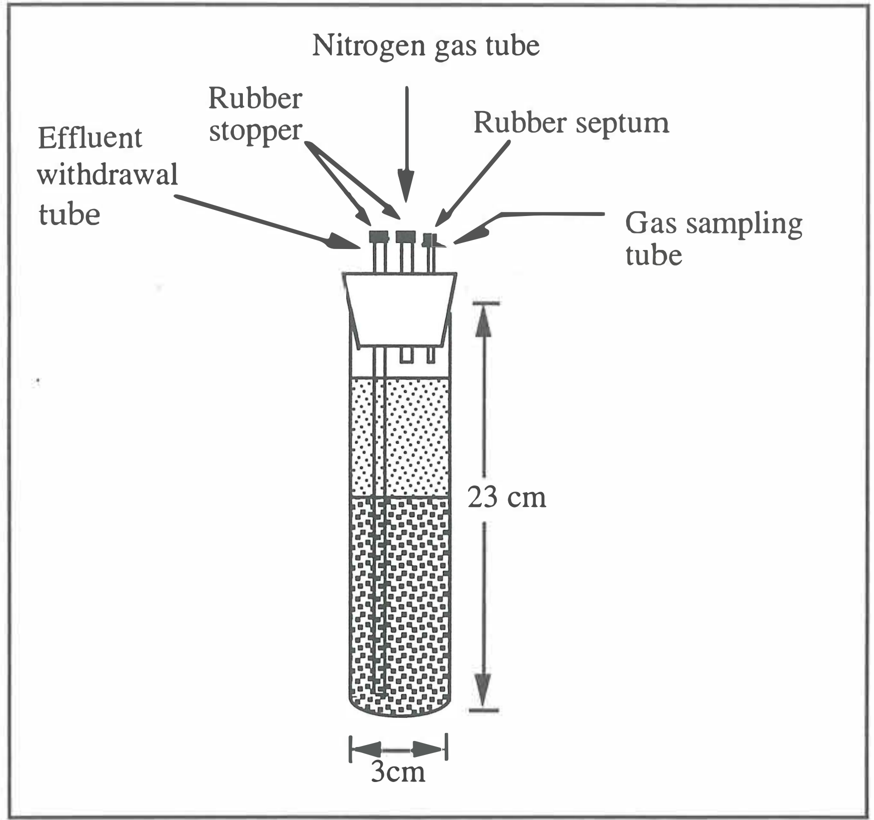


Figure 3.3.1.1 Schematic diagram of grease flocculation in mixed batch reactor

Figure 3.3.1.2 Mixing mode of mixed batch reactors

Liquid samples were taken from the reactors using a 50 ml glass syringe through the effluent withdrawal tube, with nitrogen gas added through the nitrogen gas tube, to replace the removed sample. The gas volume produced from each reactor was measured by water displacement as shown in Figure 3.3.1.3.

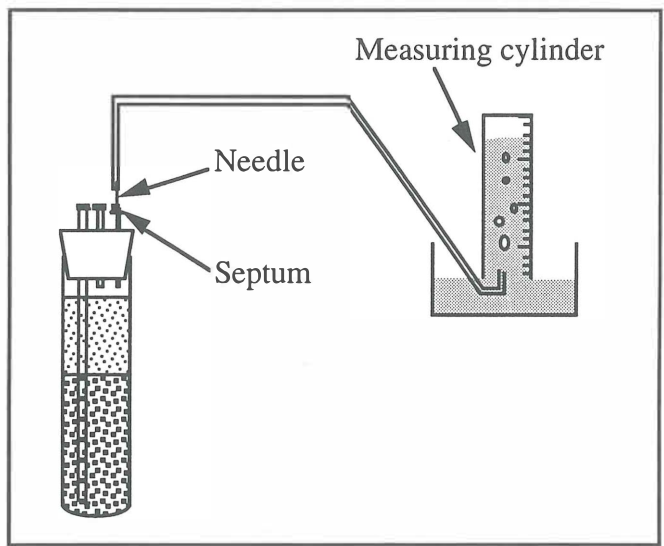


Figure 3.3.1.3 Schematic diagram of gas measurement from batch reactor

3.3.2 Single-stage anaerobic bioflocculation semi-continuous system

Two cylindrical reactors were constructed from clear glass as shown in Figure 3.3.2.1 and 3.3.2.2. One reactor without a water jacket was designed to operate at a constant room temperature of 20 °C. The other reactor with a water jacket allowed the system to operate at a higher temperature of 37 °C, by circulating water at a constant temperature from a water bath through the water jacket.

The reactors, of 1.4 litres volume, were started up by filling them with anaerobic sludge (see section 3.2.1.1). After the sludge had incubated for 24 hrs, the reactors

were fed with WSE. The WSE used as feed (see section 3.2.3) was continuously mixed by a magnetic stirrer to give a consistent feed quality during the period of experiment (some of the solids normally settled to the bottom without stirring). The hydraulic residence time was regulated by the feed inflow rate. Feed was pumped, using a peristaltic pump (Chemap AG, SA 8031) to the base of each reactor. The overflow from the reactor, running through a liquid trap, was collected in a 2 litre separating funnel. The function of the U-shape liquid trap was to maintain a liquid seal preventing gas from escaping from the reactor as well as preventing air entering the reactor from the effluent outlet.

The content in the columns was mixed by circulating the liquor from the base of the reactors to the top, using separate peristaltic pumps (Chemap AG, CH 8604) operated automatically. The flow rate of the mixing pump was set at 60 ml/min, which was sufficient to circulate the settled sludge back to the liquor without generating excessive turbulence.

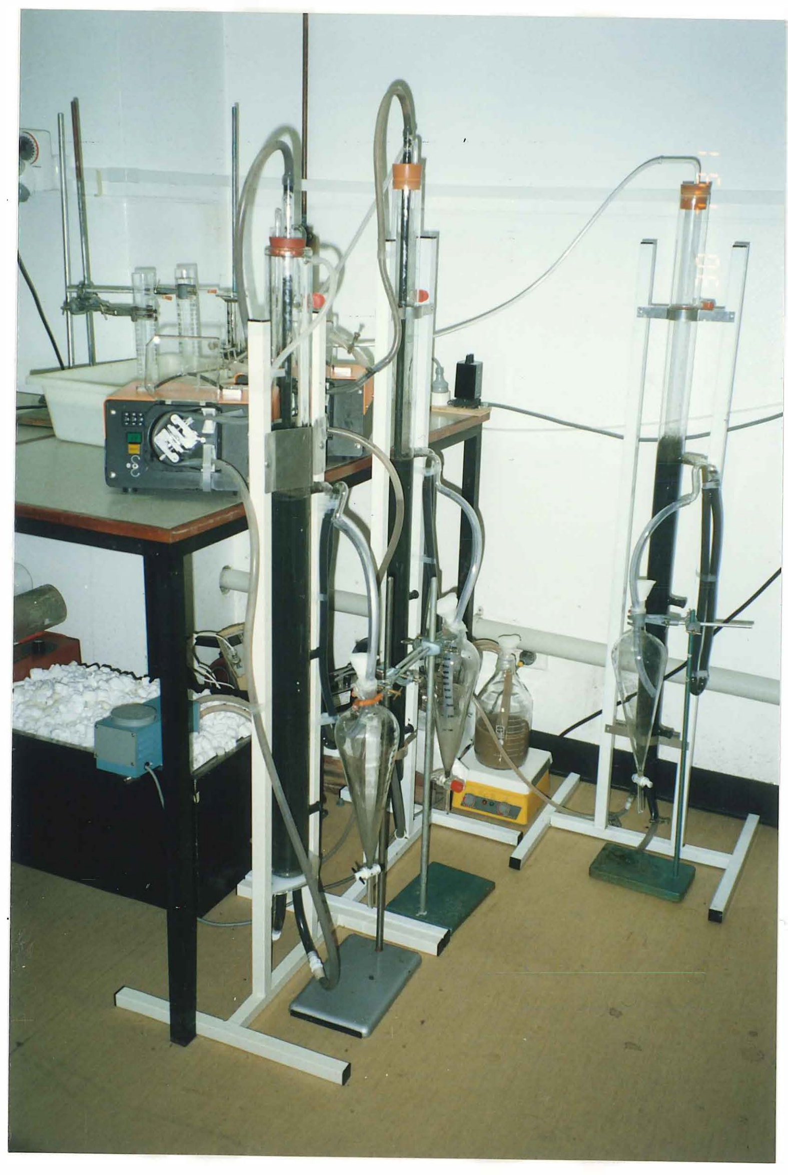


Figure 3.3.2.1 Experimental set up of single stage anaerobic bioflocculation semi-continuous system

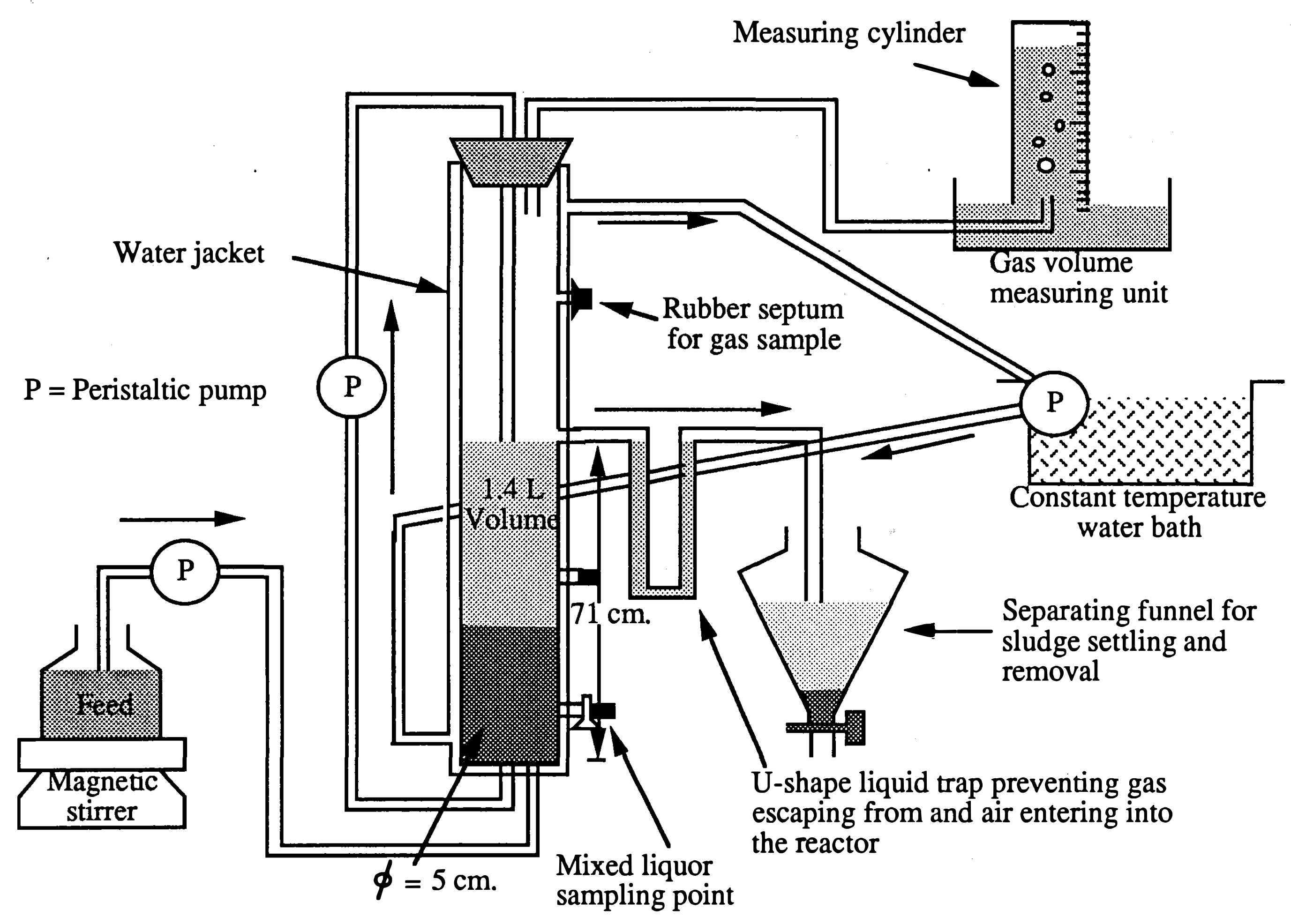


Figure 3.3.2.2 Schematic diagram of a single-stage anaerobic bioflocculation semi-continuous system

The operation of each reactor was controlled by a timer (Figure 3.3.2.3). Every hour, fresh WSE was fed to the base of the reactor for 5 min and clarified effluent from the surface overflowed. The content of the reactor was gently mixed for 15 min by the circulating pump and the mixed content was allowed to settle for the remaining 42 min period.

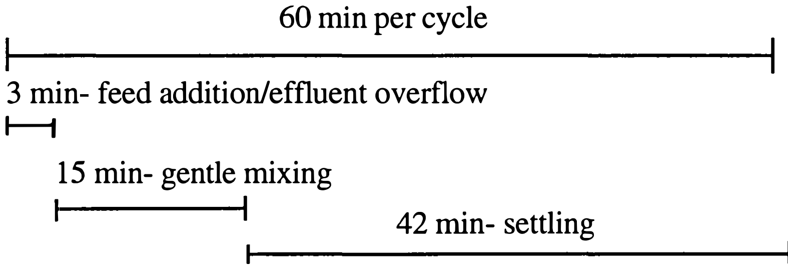


Figure 3.3.2.3 The feed/mix/settle operation cycle of the semi-continuous system

Using this method of operation, the new feed could be in contact with the microbes thus allowing the microbes to destabilise the wool grease emulsion. The destabilised grease emulsion was then provided with a settling period to form flocs and subsequently sediment before the clarified liquor at the surface overflowed during the next feed.

The gas volume produced from the reactors was measured by water displacement using an inverted measuring cylinder, which was connected directly to the top of the reactors. Gas samples were taken directly from the reactors though the rubber septum using a 1 ml plastic syringe and injected to the gas chromatograph immediately to analyse for methane.

3.3.3 Two-stage anaerobic bioflocculation semi-continuous system

The first stage of a two-stage anaerobic bioflocculation system was set up and operated in the same manner as the single-stage system (Figure 3.3.3.1), except gas volume was not measured since during the single-stage process a negligible amount of gas was produced (see section 7.2), and the water jacket was no longer required because the two-stage process was implemented at ambient temperature (in the range of 18 - 30 °C).

The second stage of this system employed a similar reactor with a smaller volume (1 litre). This reactor was also started up with anaerobic sludge taken from the sewage treatment plant (see section 3.2.1). Feed for this reactor was the supernatant of settled effluent from the first stage (from the separating funnel). Effluent from the second reactor was collected in the second separating funnel. A feed/mix/settle mode of operation was implemented, with timings as for the first stage.

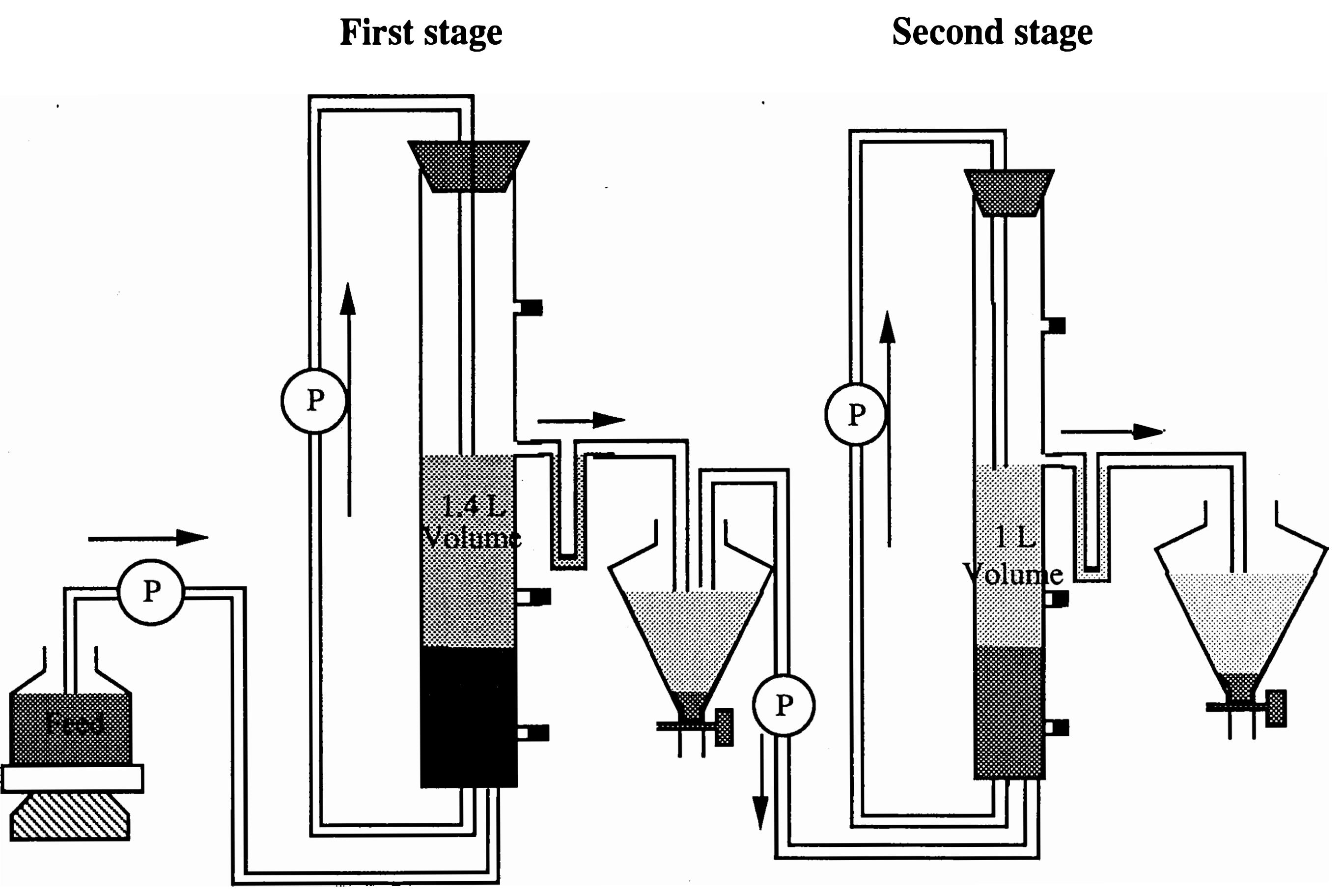


Figure 3.3.3.1 Schematic diagram of two-stage anaerobic bioflocculation semi-continuous system

3.3.4 Aerobic post treatment system

An aerobic reactor was modified from a CSIRO bioreactor, air-lift fermentor type with baffle for internal sludge recycle (Cord-Ruwisch *et al.,* 1990) (Figure 3.3.4.1). Total volume of the reactor was 3 litres, with a 2 litre aeration chamber and a 1 litre settling chamber. Two adjustable baffles were used to separate the settling chamber from the aeration chamber. The function of the internal baffles was to form an internal settling chamber, for the retention of flocculated biomass, thereby giving a clarified effluent overflow from the system as well as increasing the biomass concentration in contact with the influent.

The reactor was started up by filling the reactor with aerobic sludge obtained from the municipal wastewater treatment plant (see section 3.2.2). After the sludge was incubated for 24 hrs, the reactor was then fed with the supernatant of the effluent from the anaerobic bioflocculation process without any nutrient supplementation. A feed pump (Chemap AG, SA 8031) was operated continuously. The hydraulic residence time was set at 3 days. The effluent (overflow) was collected in a 2 litre separating funnel. The system was operated at ambient temperature (in the range of 18-30 °C). Oxygen was supplied continuously via compressed air pumped through an air-sparging stone, at the rate of 15 L/hr.

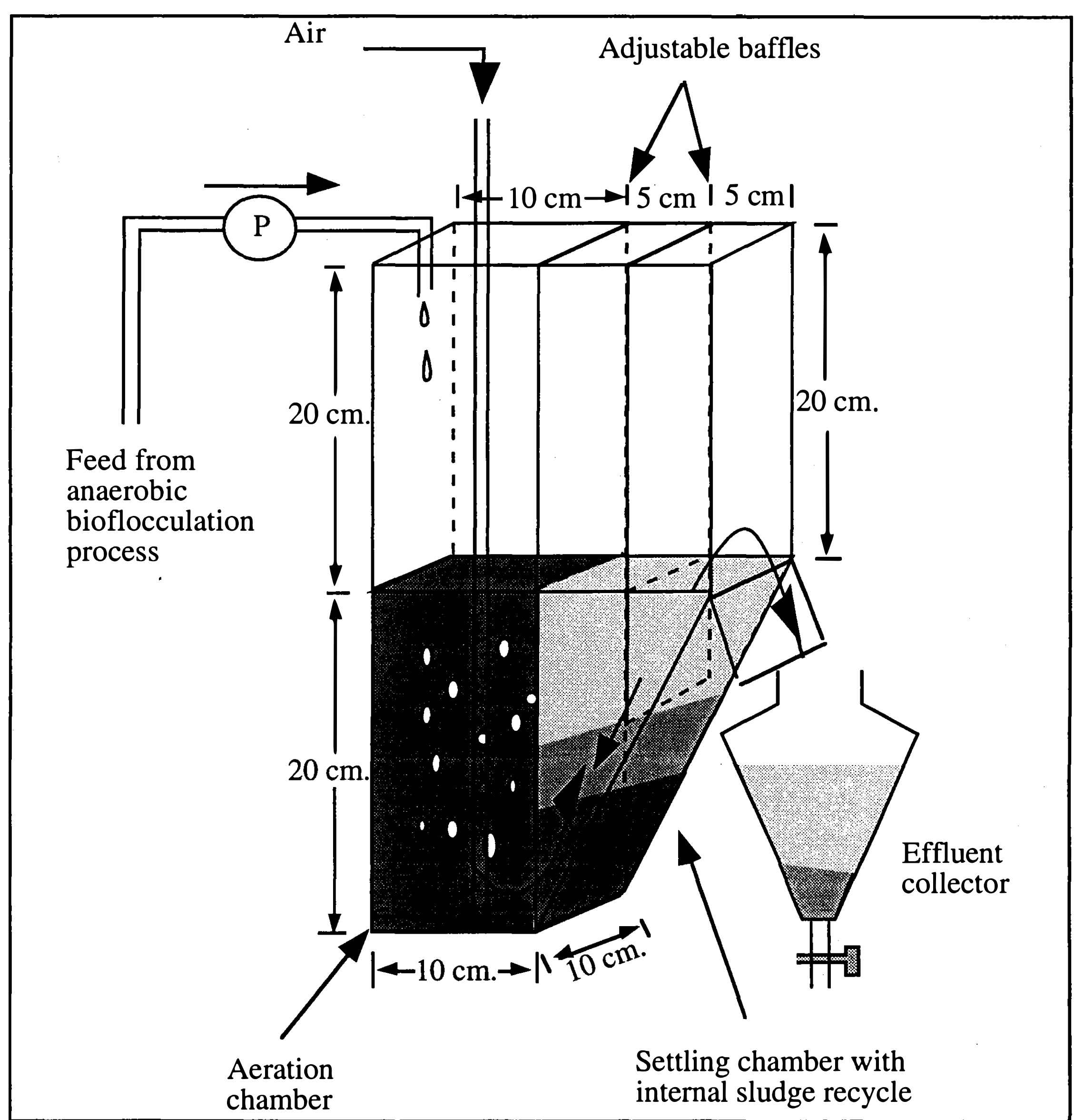


Figure 3.3.4.1 Schematic diagram of post-treatment aerobic system

3.4 The investigation of bacteria involved in bioflocculation process

3.4.1 Preparation of culture media

Three types of agar media, anaerobic sludge supernatant, WSE, and tryptic soy agar plates were used in the experiment. The preparation of these media was as follows:

- Anaerobic sludge supernatant agar was prepared by autoclaving fresh anaerobic sludge from municipal wastewater treatment plant (see section 3.2.1.1) and centrifuged first at 10 000 rpm for 15 min and then at 20 000 rpm for 10 min. The supernatant was then supplemented by 0.1% yeast extract for the preparation of agar plates.
- WSE agar was prepared from fresh WSE without nutrient supplement.
- Tryptic soy agar was obtained from Difco Laboratories, Detroit, Michigan, USA. It was prepared as broth following the supplied instructions.

All plates made from the above media used 1.5% bacteriological agar (obtained from Life Technology Ltd., Paisley, U.K).

3.4.2 Isolation of bacteria from anaerobic bioflocculation process

The supernatant from flocculated WSE was used as an inoculum. This was diluted in 10 fold dilution steps to 10^{-8}. A sample of 100 µl of the 10^{-6} to 10^{-8} dilution was spread aseptically onto the sludge supernatant and WSE agar plates. Duplicates were made of each dilution. One complete set of duplicates were incubated at 30 °C aerobically and the other set incubated anaerobically using an anaerobic jar (BBL® GasPak System with BBL GasPak Plus™ Anaerobic system envelope, obtained from Becton Dickinson and Company, USA.). Aerobic plates were checked for visible bacterial colonies after 24 hrs, and anaerobic plates were checked after 96 hrs. Different bacterial colonies were isolated and purified by sampling from each colony

on to new plates. Once pure strains were obtained they were maintained in nutrient Tryptic Soy broth (soybean-casein digest medium) and stored at 4 °C.

Sterilisation was carried out by autoclaving at 121 °C for 15 min.

3.4.3 Viable bacterial counts

The bacterial population was estimated by the plate count method as follows. Serial 10-fold dilutions of 1 ml samples were made in sterile distilled water on the day of sampling. Liquors of 100 μl of each dilution in the range from 10^{-1} to 10^{-9} were spread aseptically onto WSE plates in duplicate. The agar plates were then incubated aerobically for 24-48 hrs, or until the colonies were clearly observed at 30 °C. The numbers of bacterial colonies were determined using a colony counter at a magnification of 10x.

It should be noted that although the bacteria were isolated from an anaerobic process, the viable bacterial counts were obtained by plate count method under aerobic condition. This was due to the result obtained in Appendix 2, that under anaerobic conditions bacteria fail to form discrete, distinct colonies, which therefore precluded the possibility of bacterial counts. Aerobic plate counts were considered to be more reliable.

3.5 Analyses of samples

All samples were analysed in duplicate as follows:

<u>Total solids (TS).</u> A sample (5 ml.) in a tared crucible was evaporated to dryness at 105 °C overnight. TS was defined as the solids residue left in the crucible.

Volatile solids (VS), or organic materials, was measured as the decrease in mass when TS was ashed in a muffle furnace at 550 °C for 20 min.

Suspended solids (SS). A sample was filtered through a weighed standard glass microfibre filter (GF/C, Whatman). The residue retained on the filter after being dried at 105 °C for 12 to 24 hrs was defined as SS.

Volatile suspended solids (VSS) was determined as the decrease in mass when SS were ashed in a muffle furnace at 550 °C for 20 min..

Grease (solvent extractable material) was determined by vigorously mixing ten to twenty millilitres of sample with equal proportions of ethanol and trichloroethane in a homogeniser for 1 minute. After extraction of the grease into the trichloroethane layer, the mixture was centrifuged and the trichloroethane was separated and evaporated to give the grease content. This was according to the method of McLachlan *et al.* (1978b). Laboratory studies showed that this method gave similar results to the Soxhlet extraction method in Standard Methods (APHA, 1989).

Biomass was calculated as volatile suspended solids minus grease.

Biochemical Oxygen Demand (BOD_5) is a measure of the oxygen required for the biochemical oxidation of organic material. The manometric method in Standard Method (APHA, 1989) was used. The method consists of placing a sample in an airtight bottle and incubating at 20 °C for 5 days. Carbon dioxide evolved is absorbed on to a KOH solution. The BOD_5 is then computed from the difference between the initial and final manometer reading.

Chemical Oxygen Demand (COD) is a measure of the oxygen equivalent required for the chemical oxidation of the organic materials, both degradable and refractory,

present in a sample. The determination of COD followed the closed reflux microtitrimetric method in Standard Methods (APHA, 1989). The organic materials are oxidised by potassium dichromate. After digestion, the remaining unreduced potassium dichromate is back tritrated with ferrous ammonium sulphate.

The main organic material in WSE was wool grease which was present as an emulsion rather than in dissolved form in the liquor. In some samples, wool grease was flocculated. Thus particular care had to be taken to obtain representative samples and accurate dilutions of samples for COD analysis. Samples were thoroughly mixed prior to sampling and all dilutions involved a homogenising step.

Methane concentration in biogas. Methane gas was determined directly by gas chromatography (Varian Model 3700) using the following parameters:

Column:	Glass, 1.8 m * 2 mm ID
Packing material:	Poropack QS, 80/100 mesh
Column temperature:	100 °C
Carrier Gas:	Nitrogen
Injection mode:	On column
Injection temperature	200 °C
Sample volume:	0.5 ml.
Detector:	Flame ionisation detector
Detector temperature:	200 °C

Volatile fatty acids (VFA) Samples were centrifuged at 15 000 rpm for 10 min to remove suspended solids. The supernatant was acidified by the addition of 0.5% v/v concentrated phosphoric acid to ensure that VFA were converted to the free acid form. In many cases a second centrifuging step was required after acidification to remove some precipitated suspended material. The samples could then be analysed for the presence of VFA by gas chromatography, which was operated as described

for methane gas analysis, except the column temperature was 190 °C and the sample volume was 5 μl.

The samples were stored frozen at -20 °C if volatile fatty acid analysis could not be carried out at the time of sampling.

Non-ionic surfactant (NPnEO) analysis was modified from Ahel & Giger (1985a) as follows; 10 ml of sample was extracted in 10 ml of ethyl acetate. After vigorously shaking and centrifuging at 1 500 rpm for 15 minutes, 8 ml of the top layer (ethyl acetate) was withdrawn and then evaporated to dryness. This residue was redissolved in approximately 1 ml of dichloromethane. The purification of the extract was performed using a glass column (10 mm i.d.) equipped with a Teflon stopcock and filled with 6 g of aluminium oxide which had been packed into the column as a suspension in dichloromethane. The sample was applied to the top of the aluminium oxide which was then eluted with 35 ml of dichloromethane to remove nonpolar co-extracted compounds. The detergent was subsequently eluted with 25 ml of methanol. This methanol fraction was evaporated to dryness. The residue was redissolved in (i) 2 ml of methanol when analysed by reversed-phase High Performance Liquid Chromatography (HPLC) for phenolic group content of NPEO, and (ii) 1 ml hexane/2 propanol (95:5 v/v mixture) when analysed by normal-phase HPLC to determine ethoxylate chain distribution.

The HPLC instrument used in the experiment was a Varian Model 2010 HPLC, equipped with Varian Model 2050 UV Detector and Varian 4290 Integrator. For normal-phase operation gradient elution was applied; reversed phase analysis was run isocratically. Normal-phase HPLC was performed with spherical 5 μm LiChrospher-NH_2 (250 mm * 4.6 mm i.d., Merck) column packing. The eluents A and B (refer below) were mixtures of n-hexane/2-propanol (95:5 v/v mixture) and

methanol, respectively. Gradient elution was carried out with a linear program as follows:

Time (min)	% A	%B	Flow rate (ml/min)
0	97	3	1.0
5	96	4	2.0
15	87	13	2.0
29	80	20	2.0
44	74	26	2.0

Reversed-phase HPLC was carried out with a 5 µm LiChrospher® 100 RP-18 column (125 mm * 4 mm i.d., Merck), a methanol/water eluent (9:1 v/v mixture) and employing a flow of 1.5 ml/min. The UV detector wavelength was 277 nm for both normal and reversed phase.

Detergent standards were prepared by diluting appropriate amounts of commercial detergent TN 450 in distilled water. Standard dilutions were prepared as for experimental samples, prior to HPLC analysis.

Free (dissolved) detergent was obtained by centrifuging the samples at 10 000 rpm and filtering the supernatant through 0.2 µm membrane filter.

Chapter 4 In-plant Survey of Liquor streams at a Wool Scouring Plant

4.1 Introduction

As part of an investigation into the development of wastewater treatment processes for wool scouring effluent, an in-plant survey of a local wool scourer was conducted to provide baseline information about the quantity and characteristics of waste streams within the plant. The data obtained from this survey would then give an overall picture of a wool scouring plant effluent production, which would be useful for the design of a subsequent treatment process.

Jandakot Wool Scouring Co. Pty. Ltd. was chosen to provide all samples for this study since it is the biggest and most modern wool scouring plant in Western Australia utilising the aqua scouring process. This plant is also one of the largest and most efficient in the world with regard to its reputation for quality and quantity of scoured wool. Maximum annual production of the plant is 20 000 tonnes of wool. Wool grease produced from the plant can be up to 1 300 tonnes per year (value $1.2 million), which represents 20% of wool grease produced in Australia (Foley, 1991).

The plant was established in 1927 and is located at Hammond Rd, Jandakot, Western Australia. It has been improved and modified continually from a conventional system to the present Wool Research Organisation of New Zealand (WRONZ) system (see section 2.2). Each scouring train consists of a series of five open bowls as in Figure 4.1.1. The greasy wool passes sequentially through bowl 1 to 5. Water is added to the last bowl and flows countercurrently to bowl 1. Detergent is added to the first three bowls, containing 0.3%, 0.2% and 0.1% w/v detergent respectively. The last two bowls serve to rinse the fibre clean. Scour liquor from side bowl 1 as

well as in the downflow from the first two bowls is pumped through three-stage centrifugation (after settleable solids have been removed in the settling tank) to recover saleable wool grease. Effluent from the centrifuges, settling tank, and excess water from rinse bowls is then discharged. A series of anaerobic and facultative lagoons are employed to treat the effluent from the process. Recently, the plant was modified to discharge rinse effluent (6) and main washing effluent (5) separately to different anaerobic lagoons, which are used as a treatment process.

Since WSE depends greatly upon the scouring process being used (as discussed in section 2.2), the objectives of this survey were: (1) to provide baseline information about the characteristics (quantity and quality) of the waste streams from the selected wool scouring plant, which would form the basis for the subsequent study; (2) to confirm the hypothesis that some of the wool grease emulsions in WSE are flocculated during the treatment in anaerobic lagoon; (3) to determine the mass balance of the primary centrifuge which would give an indication of the accuracy of the sampling and analytical methods used.

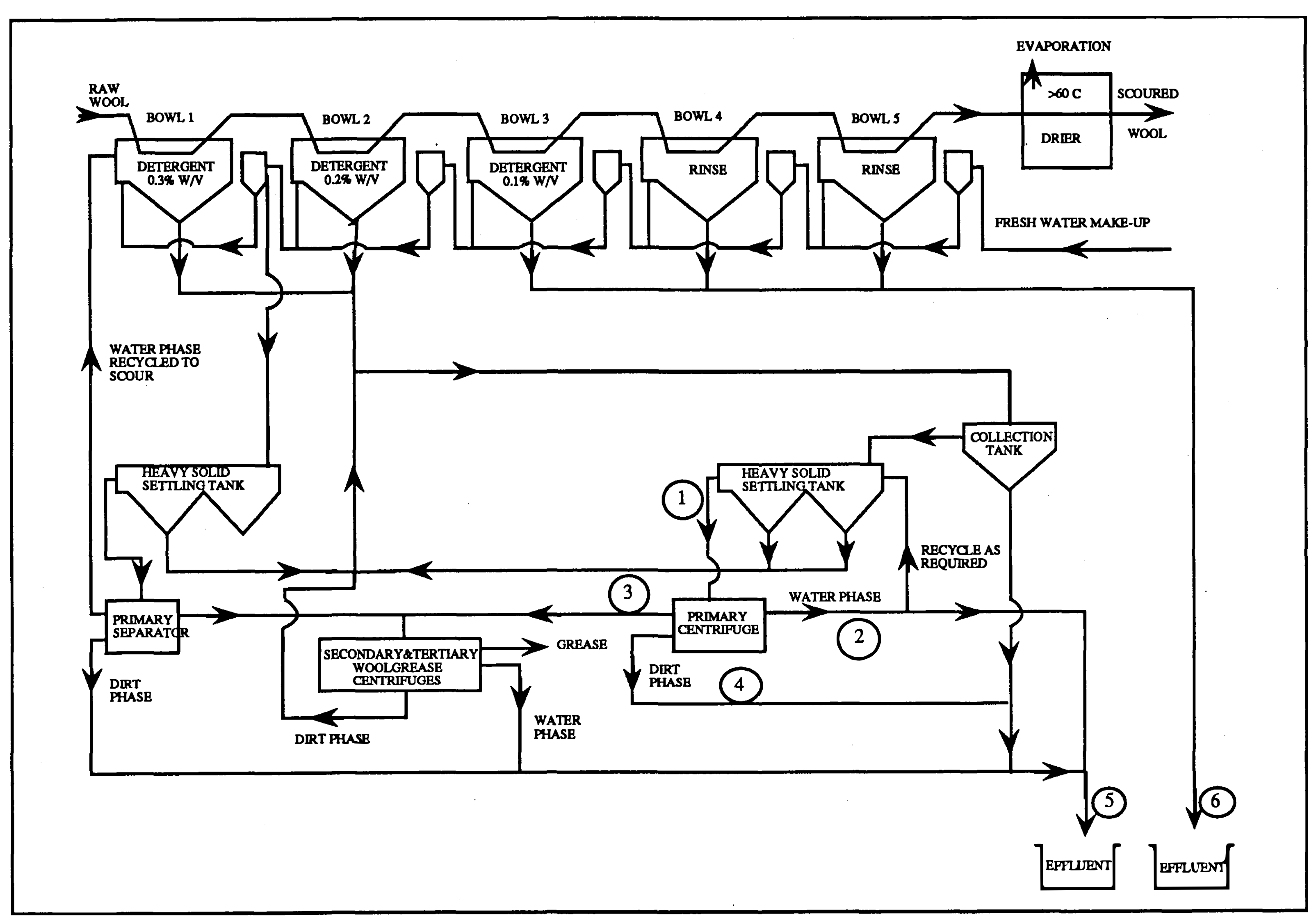


Figure 4.1.1 Schematic diagram of Jandakot wool scouring plant and sampling sites

4.2 Sampling points

Figure 4.1.1 shows the liquid flow in the wool scouring plant. It can be seen that the highly polluted flows from the 1st and 2nd bowls are passed through the in-plant wool grease recovery unit (a series of centrifuges) before discharge (stream 5) to the first anaerobic lagoon, whilst the diluted stream from the rinse bowls is discharged directly (stream 6) to the fourth lagoon. At the primary centrifuge, wool scouring liquor (stream 1) is separated into three distinct phases: a cream phase (stream 3), from which wool grease is recovered; an aqueous phase (stream 2), which is occasionally returned to the scouring machine as required or discharged as an effluent; and a sludge phase (stream 4), which is mixed with the aqueous effluent.

In the scouring plant, samples were taken from six different streams, as shown in Figure 4.1.1, every 2 hours commencing at 10.00 am. to 2.00 pm. on the 10 January 1991, and then preserved according to requirements of particular analyses following Standard Methods (APHA, 1989). Flow rate of the liquor was measured by bucket, measuring cylinder and stop watch, except for stream 1 which was calculated from the rate of stream 2+3+4.

From the anaerobic lagoon, two sludge samples were taken from the opposite sides of the first anaerobic lagoon (the method of collection is described in section 3.2.1) and then mixed well in the bucket before the analysis.

4.3 Mass balance of primary centrifuge in wool scouring plant

It can be seen from Table 4.3.1 and Figure 4.3.1 that liquor from wool washing contained an extremely high contaminant content, especially wool grease and solids. The use of the in-plant centrifuge was found to be able to recover approximately

50% of wool grease (confirmed the average figure of grease recovered by WRONZ system as shown in Table 2.2.1).

Table 4.3.1 Characteristics of liquor streams to and from the primary centrifuge at Jandakot Wool Scouring Plant.

	Flow (l/hr)	TS (g/L)	VS (g/L)	SS (g/L)	COD (g/L)	Grease (g/L)
Stream 1 (wool scouring liquor before being fed to primary centrifuge)						
Time=10.00	6 360	41.9	30.2	23.0	70.0	12.0
Time=12.00	5 130	36.8	24.3	19.7	61.5	11.4
Time=14.00	6 660	41.0	29.0	22.8	65.8	12.1
Average	6 050±570	39.9±2.2	27.8±2.5	21.8±1.5	65.8±3.5	11.8±0.3
Stream 2 (Aqueous phase from primary centrifuge)						
Time=10.00	5 500	29.6	19.2	12.5	39.0	6.9
Time=12.00	4 280	29.9	18.4	12.4	39.0	6.9
Time=14.00	5 670	29.6	18.2	12.6	40.0	6.9
Average	5 150±619	29.7±0.1	18.6±0.4	12.5±0.8	39.3±0.5	6.9±0.0
Stream 3 (cream phase from primary centrifuge)						
Time=10.00	330	122.9	114.2	100.3	536.0	115.9
Time=12.00	230	165.8	155.2	131.9	540.0	128.9
Time=14.00	400	103.6	93.4	77.7	331.0	67.8
Average	320±69	130±26	121±25	103±22	469±97	104±26
Stream 4 (sludge phase from primary centrifuge)						
Time=10.00	530	52.2	32.0	37.9	54.0	7.5
Time=12.00	620	53.9	33.1	36.7	58.0	6.8
Time=14.00	590	52.9	32.2	40.3	56.1	6.2
Average	580±37	53.0±0.7	32.4±0.5	38.3±1.5	56.0±1.6	6.8±0.6

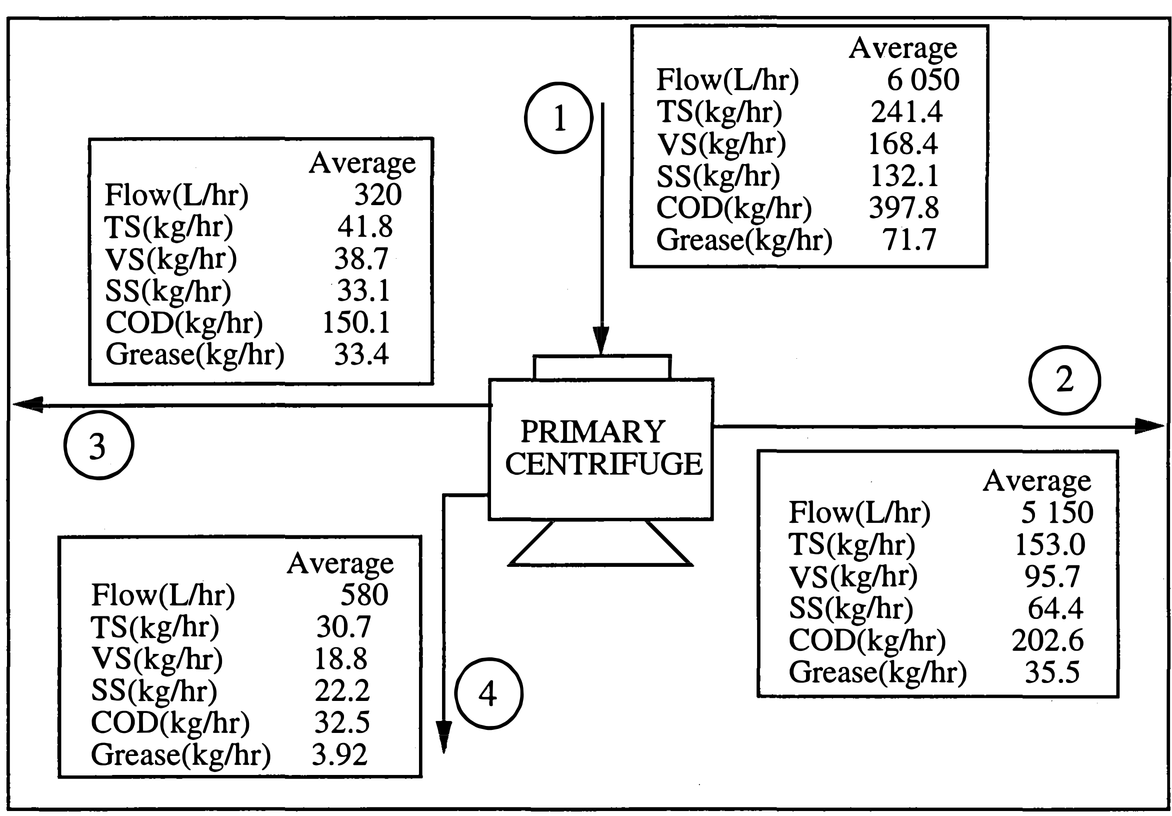


Figure 4.3.1 Liquor-flow diagram and material balance at primary centrifuge at wool scouring plant

Table 4.3.2 presents the material balance around the primary centrifuge. The material balance shows that the agreement between the sum of input and output streams is within 12% which indicates that the sampling and analytical procedures produce acceptable results.

It is noticeable that the input has a higher value than output in most of the parameters. This may be the effect of the flow rate measurement since the flow rate of stream 1 was the estimated figure calculated from stream 2+3+4. The sum of these streams was speculated to give a positive error to stream 1, resulting in an over estimated figure of the stream.

Table 4.3.2. Material balance at primary centrifuge.

Parameters	In put	Out put	% differences
Flow (L/hr)	6 050*	6 050	-
Total solids (kg/hr)	241.4	225.5	-7.9
Volatile solids (kg/hr)	168.4	153.2	-10.8
Suspended solids (kg/hr)	132.1	119.6	-11.2
COD (kg/hr)	397.8	385.1	-4.9
Grease (kg/hr)	71.7	72.8	+1.1

* Assumed equal to sum of out puts.

4.4 Characteristics of the effluent

From Table 4.4.1 it can be seen that although half of the wool grease was recovered by the in-plant centrifuge, the main effluent from the wash stream still contained

remarkably high levels of grease and volatile solids, which resulted in the extremely high COD and BOD_5 values in the waste liquor.

By contrast, the pollutants in the rinse stream were noticeably lower for all parameters, particularly grease which was an order of magnitude less. However, the contaminants in both streams are still so high as to require appropriate treatments before they can be safely discharged to the receiving environment.

Table 4.4.1 Characteristics of wastewater from Jandakot Wool Scouring Plant.

Parameters	Wash stream (No. 5)	Rinse water (No. 6)	Total
Flow rate (L/hr)	12 600	4 693	17 293
Total solids (mg/L)	56 000	8 400	43 082
Volatile solids (mg/L)	33 400	4 300	25 503
Suspended solids(mg/L)	34 800	4 200	26 496
BOD_5 (mg/L)	19 750	2 800	15 150
COD (mg/L)	67 000	9 500	51 396
Grease (mg/L)	8 520	800	6 425

Note that the BOD_5 of WSE accounts for less than one third of the COD value, which fails to estimate the true biological load of the samples (see also section 2.3.6). Therefore grease and COD were mainly used to determine the pollution load of WSE in this study.

Table 4.4.2 compares the characteristics of WSE in the survey to literature values. It can be seen that the total pollution load of WSE in present survey is noticeably lower

than average from the literature, considered that a WRONZ system is employed. The Jandakot wool scourer effluent can be regarded to have an average to low grease content . Although the variation of pollutant concentrations during the survey (Table 4.3.1)was not great (since the same type of wool was scoured at the time), the fluctuation of pollution load can be extreme when different types of wool are scoured. Grease content in the effluent can vary from as high as over 20 g/L to as low as 5 g/L depending on the quality of wool being scoured (John Sheehan, personal communication). As discussed in section 2.3, apart from the scouring process, the characteristics of WSE depend greatly on the type and quality of wool, therefore the fluctuation of pollution load within the same scouring plant cannot be avoided. Any proposed treatment process, therefore, should be flexible enough to cope with the extreme ranges in the quality of this effluent.

Table 4.4.2 Comparison of effluent characteristics of the present survey to other authors.

Parameters	Present survey			Townsend,	Genon,	Cail,	Christoe,
	Wash	Rinse	Total	1989	1984	1986	1976
Total solids (mg/L)	56 000	8 400	43 082	10 000-30 000	-	-	10 000-80 000
Volatile solids (mg/L)	33 400	4 300	25 503	-	-	-	-
Suspended solids (mg/L)	34 800	4 200	26 496	-	15 000	9 000	3 000-8 000
BOD_5 (mg/L)	19 750	2 800	15 150	-	22 000	8 000	3 000-25 000
COD (mg/L)	67 000	9 500	51 396	50 000	45 000	25 000	15 000-120 000
Grease (mg/L)	8 520	800	6 425	10 000-20 000	5 000	7 000	3 000-10 000

4.5 Anaerobic lagoon treating WSE

At the Jandakot wool scouring plant, a series of 15 anaerobic-facultative lagoons are employed to treat the effluent. The first four lagoons were designed to receive the effluent from the wash stream (No. 5), which contained an extremely high pollution load, particularly solids and grease as discussed in section 4.4. These lagoons were believed to be anaerobic since the surfaces were almost completely sealed by a layer of wool grease and other floating materials. Rinse water (stream 6) from the plant was discharged to the fifth lagoon, combined with the overflow from the fourth lagoon, and then further treated through another 11 lagoons. No scum was observed on the surface of lagoons 5 to 15, which were hence considered to be facultative.

The efficiency in terms of grease and COD reduction is shown in Table 4.5.1. Under anaerobic conditions (the first 4 lagoons), about 80% of grease was removed from the liquor at the residence time of approximately 18 days. Further purification by the facultative lagoons reduced grease and COD to less than 5% of the original contaminant levels. Grease content in the last lagoon averaged 200-300 mg/L.

To investigate whether the removal of grease was due to complete degradation or partly a result of flocculation, sludge samples from the bottom of the first lagoon were examined.

Table 4.5.1 The efficiency of the lagooning system at Jandakot wool scouring plant.

	The first 4 lagoons	Total of 15 lagoons
HRT (days)	18	>45
% grease reduction	78-80	95-98
% COD reduction	80	95

Table 4.5.2 shows the characteristics of the sludge sampled from the bottom of the first anaerobic lagoon compared to a typical anaerobic sludge sampled from the sewage treatment plant. Since there is no mixing in the lagoon, a large proportion the non-dissolved materials had settled to the bottom of the lagoon forming a thick layer of sludge which contained a rather high total solid content with a high percentage of inorganic solids.

Grease content of this lagoon sludge was as high as 36.4 g/L which comprised about one third volatile suspended solids, accounting for more than half (64%) of the total VSS. The accumulation of grease in the sludge was speculated to be the result of wool grease emulsions which had settled during the treatment process. Thus the high efficiency of lagooning in the treatment of WSE, as shown in Table 4.5.1, was the result of not only biodegradation but also partial flocculation of the wool grease emulsion.

Table 4.5.2 Characteristics of anaerobic sludge from the bottom of the first anaerobic lagoon at Jandakot wool scourer, compared with anaerobic sludge from a sewage treatment plant.

Parameter	Lagoon sludge	Sewage sludge
Total solids (g/L)	322.1	23.6
Volatile solids (VS) (g/L)	93.2	17.2
Grease (g/L)	36.4	1.2
% Water content	73.0	97.9
% Grease in VSS	39.0	7.0
Grease : VSS	1 : 1.6	1 : 13.3

4.6 General discussion

The results of this survey present the current flow of contaminants in the wool scouring liquor streams of a wool scouring plant effluent treatment process. The use of in-plant centrifugation was found to remove approximately 50% of wool grease from the wash stream liquor. In the anaerobic lagoons, 80% of wool grease from the effluent could be removed within the 18 day residence time, and a further 15% can be removed within the facultative lagoons.

It is interesting to compare these results to those obtained from the anaerobic bioflocculation processes presented in section 5.3 and section 7, in which the same grease removal result was obtained with a considerably shorter residence time of 4 days. This can potentially reduce the size of the required treatment facility to less than a quarter of that currently used, and therefore reduce the capital cost of treatment facilities.

It should be noted that the segregation of the rinse stream from the wash stream, as practised at the Jandakot plant, for separate treatment can considerably improve the performance of the proposed bioflocculation process. Since destabilisation of wool grease emulsion was enhanced by increased concentration of the liquor (Cord-Ruwisch *et al.*, 1990; Wood *et al.*, 1979), the concentrated wash stream would be appropriately treated by anaerobic bioflocculation. The rinse stream may be discharged with the effluent from the anaerobic bioflocculation system to the aerobic post-treatment system. This would considerably reduce the amount of influent to the bioflocculation system, resulting in a corresponding cost reduction of treatment facilities.

Chapter 5 Investigation of bioflocculation mechanisms by batch experiments

5.1 Introduction

The flocculation of wool grease emulsion in WSE was clearly observed in aerobic treatment processes (Cord-Ruwisch *et al.*, 1990; Roth *et al.*, 1989). However, the basic mechanism of the process was not yet clear. In the aerobic process, Poole (1991) suggested bacterial cell adherence to the grease emulsion resulting in the flocculation of bacteria and grease. In anaerobic processes treating WSE, all studies (see section 2.4.3.2) reported grease and COD removal efficiency based on the difference between grease in the influent and effluent without determining whether the removal of grease was due to purely oxidation or partly flocculation and adsorption onto biological sludge. Therefore, the bioflocculation of wool grease emulsion which may have taken place concurrently had been overlooked.

This chapter aims to investigate the destabilisation/flocculation of wool grease emulsion by anaerobic microbial activities. This involved a series of eleven batch experiments which were conducted sequentially as discussed in section 5.2 to 5.12. General microscopy and viable bacterial counts were employed to estimate bacteria populations (see section 3.4.3).

5.2 Confirmation of bioflocculation as a biological process

Although wool grease emulsions were found to flocculate under anaerobic conditions as observed in anaerobic lagoons, there was no evidence as to the mechanisms of this process. Since emulsions can be destabilised in a number of

ways (see section 2.3.4.4), the first experiment was designed to investigate whether grease removal under anaerobic condition was due to:

(1) degradation (converted to CO_2 + CH_4) or flocculation,

(2) if flocculation took place, what the mechanisms would be (physico-chemical adsorption/flocculation to the anaerobic sludge or the biological activities of anaerobic microbes on the wool grease emulsion).

This experiment therefore involved the separate monitoring of grease reduction achieved using both viable and dead anaerobic sludge.

Experimental set up

The experiment was set up at ambient temperature using four of the mixed batch reactors as described in section 3.3.1. Two reactors were filled with 75 ml of anaerobic sludge (see section 3.2.1) and 75 ml WSE (see section 3.2.3). As control, another two reactors were filled with the same mixture, except the anaerobic sludge was sterilised before use by autoclaving at 132 °C for 15 min. To inhibit bacterial activity 150 µg/L of penicillin was added in this trial.

After air in the head space was displaced by nitrogen gas, all reactors were completely sealed and placed on a Coulter mixer for gentle mixing. This operated automatically for 15 min/hr, enough mixing time for settled sludge to come in contact with WSE, with the settling time allowing flocculated grease to form larger flocs and settle with the sludge.

A sample of 20 ml mixed liquor was taken from each reactor, initially after 15 min of mixing and then daily for 5 days. Low gravity centrifuging (105 G for 5 min) was used immediately to separate the bulk sludge from WSE.

The centrifuged supernatant was then divided into two portions. The first portion was analysed for total grease content while the second portion was kept undisturbed for 24 hrs to observe any further flocculation. The supernatant of the second portion was then withdrawn and analysed for grease content.

The gas volume produced from each reactor was measured daily by water displacement (Figure 3.3.1.3).

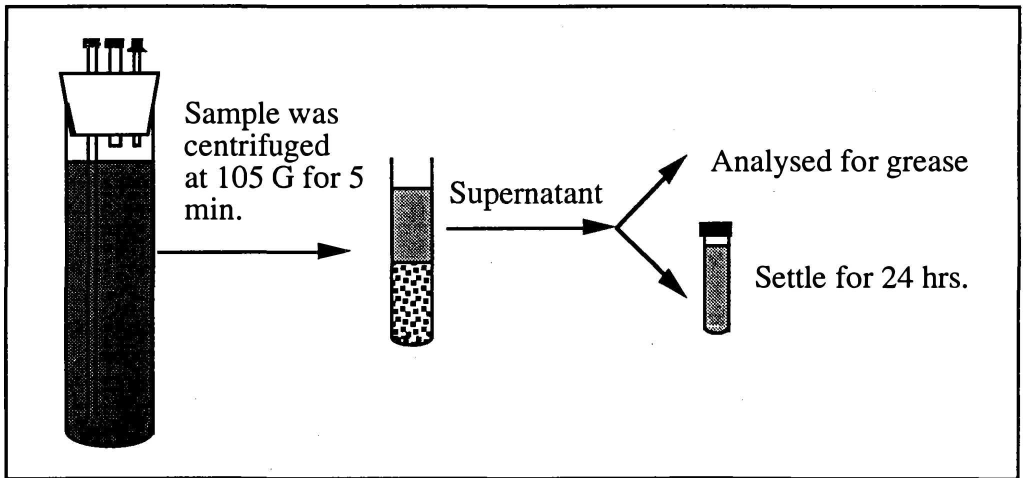


Figure 5.2.1 Schematic diagram of the experiment section 5.2

Results and Discussion

Table 5.2.1 presents sludge and WSE characteristics used in the experiment. The grease content of the initial digested sludge was less than 10% of the WSE. After mixing the sludge with WSE in equal amounts, grease content in the mixed liquor was 5.8 g/L at the start of the experiment.

Table 5.2.1 Characteristics of anaerobic digester sludge and WSE used.

	Total solids g/L	Volatile solids g/L	Grease g/L	% Grease in total solids
Sludge	23	17	0.9	4
WSE	36	22	10.8	30

During mixing for 15 minutes, some of the grease in the WSE was reduced by adsorption or destabilisation/flocculation, decreasing the emulsified grease level from 5.9 g/L to 4.9 g/L and 4.1 g/L in the sterilised and non-sterilised systems respectively (Figure 5.2.2). This immediate reduction of grease emulsion from WSE was postulated to be the result of adsorption of grease emulsion to the sludge phase rather than by biological destabilisation.

The active sludge showed a higher capacity to adsorb grease from WSE resulting in the lower grease content in the supernatant than that from the sterilised system during the five day period of the experiment (Figure 5.2.2). Grease in the supernatant from the system with active sludge gradually decreased from 4.1 g/L at day zero to 2.4 g/L at day five, or 41.5% grease reduction. In the sterilised system grease in the supernatant decreased only slightly from 4.9 g/L at day zero to about 4.2 g/L at day five (14.3% grease reduction).

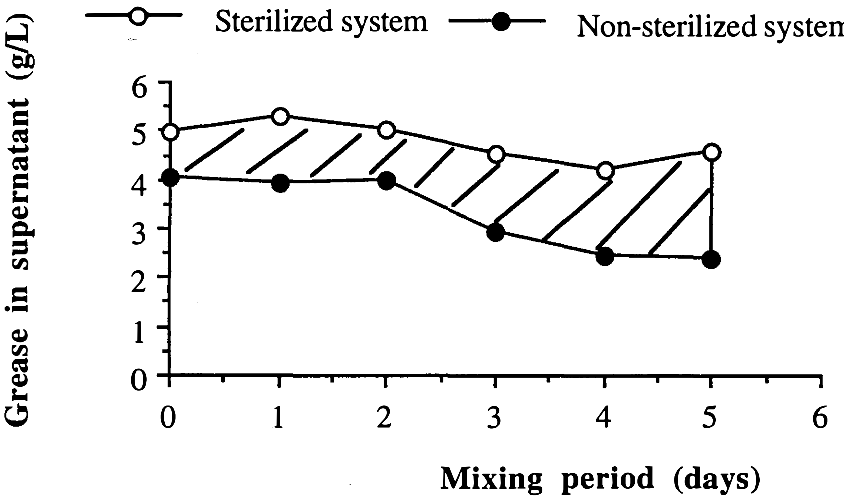


Figure 5.2.2 Removal of emulsified wool grease from the supernatant of WSE obtained by viable and sterilised anaerobic sludge after a period of mixing

It was further observed that there were interactions between active anaerobic sludge and WSE which resulted in the destabilisation of the emulsified wool grease in the WSE. After the bulk sludge was removed from the mixed liquor by low gravity centrifugation (105 G for 5 min), floc formation was clearly observed in the supernatant when it was left undisturbed for a further 24 hrs. The flocs increased in size and finally settled resulting in clarification of the supernatant due to the further removal of emulsified grease (Figure 5.2.3). Microscopical examination showed the formation of flocs of grease emulsion, dirt, bacterial cells and possibly other contaminants in WSE (Figure 5.2.4).

About 65% of grease was removed from the supernatant by biologically active anaerobic sludge (adsorption and flocculation), while approximately 20% could be accounted for by physical adsorption to and flocculation by the sterilised sludge. Microscopic examination of the samples revealed the development of large numbers

of motile and non motile bacteria (approximately 10^8-10^9 cells/ml) in the supernatant of WSE flocculated by biologically active sludge.

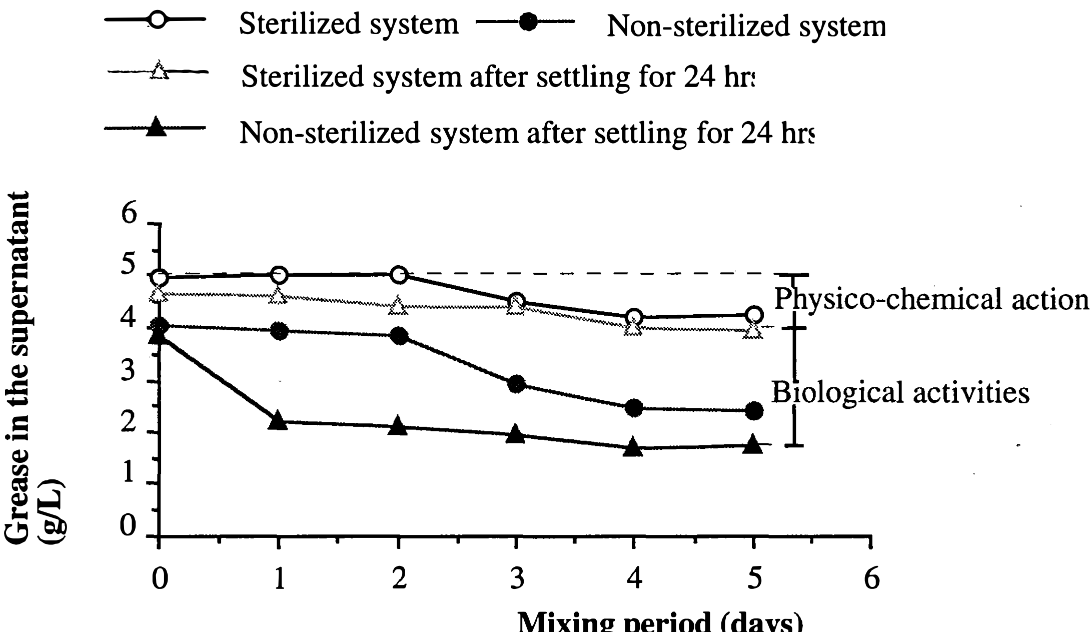
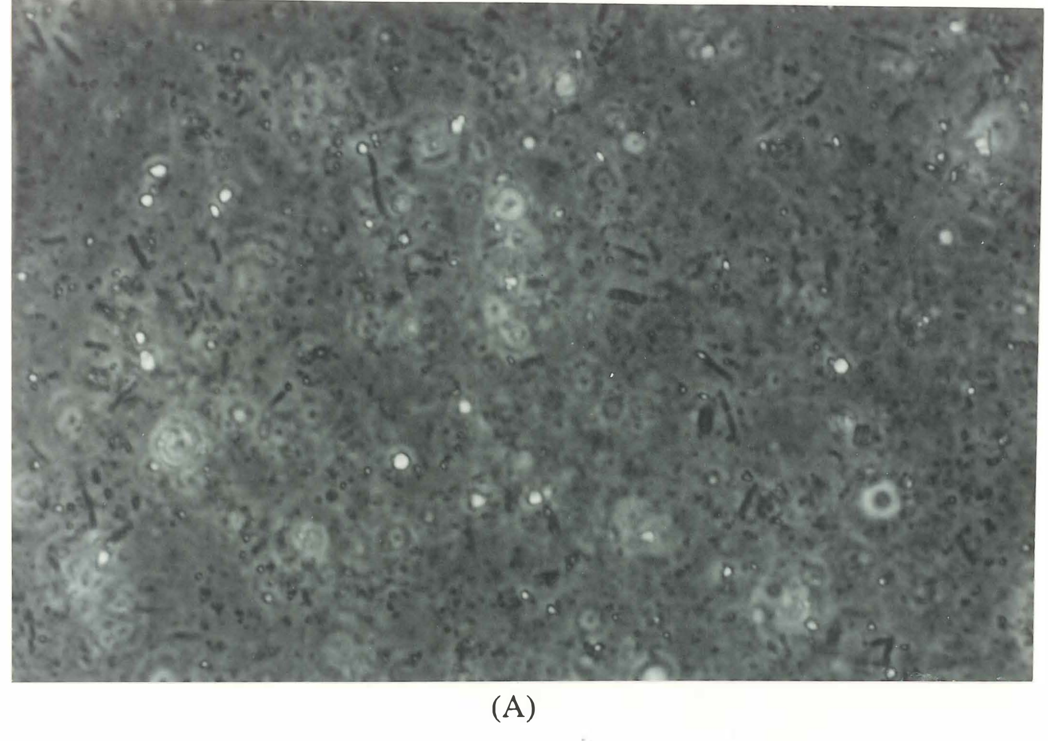
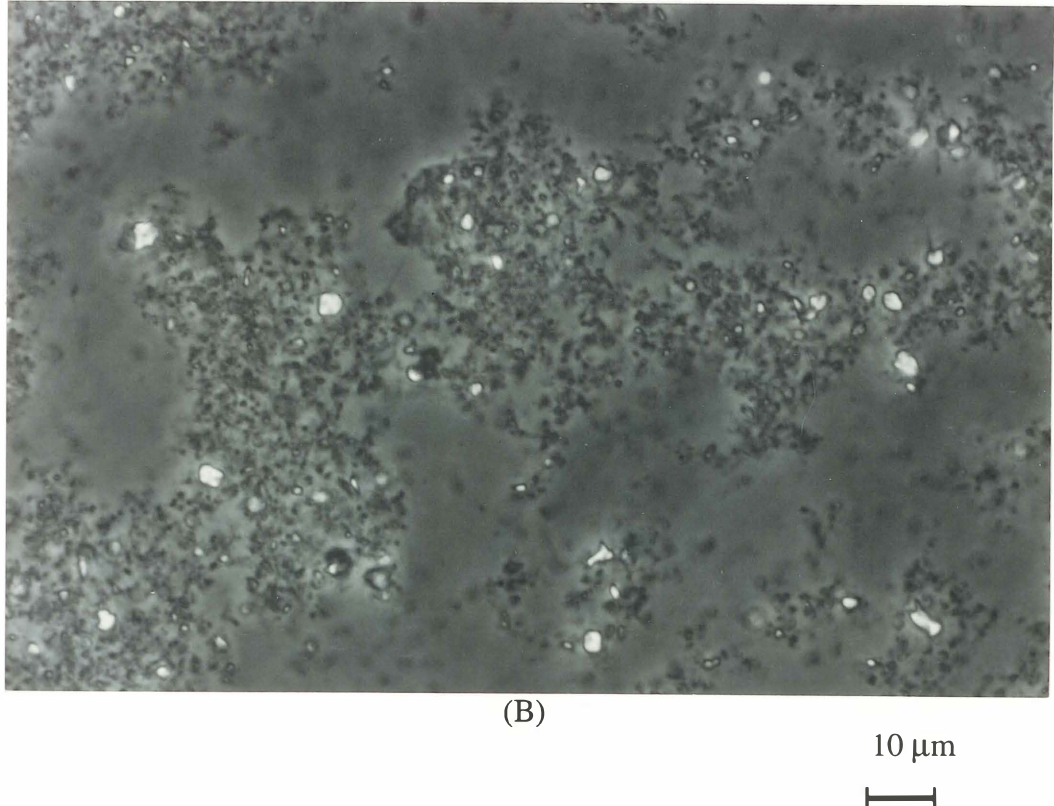


Figure 5.2.3 Removal of emulsified grease by flocculation in the supernatant incubated with active and sterilised sludge



(A)



(B)

10 μm

Figure 5.2.4 Photos show the formation of flocs during anaerobic bioflocculation process; (A) WSE before anaerobic bioflocculation process; (B) after anaerobic bioflocculation process

Thus the flocculation of wool grease is mainly due to biological activity rather than physical or chemical interactions between anaerobic sludge and WSE.

The total mass balance showed no significant degradation of grease and other organics as measured by volatile solids (Table 5.2.2). Hence the activity of anaerobic bacteria did not cause any significant biodegradation but merely a transfer of grease from the emulsified form into the sludge phase (Figure 5.2.3), resulting in grease reduction in the supernatant. This is supported by the fact that gas production was negligible (Figure 5.2.5). Only 0.13 L of gas was produced per litre of sludge over the five days. Assuming 50% CH_4 content in the gas this would account for approximately 0.17 g of COD (based on 1 g COD producing 382 ml CH_4 at 20 °C). In WSE, about 1.4 g COD corresponded to 1 g volatile solids (Cord-Ruwisch *et al.*, 1990). Accordingly, over the experimental period of five days only 0.12 g of volatile solids were converted into biogas.

Table 5.2.2 Characteristics of mixed liquor of WSE and anaerobic sludge before and after 5 days incubation.

Mixed liquor from	Total solids g/L	Volatile solids g/L	Grease g/L
Before incubation	29.5	19.5	5.8
After incubation for 5 days			
- Sterilised system	29.6	19.8	5.3
- Non-sterilised system	30.3	20.4	5.5

Table 5.2.3 Distribution of grease in 3 different fractions of the mixed liquor of WSE after anaerobic flocculation for 5 days.

	Mixed liquor (g)	Supernatant (g)	Sludge (g)
Sterilised system	5.3	4.5	0.8
Non-sterilised system	5.5	2.4	3.1

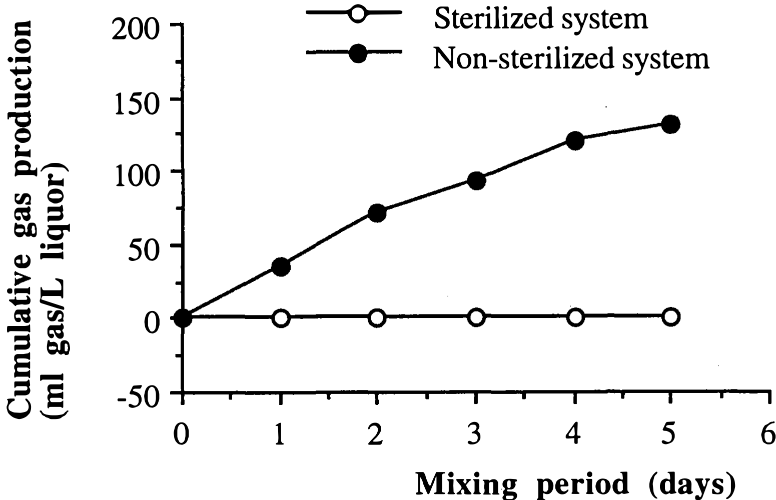


Figure 5.2.5 Gas production during bioflocculation process using active and sterilised sludge

5.3 The effect of mixing and settling periods on grease removal

From the previous set of experiments, it was shown that viable anaerobic bacteria caused grease flocculation during an appropriate mixing period and also after mixing during a settling period. Since mixing requires energy and a longer settling period means larger sedimentation tanks, to minimise operation cost, this experiment was

designed to determine the minimum required length for mixing and settling periods of this process. This information would then be used in subsequent experiments.

Experimental set up

The experiment was set up in a similar way to that in section 5.2. At ambient temperature, twelve batch reactors (see section 3.3.1) were initially set up by mixing 75 ml of anaerobic sludge with 75 ml WSE. After air in the head space was displaced by nitrogen gas, all reactors were completely sealed and placed on a Coulter mixer for gentle mixing.

The two reactors were sampled after 15 min of mixing and then daily for 5 days. The mixed liquor was centrifuged at 105 G for 5 min, then 20 ml of the supernatant was taken for grease analysis. The rest of the supernatant was divided into 5 portions in different capped test-tubes (Figure 5.3.1). After displacing the head space by nitrogen gas, these test-tubes were sealed and left undisturbed for a period of 1-5 days to observe further flocculation as a function of settling time. Each day one test-tube was taken and the supernatant was analysed for grease content.

Since gas production was found to be negligible in the previous experiment, no gas measurement was performed in this and subsequent experiments. Duplicated controls used distilled water instead of sludge.

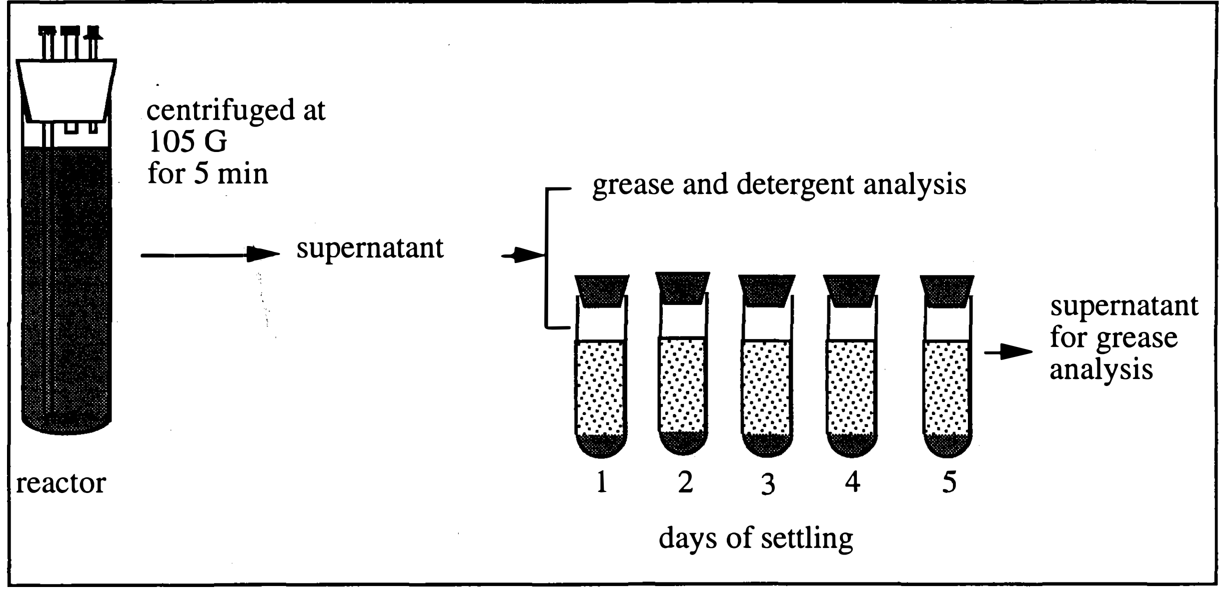


Figure 5.3.1 Schematic diagram of the experiment section 5.3

Results and Discussion

Figure 5.3.2 (a) and (b) present grease flocculation as a function of the mixing time and settling time respectively. In the control where WSE had no previous contact with anaerobic sludge, the grease content was almost constant during the settling period of 5 days. In comparison, when the WSE had been mixed with anaerobic sludge and then followed by settling, the floc formation was clearly visible, even after a contact time of only 15 min. Increasing the mixing time to 2 days greatly increased grease removal (Figure 5.3.2 a). Further increasing mixing to more than two days, however, did not increase flocculation significantly. This suggests that the mixing period of the sludge with WSE provided contact time for anaerobic bacteria to adsorb some of the grease. Since prolonging of this period did not significantly increase the reduction of grease, this step was believed to be rate limiting. That is, only limited amounts of emulsified grease can be adsorbed by the sludge. The settling period provided appropriate time for destabilised grease to form clusters which then settled by gravity. During the settling period high numbers of anaerobic

bacteria (~ 10^9 cells/ml) developed in the supernatant. It was therefore speculated that the settling process not only represents physical gravity separation of flocs formed, but could also involve the biological activity of anaerobic microbes. From the results of this experiment, it can be deduced that the destabilisation and flocculation of wool grease requires less mixing, but longer settling time, during which time there is considerable anaerobic microbial activity.

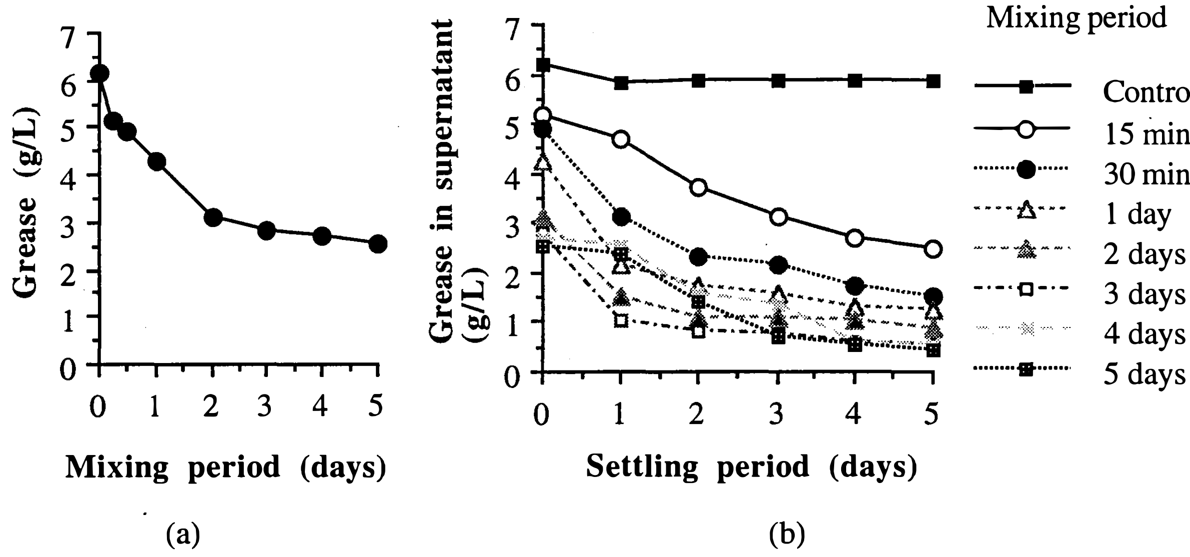


Figure 5.3.2 Grease content in the supernatant after (a) mixing alone and (b) mixing plus settling, during the period of experiment

At the beginning of this experiment, the grease content in the mixed liquor was 6.2 g/L. As shown in Figure 5.3.2 (b), it reduced to 1.7 g/L after 1 day of mixing and 2 days settling, similar to the grease content (1.5 g/L) in the supernatant after 2 days of mixing and 1 day settling. This suggests that to obtain approximately 75% grease flocculation, a total treatment time of three days would be required (two days mixing and one day settling, or one day mixing and two days settling). A reduction of 90% grease from the supernatant can be obtained with a much longer period of 8-9 days

(Figure 5.3.2b). In comparison to the anaerobic lagooning system (80% grease reduction at HRT of 18 days- see section 4.5), the efficiency of this process was found to be much greater.

5.4 The effect of temperature on grease removal in anaerobic bioflocculation process

From section 5.2 and 5.3, it was clearly shown that the destabilisation and flocculation of grease emulsion from WSE was due to some activities of anaerobic microbes, particularly during the settling period where high numbers of bacteria developed. Biological processes normally require an optimum temperature for maximum reaction rate on cell growth. In anaerobic digestion, the rate of the process is greatly influenced by temperature. The microbial activity for the degradation of organic matter increases with increasing temperature until the optimum value is attained (Forday & Greenfield, 1983).

Customarily, the bacteria are divided into three categories according to the temperature at which they experience their shortest generation time; (i) psychrophiles grow well at a low temperature range of 0 °C to 20°C; (ii) mesophiles thrive at the middle range of 20 °C to 40°C; and (iii) thermophiles multiply best at high temperatures of 40 °C to 90 °C (Alcamo, 1990).

Although the anaerobic bioflocculation process relies on anaerobic microbes, it is not known whether the optimum temperature for these microbes is the same as anaerobic digestion generally. Thus an experiment was designed to determine the optimum temperature for this process.

Experimental set up

Ten batch reactors (see section 3.3.1) were initially set up by mixing 75 ml of anaerobic sludge (biomass concentration of 17 g/L) with 75 ml WSE. Duplicated controls were provided using sterilised distilled water instead of sludge. After air in the head space was replaced by nitrogen gas, all reactors were completely sealed and placed on a Coulter mixer for 30 min. The mixed liquor was then divided into 5 portions in different capped test-tubes. After the head space was replaced with N_2, these test-tubes were sealed and left undisturbed for a period of 1-5 days to observe flocculation at different temperatures (10 °C, 20 °C, 30 °C, 40 °C, and 50 °C, duplicate trial at each temperature). Each day one test-tube from each trial was taken and the supernatant was analysed for grease content.

Results and discussion

Figure 5.4.1 compares grease reduction in the supernatant of WSE during the bioflocculation process at different temperatures. Anaerobic bioflocculation did not take place at 10 °C. At 20 °C only about 20% grease flocculated. At temperatures of 30 °C or over, around the optimum temperature for mesophilic anaerobic microbes, the flocculation of wool grease was clearly observed.

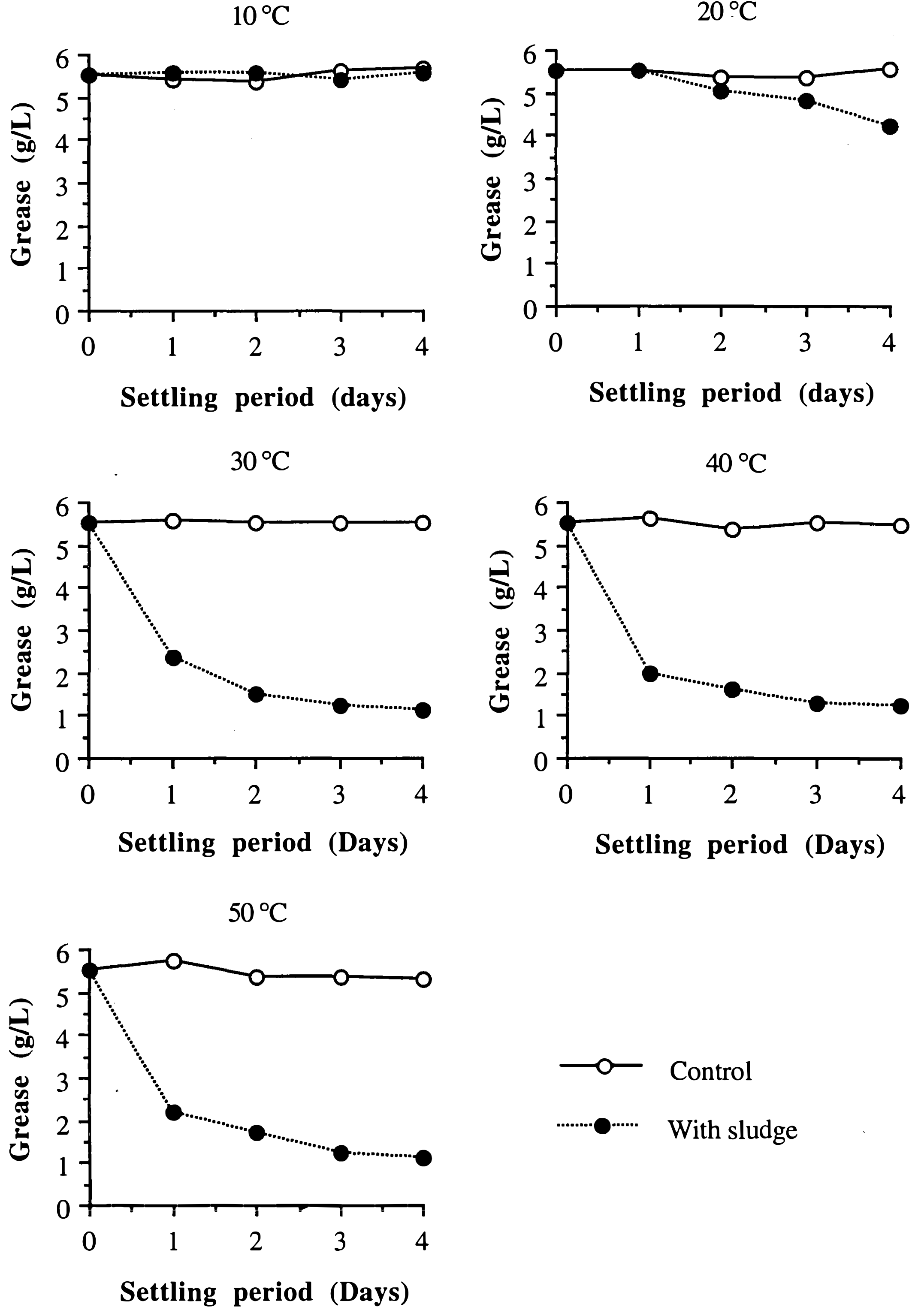


Figure 5.4.1 Grease content in the supernatant of WSE at different temperatures

(50% inoculum, 30 min gentle mixing)

Increasing the temperature over 30 °C did not increase the bioflocculation efficiency, as the same amount of wool grease was destabilised from the liquor (expressed as the same grease content in the supernatant in Figure 5.4.2). However, at higher temperatures the total volume of precipitated grease was found to be smaller (Figure 5.4.3; note that at 20 °C no clear boundary between sludge and liquor phase was observed). It is postulated that the greater compaction of sludge (flocculated grease) obtained is the result of the physical acceleration of sludge settling at the higher temperatures rather than increased microbial activity. The melting point of wool grease is about 40 °C and above this temperature it could be expected that upon settling the mixture of grease and dirt would occupy a smaller volume.

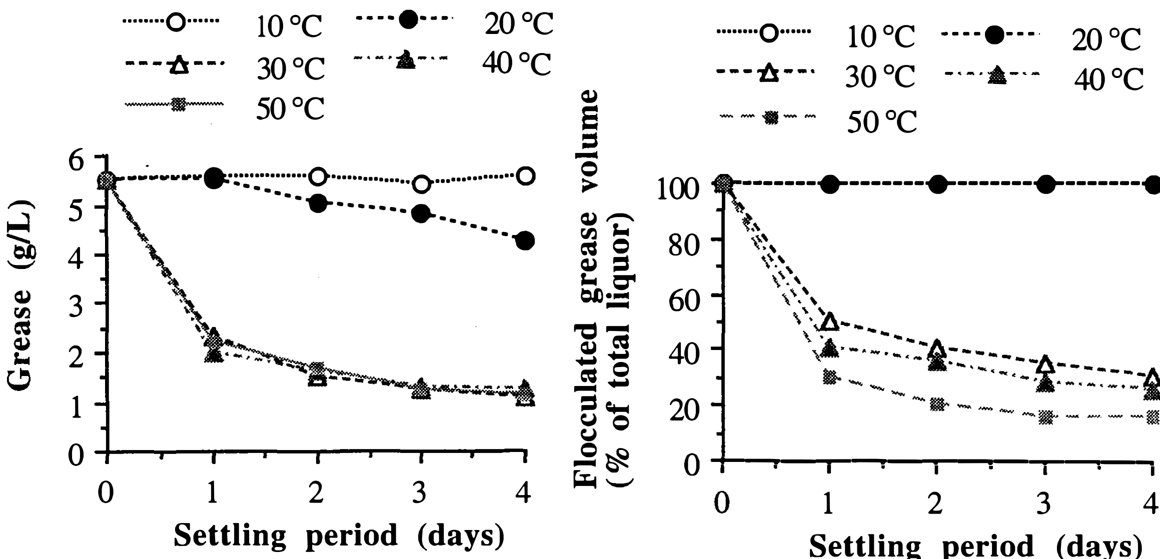


Figure 5.4.2 The effect of temperature on anaerobic grease flocculation in WSE

Figure 5.4.3 Comparison of flocculated grease volumes at different temperatures

As with other biological treatment processes, the sludge settling and compaction characteristics during the bioflocculation process are considered to be important factors affecting successful operation (Eckenfelder, 1980). In a continuous system, a

poor settling of sludge results in solids carryover in the effluent which will contribute to increased BOD and COD. The poor compaction of sludge normally results in increased difficulty and thus an expensive sludge dewatering and disposal procedure.

In the anaerobic bioflocculation process, the compaction of sludge was found to vary between 85% to 92% depending on the operating temperature (Table 5.4.1). It was found that the higher the operation temperature, the more compact the sludge. With an increase in the operating temperature of 20°C (from 30°C to 50°C), water content in the sludge could be reduced from 92% to only 85.7%. This sludge however still contained too high a water content and would require some form of sludge dewatering before disposal.

In comparison to the sludge from the anaerobic lagoon which contained only 73% water (Table 4.5.2), the water content in the sludge from the anaerobic bioflocculation process was found to be considerably higher. This could be due to a much longer settling period in the lagoon compared to only 4 days in the experiment.

Table 5.4.1 Characteristics of bioflocculated sludges which had been incubated at different temperatures.

Incubating Temperature	Grease (g/L)	TS (g/L)	% Water content
30 °C	14.8	85.3	92.8
40 °C	17.7	102.4	91.4
50° C	29.5	170.7	85.7

It should, however, be noted that longer sludge residence times could result in variations in steady-state acclimatised cultures at each temperature (Eric Hall,

personal communication) . It is possible that the results presented are transient responses of single mesophilic cultures, and should be interpreted with this in mind.

5.5 The role of solid and liquid phase of anaerobic sludge on the bioflocculation process

In the experiment presented in section 5.3 the flocculation of wool grease, which took place during the settling period, occurred even if the bulk biomass was removed after a short contact time with anaerobic sludge. In contrast to other biological (aerobic and anaerobic) digestion processes where the amount of biomass and contact time between biomass and substrate normally dictate the effectiveness of the digestion process (see section 2.4.3), the effectiveness of anaerobic bioflocculation did not greatly improve with an increased contact (mixing) period. This suggested that the bulk of the anaerobic digestion sludge added may not be necessary for bioflocculation.

This experiment aimed to determine the importance of maintaining high biomass concentration in the system (i.e. whether the bulk of anaerobic biomass was necessary, or a smaller inoculum of anaerobic bacteria could also cause grease flocculation). This is important for the development of a feasible biological treatment of WSE. The experiment was performed by (i) reducing the amount of sludge inoculum, and (ii) separating the sludge liquor which still contained suspended bacteria from the bulk anaerobic flocculated biomass by mild centrifugation. The capacity of these two fractions to cause grease flocculation was compared.

Experimental set up

As shown in Figure 5.5.1, anaerobic sludge was centrifuged at 105 G for 5 min. The supernatant (**liquid phase**), which contained mainly sludge liquor and suspended microbes, was withdrawn from the bulk biomass (**solid phase**). Deoxygenated sterilised distilled water was used to make up the volume of liquid and solid phase to the original volume of total sludge (to standardise the final volume of all trials) before being used to flocculate WSE.

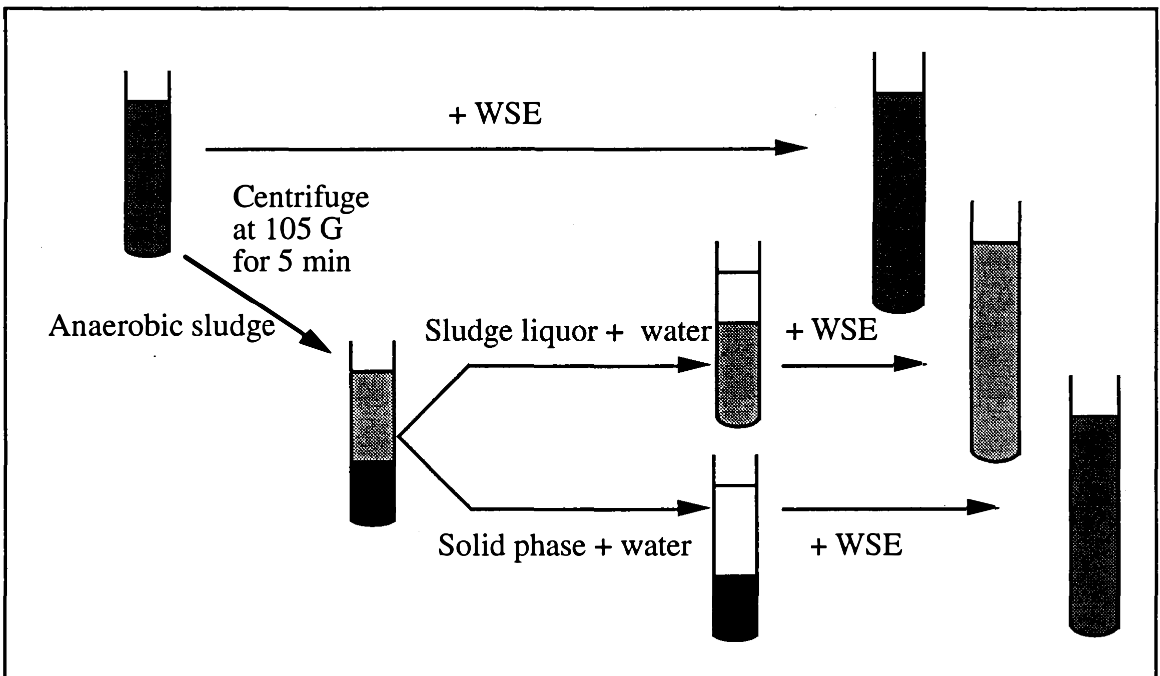


Figure 5.5.1 Schematic diagram of experimental set up of section 5.5

In this experiment, 70 ml test-tubes were used as reactors (8 reactors per trial). The **total sludge**, the **solid phase** only, and **liquid phase** only of the same sludge (Figure 5.5.1) were added to WSE at the proportion 25% and 50% sludge or its equivalent solid phase and liquid phase.

All vessels had head space replaced by N_2, were capped, and then mixed (on a Coulter mixer) for 30 min before being incubated at 30 °C. Two reactors from each trial were taken daily (for 4 days) to analyse grease content in the supernatant, with their bioflocculation ability compared to a control trial (distilled water).

To determine grease degradation during the flocculation period, an extra duplicate trial of **total sludge** was added to the experiment. Two vessels of this trial were taken daily for analysis of grease in the mixed liquor. This was achieved by vigorously mixing the liquor for 5 min before samples were taken for grease analysis.

Results and discussion

Figure 5.5.2 and 5.5.3 compare the effectiveness of anaerobic bioflocculation under varying doses of anaerobic digestion sludge. Although the flocculation of grease in WSE increased with the increase in sludge inoculum, only approximately 10% difference in grease reduction between the 50% and 25% inoculum trials occurred. Thus unlike the anaerobic digestion, where the quantity of biomass determines the effectiveness of the process, the efficiency of bioflocculation was not greatly influenced by the amount of anaerobic bacteria added as inoculum. On this basis, it may be deduced that anaerobic bioflocculation is a rate limiting process. Only a certain amount of grease emulsion in WSE can be easily destabilised with subsequent flocculation. The increase of another 100% sludge inoculum did not increase the process efficiency proportionally.

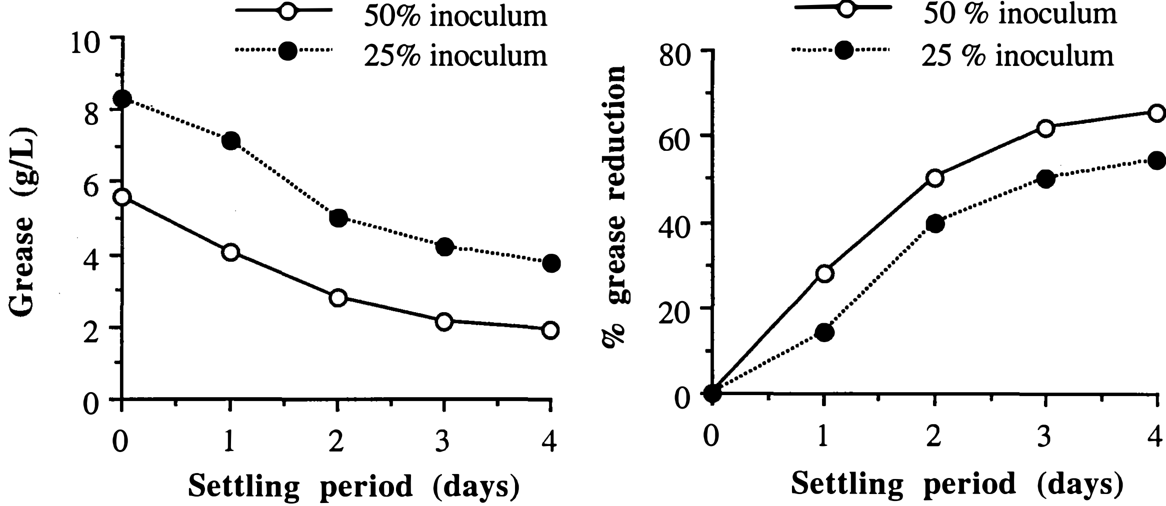


Figure 5.5.2 Grease content in the supernatant of WSE from the trial using 50% sludge inoculum compared to 25% sludge inoculum

Figure 5.5.3 % Grease reduction in the supernatant of WSE from the trial using 50% sludge inoculum compared to 25% sludge inoculum

Absence of biodegradation was also confirmed by monitoring grease content in the mixed liquor (supernatant and flocculated grease), and comparing this to the grease in the supernatant only (Figure 5.5.4). It is clear that during the process of bioflocculation there was no significant degradation of wool grease. The grease was simply flocculated and settled, thus a lower grease content was obtained in the supernatant.

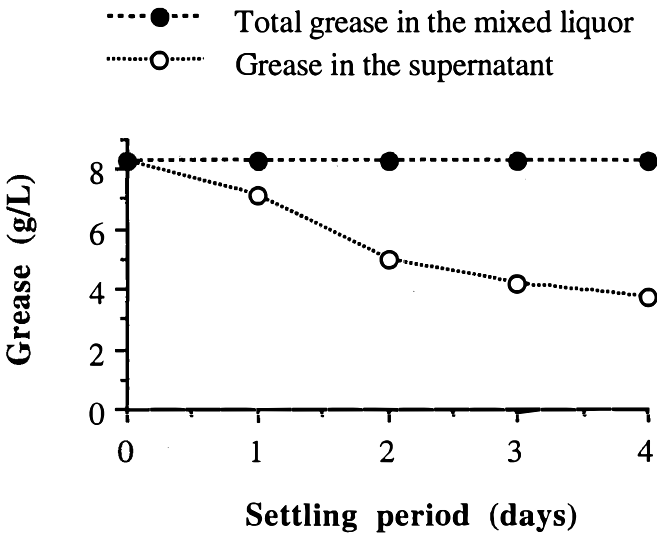


Figure 5.5.4 Emulsified grease content in the supernatant compared to total grease (emulsified grease plus flocculated grease) in the mixed liquor during the bioflocculation process

The liquid phase from fractionated anaerobic sludge, with only a small amount of suspended anaerobic micro-organisms, was more efficient in causing the flocculation of WSE (Figure 5.5.5 and 5.5.6). The flocculation caused by the bulk of the biomass (solid phase) was slower (Figure 5.5.7 and 5.5.8) and less complete than flocculation by the liquid phase. The difference between solids and liquid seems to indicate the presence of extra-cellular proteins which contribute to the bio-coagulation /flocculation. Figure 5.5.6 also supports the biopolymer theory as coagulation/flocculation was seen to increase using solids only when solids were allowed to metabolise. However, the process is also affected by the presence of bulk sludge (synergistic effect) as shown by the highest efficiency with total sludge (Figure 5.5.5 and 5.5.6).

A general conclusion from this experiment is that successful bioflocculation does not require a high concentration of bacteria as the inoculum. Thus the bulk biomass, which is normally required in an anaerobic digestion process, was not necessary in the flocculation process. The liquor of the sludge was more important in this process. Since the liquor phase of anaerobic sludge contains (i) suspended anaerobic microbes and (ii) the sludge liquor, it can be hypothesised that:

(i) the flocculation of grease emulsion was due to some chemical substances such as bioflocculant (biopolymer) in the liquor (see section 2.4.3.3). (Note that these substances were destroyed during autoclaving, as evident by the lack of flocculation ability in section 5.2.)

or (ii) the flocculation of grease emulsion was due to some specific anaerobic microbes suspended in the sludge liquor.

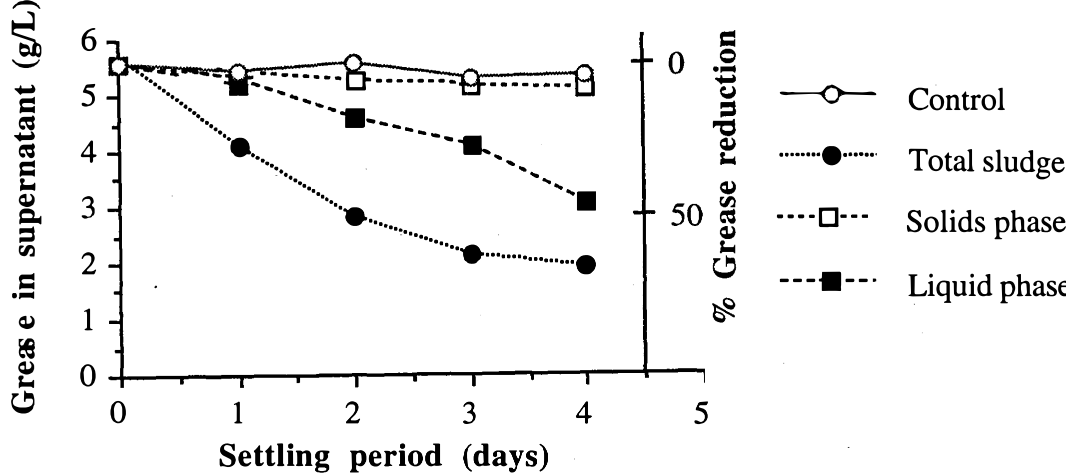


Figure 5.5.5 The effect of sludge fractions (total sludge, solid phase, and liquid phase) on anaerobic grease flocculation in WSE (**50% inoculum**, 30 min gentle mixing, incubation temperature 30 °C)

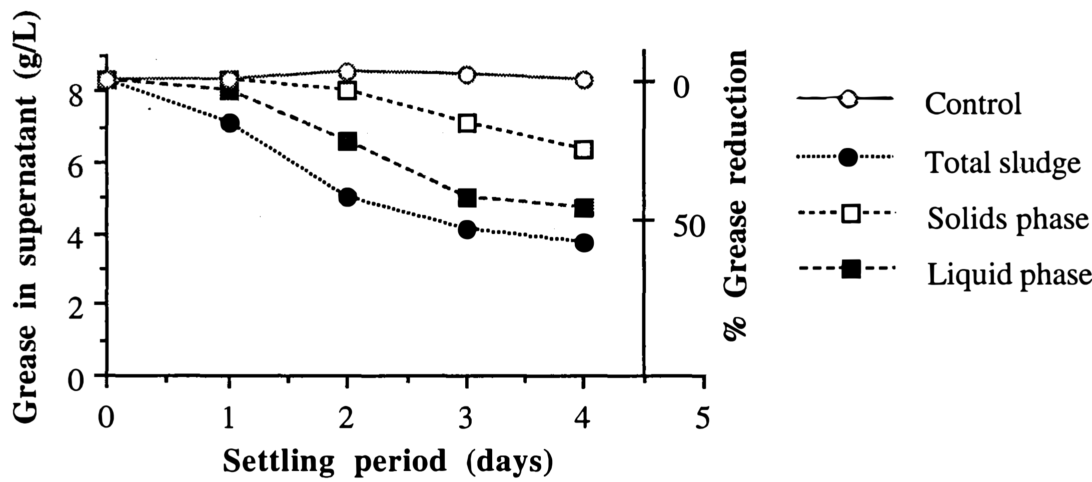


Figure 5.5.6 The effect of sludge fractions (total sludge, solid phase, and liquid phase) on anaerobic grease flocculation in WSE (**25% inoculum**, 30 min gentle mixing, incubation temperature 30 °C)

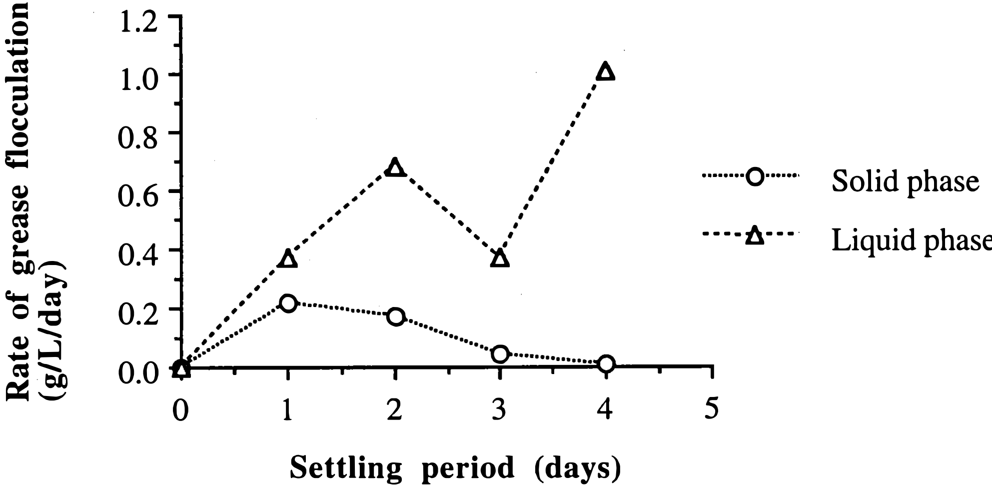


Figure 5.5.7 Rate of grease flocculation of WSE using fractionated solid and liquid phase from anaerobic sludge (50% sludge inoculum)

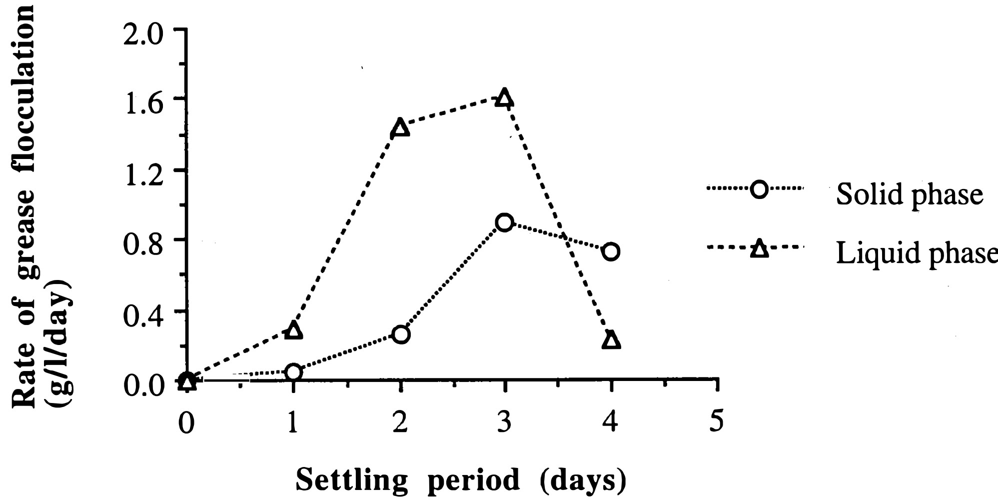


Figure 5.5.8 Rate of grease flocculation of WSE using fractionated solid and liquid phase from anaerobic sludge (25% sludge inoculum)

5.6 The role of suspended anaerobic microbes and sludge liquor on the bioflocculation process

The results from section 5.5 led to subsequent investigations, involving further separating of the sludge liquor into **suspended viable microbes** and **sterile sludge liquor**.

Experimental set up

Sterile sludge liquor was produced by centrifuging 12.5 ml of the sludge **liquid phase** at 10 000 G for 10 min and filtering the supernatant through a cellulose nitrate membrane filter (0.2 μm) under sterile conditions. Deoxygenated sterilised distilled water was used to make up the sterile filtrate to a total volume of 12.5 ml., and also used to wash and resuspend the microbes from the centrifuge tube and filter paper, also making up to the total volume to 12.5 ml (to standardise the final volume).

The experiment employed 70 ml test-tubes as reactors. The prepared sterile filtrate, and suspended microbes (from the filter paper and centrifuge pellet) were added to 37.5 ml WSE (containing 25% sludge equivalent). The bioflocculation ability of these trials was then compared to a control trial which used sterile distilled water.

All reactors had their head space replaced by N_2, were capped, and then mixed (on a Coulter mixer) for 30 min then maintained undisturbed at 30 °C. Two reactors from each trial were taken daily (for 6 days) to analyse grease content in the supernatant.

Results and discussion

The sterile filtrate was found to cause 12 % grease flocculation relative to the control while suspended microbes were able to flocculate 47 % grease. These results show

that the flocculation of grease from WSE was predominantly the consequence of anaerobic microbial activities rather than the physico-chemical properties of the sludge supernatant (Figure 5.6.1). However, some substances in the sludge liquor were also acting as flocculants. These flocculating substances may originate from (1) metabolism of lysis of microorganisms (proteins, DNA, polysaccharides and lipids), and/or (2) from the wastewater itself such as cellulose, humic acid, etc. (Urbain *et al.*, 1993).

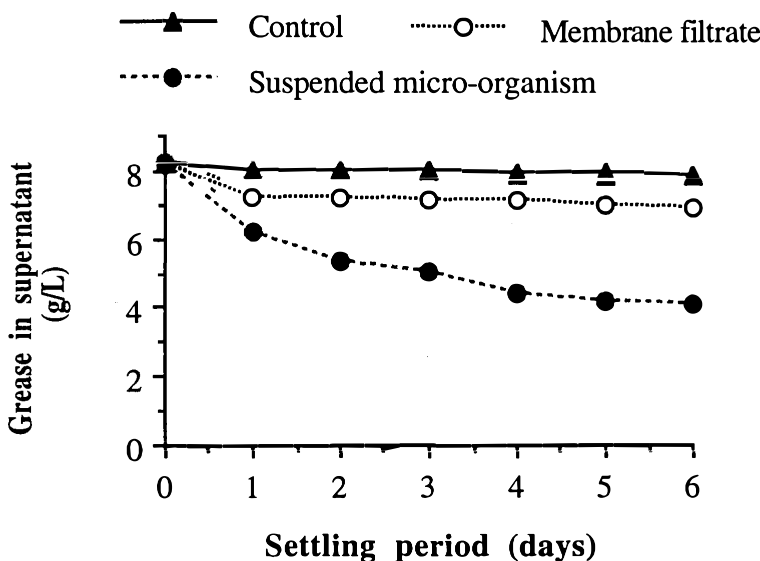


Figure 5.6.1 Grease content in the supernatant of the trial using suspended micro-organisms and membrane filtrate (of sludge liquor) (25% inoculum, 30 min. gentle mixing, incubation temperature 30 °C)

From the results of this experiment, it was concluded that although the suspended bacteria fraction contained about 100 times less bacteria than the actual bulk sludge (as measured by VSS in Table 5.6.1), it was more efficient in grease flocculation than the bulk biomass. This suggested that the flocculation of wool grease emulsion did not rely on the same microbes as in the anaerobic digestion process, but may

require specific microbes which were suspended in the liquor. When these microbes were inoculated to WSE, they had the ability to survive and successfully grow. Thus their properties and/or activities resulted in the destabilisation and subsequent flocculation of grease emulsion.

Table 5.6.1 Volatile suspended solids (VSS) of anaerobic sludge and sludge fractions used in the experiment.

	Volatile suspended solids (mg/g sample)
Total sludge	170
Solid phase or bulk biomass	370
Suspended microbes in the sludge liquor	3.2

5.7 Efficacy of treated effluent extracts in flocculating raw WSE

The previous experiment concluded that the flocculation of grease in WSE is the result of the activity of suspended anaerobic microbes (from anaerobic digestion sludge) which can successfully grow in WSE. To confirm this hypothesis as well as to determine the ability of these microbes to grow and flocculate fresh WSE, the supernatant phase and the flocculated grease of bioflocculated WSE were tested for their capability to cause new flocculation when added to fresh WSE.

Experimental set up

Three 170 ml test-tubes were filled with 75 ml WSE and 75 ml sludge supernatant (see section 3.2.1.3). After all the test-tubes had their head space replaced by N_2, were capped with rubber stoppers and gently mixed on a Coulter mixer for 30 min.,

the samples were left standing undisturbed at 30 °C for three days. The flocculation of wool grease was monitored and the supernatant from this process was separated from the flocculated grease. Both portions, **bioflocculation supernatant** and **flocculated grease**, were used to further flocculate grease emulsion from fresh WSE, and compared to the effect caused by the original sludge supernatant. Using 70 ml test-tubes as reactors, three separate trials involving 25% of (i) the original sludge supernatant, (ii) the bioflocculation supernatant, and (iii) flocculated grease were added to 75% WSE.

All reactors had their head space replaced by N_2, were capped, and then mixed (on a Coulter mixer) for 30 min before being left undisturbed at 30 °C. Two reactors from each trial were taken daily (for 4 days) to analyse grease content in the supernatant, with their bioflocculation ability compared to a control trial (using distilled water).

Results and discussion

The results show that the bioflocculation with the supernatant phase from the flocculated WSE and the original sludge supernatant gave very similar grease reductions (Figure 5.7.1). Previously flocculated grease, which started with a higher grease content in the mixed liquor, produced a slightly faster grease reduction, with the same degree of grease removal in two days as the supernatant did in three to four days. However, at the end of the fourth day the supernatant grease content of all trials was approximately the same.

These results lead to the conclusion that the anaerobic microbes responsible for the bioflocculation process could be transferred from flocculated WSE to further flocculate fresh WSE. This suggests that the bioflocculating bacteria were capable of multiplying and utilising some organic compounds in WSE as an energy and carbon source. When the flocculating bacteria are able to multiply, a continuous

seeding with digester sludge might not be necessary. This should allow the development of the anaerobic bioflocculation process as a continuous system.

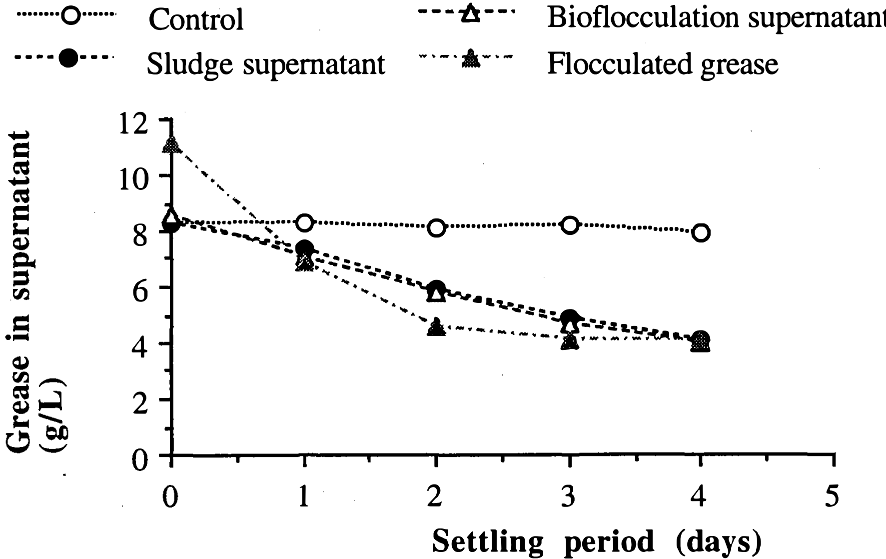


Figure 5.7.1 Supernatant grease content for the trials using original anaerobic sludge supernatant, compared to the trials using supernatant and flocculated grease from a previous bioflocculation (25% inoculum, 30 min. gentle mixing, incubation temperature 30 °C)

5.8 Bioflocculation of wool scouring effluent using supernatant of different sludges

Section 5.6 indicated that the bioflocculation of wool grease emulsion in WSE required the activity of bacteria, which can be found in the liquor of anaerobic digester sludge. Anaerobic lagoons treating WSE at a local wool scourer were suspected to have such anaerobic microbes. Due to a long period of acclimatisation to WSE, these bacteria were expected to have a better ability to flocculate grease emulsion . This experiment was therefore designed to examine the flocculation

ability of the suspended microbes from these lagoons compared to those from the municipal wastewater treatment plant.

Experimental set up

Three different anaerobic sludge supernatants were obtained from the sludges collected from the primary anaerobic digester at a municipal wastewater treatment plant and the 1st and 2nd anaerobic lagoons treating WSE at a wool scouring plant. The collection and preparation of these sludge supernatants was as detailed in section 3.2.1.

The experiment employed 70 ml test-tubes as reactors, and 25% of the sludge supernatants were used as inoculum. All reactors had their head space replaced by N_2, were capped, and then mixed (on a Coulter mixer) for 30 min before being left undisturbed at 30 °C. Two reactors from each trial were taken daily (for 4 days) to analyse the grease content in the supernatant, with their bioflocculation ability compared to a control trial (using distilled water).

Results and discussion

As shown in Table 5.8.1 the sludge supernatant from the first two anaerobic lagoons of the wool scouring plant although containing a slightly higher VSS content, also contained more grease than that of the municipal wastewater treatment plant. The biomass of the three samples did not vary significantly.

Table 5.8.1 Characteristics of initial sludge supernatants

Sludge supernatant from	VSS (g/L)	Grease (g/L)	Biomass (g/L)
- Subiaco wastewater treatment plant	3.2	0.58	2.62
- 1st lagoon at Jandakot wool scouring plant	4.4	1.70	2.70
- 2nd lagoon at Jandakot wool scouring plant	3.7	1.15	2.55

Anaerobic microbes from the lagoon, as well as from the municipal wastewater treatment plant, had an ability to flocculate wool grease from WSE (Figure 5.8.1). The ability of the microbes from the lagoons was not higher than that of the microbes from the wastewater treatment plant, even though they had been acclimatised to WSE for more than a year. The performance of the microbes from both sources was similar, in fact the microbes from the sewage treatment plant gave a slightly higher efficiency (Figure 5.8.2). It is hypothesised that the acclimatisation of microbes to WSE was not essential. The reason for the variation in performance between these sources of sludge within the bioflocculation process is not clear. It is possible that sewage sludge contains more of the specific microbes required for bioflocculation. In subsequent experiments anaerobic digestion sludge from a municipal wastewater treatment plant was used as the inoculum.

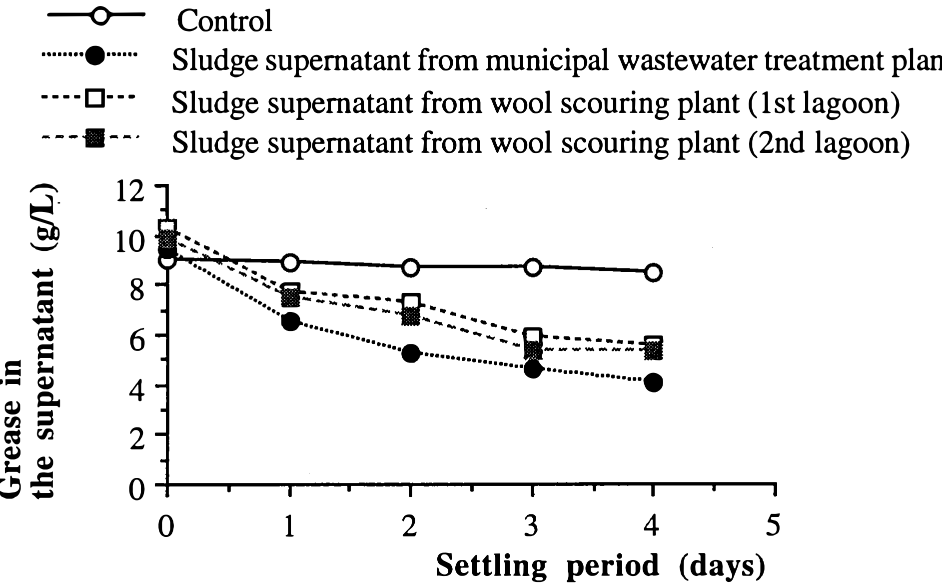


Figure 5.8.1 Grease content in the supernatant of the trial using different sources of sludge (25% inoculum, 30 min. gentle mixing, incubation temperature 30 °C)

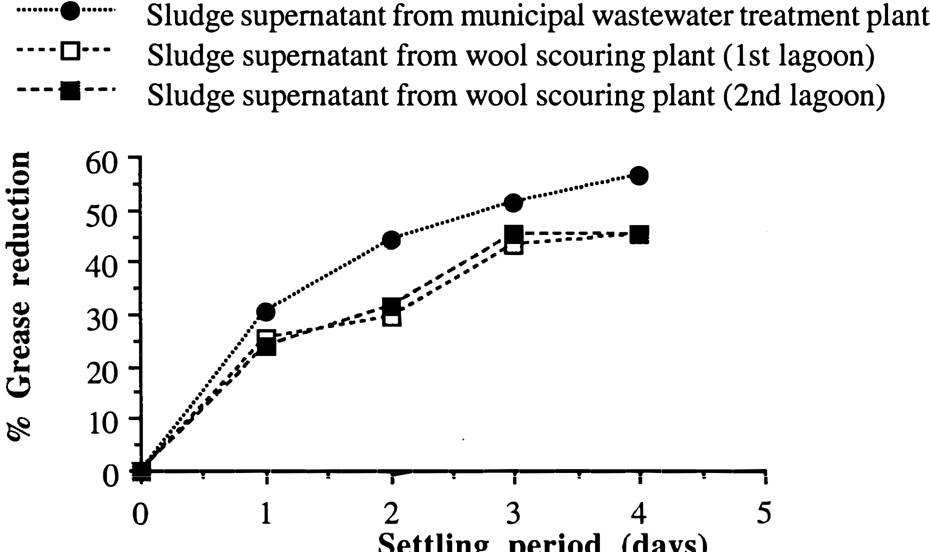


Figure 5.8.2 Grease reduction from bioflocculation process using different sources of sludge

5.9 Bioflocculation ability in different wool scouring effluents

As discussed in section 2.3, the characteristics of WSE are highly varied depending not only upon the scouring process being used but also on the characteristics of the wool itself. At the same wool scourer where the same process is used, the complexity and diversity of the composition of WSE is still unavoidable. The treatment processes are required to be flexible enough to handle this variation.

Biological processes, employing living microorganisms, are normally more sensitive to a change in the effluent being treated rather than chemical and physical processes. Since anaerobic bioflocculation of wool grease was found to be the result of anaerobic microbes, this experiment aimed to examine the consistency of the bioflocculation ability when different WSE samples were used.

Experimental set up

Four WSE samples were taken at two week intervals (from 21/1/92 to 3/3/92) from the same sampling point (stream 5, Figure 4.1.1) at the same wool scouring plant and then preserved as stated in section 3.2.3.

After the four WSE samples were analysed for grease, total surfactant, and free surfactant, they were mixed with sludge supernatant (at the proportion of 3:1 WSE : sludge supernatant) in 70 ml test-tubes which were used as reactors. All reactors had their head space replaced by N_2, were capped, and mixed (on a Coulter mixer) for 30 min before being left undisturbed at 30 °C. Two reactors from each trial were taken daily (for 7 days) to analyse grease content in the supernatant. The bioflocculation ability of the sludge was then compared to a control trial (using distilled water).

Results and discussion

Table 5.9.1 gives the grease content of the different WSE samples. Although the WSE samples were from the same scouring plant, the grease content of the samples varied dependent upon when the samples were taken.

Table 5.9.1 Characteristics of WSE samples used in the experiment

WSE sample	Date of sampling	Grease content (g/L)
Sample A	21/1/92	11.1
Sample B	4/2/92	5.9
Sample C	18/2/92	11.2
Sample D	3/2/92	10.4

The four WSE samples were compared in terms of grease and surfactant concentration, and the flocculation ability using the same anaerobic microbes as inoculum. The results show that the bioflocculation process was not easily reproducible with different WSE. The efficiency of grease reduction varied greatly (between 30% and 80% - Figures 5.9.1 and 5.9.2) with samples. Moreover, the pattern of grease reduction was dissimilar. In Figure 5.9.3, samples A and C showed rapid flocculation of wool grease during the first day, then stabilising during the remainder of the experiment. In contrast, samples B and D demonstrated slower grease removal over the first two days of incubation, gradually increasing throughout the experimental period. From these results, it was concluded that the success of anaerobic bioflocculation was highly influenced by the characteristics of WSE itself. This suggested that as far as bioflocculation was concerned, WSE might be classified as easily-to-flocculate and difficult-to-flocculate WSE. The reason for the difference

was not yet known, however a plausible hypothesis is that it is related to the variation in grease and surfactant concentrations.

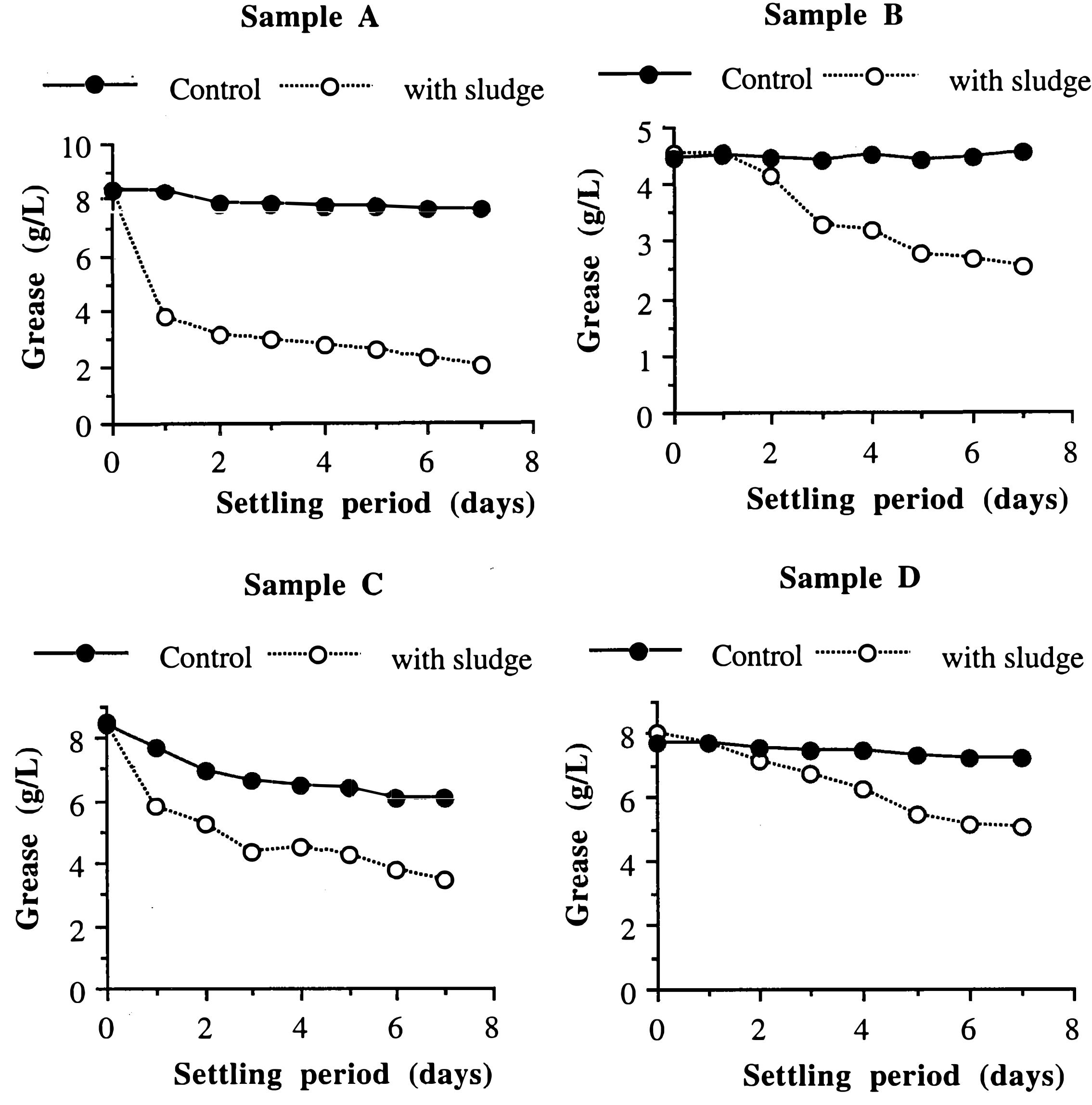


Figure 5.9.1 Grease bioflocculation ability of different WSE samples (25% inoculum, 30 min gentle mixing, incubation temperature 30 °C

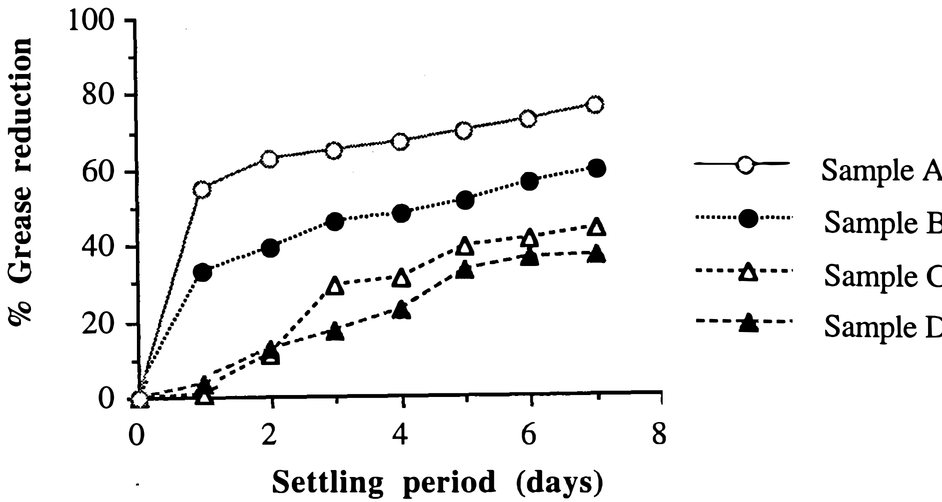


Figure 5.9.2 Grease reduction in different WSE samples during bioflocculation process

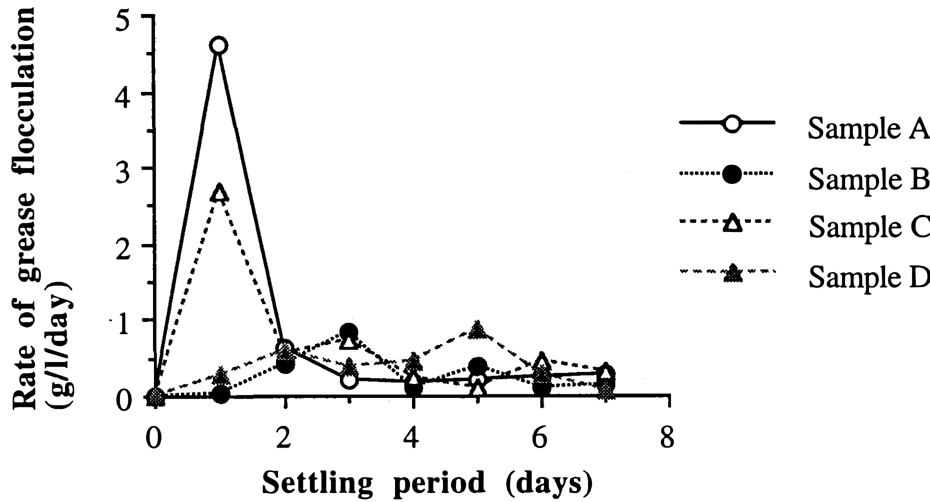


Figure 5.9.3 Rate of grease flocculation in different WSE samples during bioflocculation process

As discussed in section 2.3.4.3, the flocculation of wool grease emulsion from WSE may involve firstly the **destabilisation** of grease emulsion by reducing the repulsive force of the emulsion surface to the level where attractive forces can dominate. Secondly, the destabilised emulsion (including dirt and possibly bacteria) coagulates and forms flocs which are dense enough to settle by gravity, resulting in **flocculation**. This suggests that the destabilisation of emulsion can occur without the final result of flocculation if the flocs are not dense enough. To confirm this hypothesis, the seven-day bioflocculated WSE sample D (difficult-to-flocculate WSE) and the control (the same sample without being previously bioflocculated) in Figure 5.9.1 were further centrifuged at 3 000 G for 5 min. The grease contents in the supernatants after centrifugation were then compared.

The results (Table 5.9.2) indicate that the anaerobic bioflocculation treatment greatly increased the efficiency of subsequent centrifugation. In difficult-to-flocculate WSE, although grease reduction from the supernatant was not clearly obtained, some of the wool grease emulsion in WSE had been partially destabilised during the process of anaerobic bioflocculation. However, the density difference between the flocs formed and WSE liquor was not great enough to cause flocculation during the period of experiment. This suggests the possibility of using additional chemical or physical processes to accelerate the flocculation of grease emulsion in difficult-to-flocculate WSE.

Table 5.9.2 Comparison of grease sedimentation after centrifuging of difficult-to-flocculate WSE with and without prior anaerobic bioflocculation.

	Reduction of grease before centrifuging		Reduction of grease after centrifuging	
	g/L	%	g/L	%
-WSE (sample D) after 7-day bioflocculation	5.09	36.6	2.33	71.0
-Control - WSE (sample D) plus distilled sterile water and settling for 7 days	7.24	6.4	4.94	36.1

5.10 Surfactant in WSE and bioflocculation ability

Total surfactant concentrations in WSE samples tested in the previous experiment (section 5.9) were between 200 and 547 mg/L (Table 5.10.1). In general, non-ionic surfactant, which is added during the scouring process, is predominantly adsorbed to the surface of grease droplets forming micelles suspended in the liquor (see section 2.3.4.3). If excess surfactant is added above the critical micelle concentration or c.m.c. (the concentration above which micelle formation becomes appreciable), single dissolved surfactant molecules represent "free surfactant" (Shaw, 1980).

Table 5.10.1 Surfactant concentration in different WSE

WSE sample	Grease content (g/L)	Total surfactant (mg/L)	Free surfactant (mg/L)
Sample A	11.06	426	0.0
Sample B	5.96	200	19.2
Sample C	11.25	303	22.7
Sample D	10.39	547	44.0

Results (Table 5.10.1 and Figure 5.10.1) show most of the surfactant in the samples was present in a form attached to the grease particles. After the removal of all grease and dirt particles by centrifugation and filtration, the WSE liquor contained only a small amount of free (dissolved) surfactant, except sample D in which 25% of the surfactant was present as free surfactant. Figure 5.10.2 and 5.10.3 present attempts to determine the effect of surfactant concentration on bioflocculation ability. From the results of this experiment, no relationship between grease removal and total surfactant levels could be established (Figures 5.10.2). However, a relationship between free surfactant level and grease flocculation ($R^2 = 0.774$) was obtained (Figures 5.10.3). This finding suggested the benefit of minimising surfactant use during scouring, since the excess surfactant was likely to be one of the factors reducing the bioflocculation process efficiency.

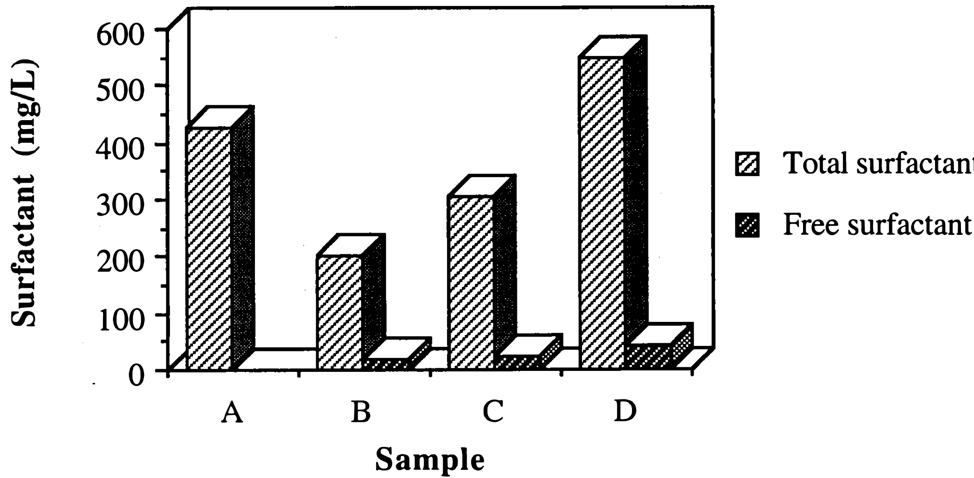


Figure 5.10.1 Total and free surfactant in the different WSE samples

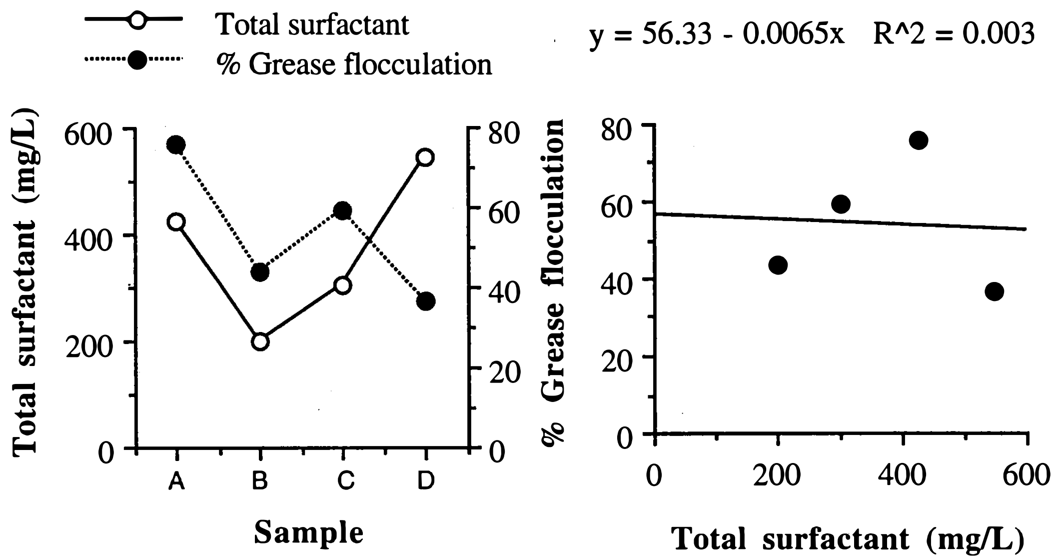


Figure 5.10.2 The relationship between the level total surfactant and bioflocculation ability of WSE

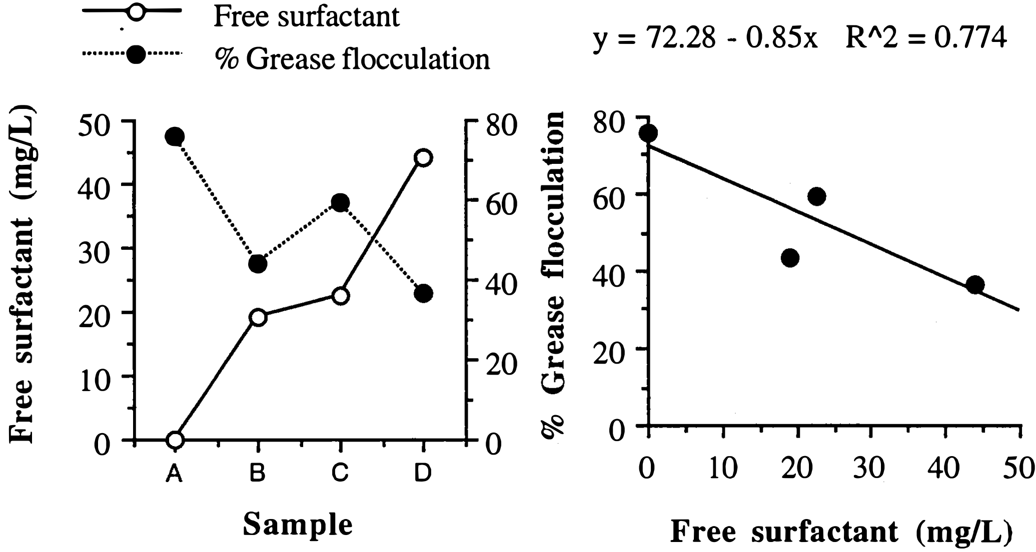


Figure 5.10.3 The relationship between the level free surfactant and bioflocculation ability of WSE

5.11 Biodegradation of non-ionic surfactant during anaerobic bioflocculation

In a general microscopy examination of WSE during bioflocculation, no bacteria cell aggregation was observed. Most of the bacteria were found in the supernatant with only a few in the sludge. The mechanism responsible for the destabilisation of wool grease was therefore believed to be a consequence of bacterial activities, particularly surfactant degradation and/or flocculation by bioflocculant, rather than cell adhesion to emulsified grease particles as found in aerobic processes (Poole, 1991).

As discussed in section 2.3.4.3, the stability of wool grease emulsion was greatly governed by the properties and concentration of non-ionic surfactant surrounding the grease droplets (Shaw, 1980). The change of this surfactant structure during the bioflocculation therefore influences the stability of the emulsion. The surfactant was also found to be able to at least partially biodegrade under anaerobic conditions (see

section 2.3.5.1). The alteration of surfactant concentration and structure during the anaerobic bioflocculation process was hypothesised to reduce the stability of the emulsion, resulting in the subsequent flocculation.

This experiment therefore aimed to investigate whether the flocculation of wool grease emulsion was a consequence of the complete or partial biodegradation of the surfactant in WSE liquor during the anaerobic bioflocculation process.

Experimental set up

The following experiment was designed to monitor the surfactant concentration as a function of time during 5 days of experimental period. By using 150 ml test-tubes as reactors, 75% of WSE was mixed with 25% sludge supernatant on the Coulter Mixer for 30 min, then left undisturbed at 30 °C. Four reactors were taken daily, two of which were analysed for grease and total surfactant in the supernatant while the other two were analysed for grease and surfactant in the mixed liquor (this was achieved by vigorously mixing the contents of the reactors for 5 min before samples were taken).

Results and discussion

Figure 5.11.1 confirms that during the process of bioflocculation there was no significant degradation of wool grease. The grease was simply flocculated and settled, and as a result, a lower grease content was obtained in the supernatant. When the surfactant level was monitored (Figure. 5.11.2), it was clearly shown that the surfactant level in the supernatant decreased from 230 mg/L to 50 mg/L during the five day experimental period. However, in the mixed liquor (mixture of the supernatant with settled grease), the surfactant level did not show any significant change during the bioflocculation process. This is evidence that the surfactant in the

liquor was not destroyed but rather flocculated with the grease particles during the bioflocculation process. Thus a close relationship between grease and surfactant concentration in the supernatant is obtained (Figure 5.11.3).

It is interesting to note, from the results in Figure 5.11.1 and 5.11.2, that approximately 75% of surfactant was flocculated while correspondingly only 50% of grease was flocculated. Thus the flocculated grease must be surrounded with higher number of surfactant molecules rather than the non-flocculated grease which remains suspended in the supernatant. This may be due to the different types of grease in the scouring liquor. The non-flocculated grease could be a highly oxidised grease which was entrapped in the swollen proteinaceous contaminants (see section 2.3.4.3 and Figure 2.3.4.3.2). Since these contaminants had low specific gravity, they do not require much surfactant to become suspended in the liquor.

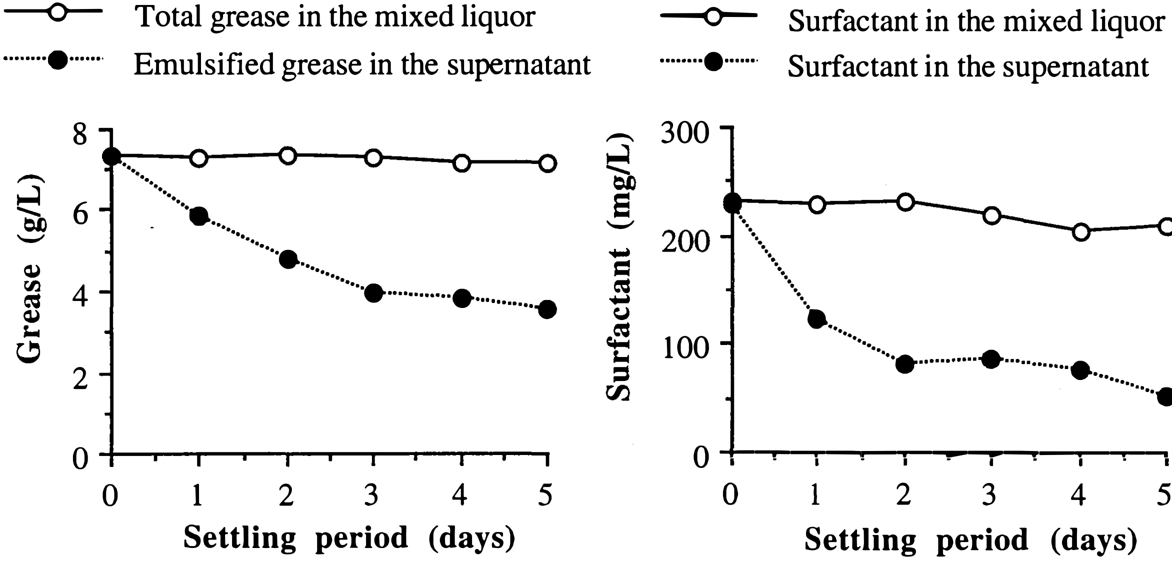


Figure 5.11.1 Emulsified grease in the supernatant compared to total grease (emulsified plus flocculated grease) in the mixed liquor during the bioflocculation process

Figure 5.11.2 Non-ionic surfactant in the supernatant and mixed liquor during the bioflocculation process

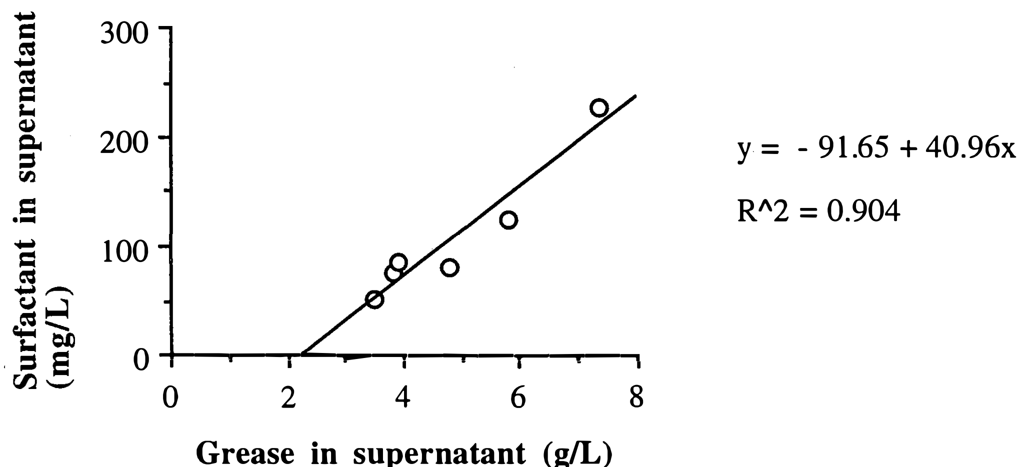


Figure 5.11.3 Correlation between surfactant level and grease content in the supernatant of WSE treated by anaerobic bioflocculation

The non-ionic surfactant (nonylphenol polyethoxylate) used in the wool scouring industry is a long hydrophobic nonyl chain linked by a phenolic group to a long hydrophilic ethoxylate chain:

$$H_{19}C_9-C_6H_4-(CH_2-CH_2-O)_n-OH$$

This surfactant is well documented as being receptive to at least partial (primary) degradation under aerobic and anaerobic conditions (see section 2.3.5.1). Due to the complexity of the structure, the dominant biodegradation pathway of NPEO is known to start with the ethoxylate chain shortening (Cain, 1977; White, 1993). However, the analysis of non-ionic surfactant by reversed-phase HPLC (see section 3.5) relies on determining the presence of UV absorbing material. In the case of the NPEO surfactant the phenolic group has this property, allowing the concurrent detection of all oligomers present (Ahel & Giger, 1985b). Hence despite the results in Figure 5.11.2 indicating no significant surfactant degradation, this does not necessarily signify that the surfactant was unaffected. These results merely show that the phenolic group of the surfactant persisted.

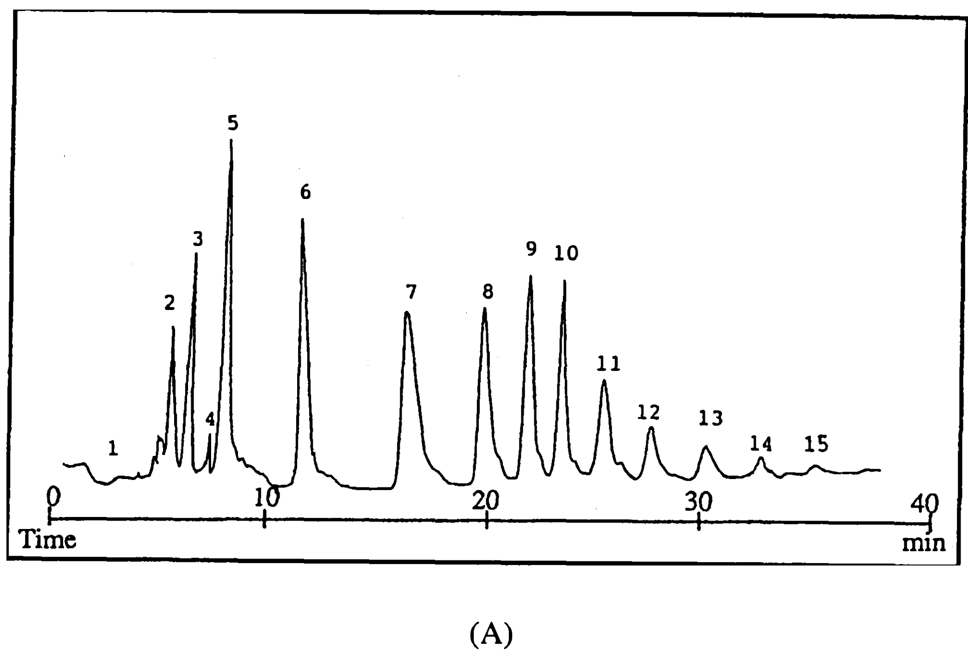
In order to determine the extent of degradation of the hydrophilic polyethoxylate chain during the bioflocculation process, normal-phase HPLC was employed (see section 3.5). This method allowed the monitoring of the polyethoxylate chain length and distribution of the surfactant (Figure 5.11.4).

The NPEO used in the wool scouring plant comprised of an ethoxylate chain of up to 15 ethylene oxide (EO) units. The distribution of EO uniis in this compound is shown in Figure 5.11.4 A. After 5 days of incubation, the distribution of NPEO oligomers had altered (Figure 5.11.4 B). Some of the longer chain EO oligomers were transformed to shorter EO oligomers. The shoulder peaks between oligomers

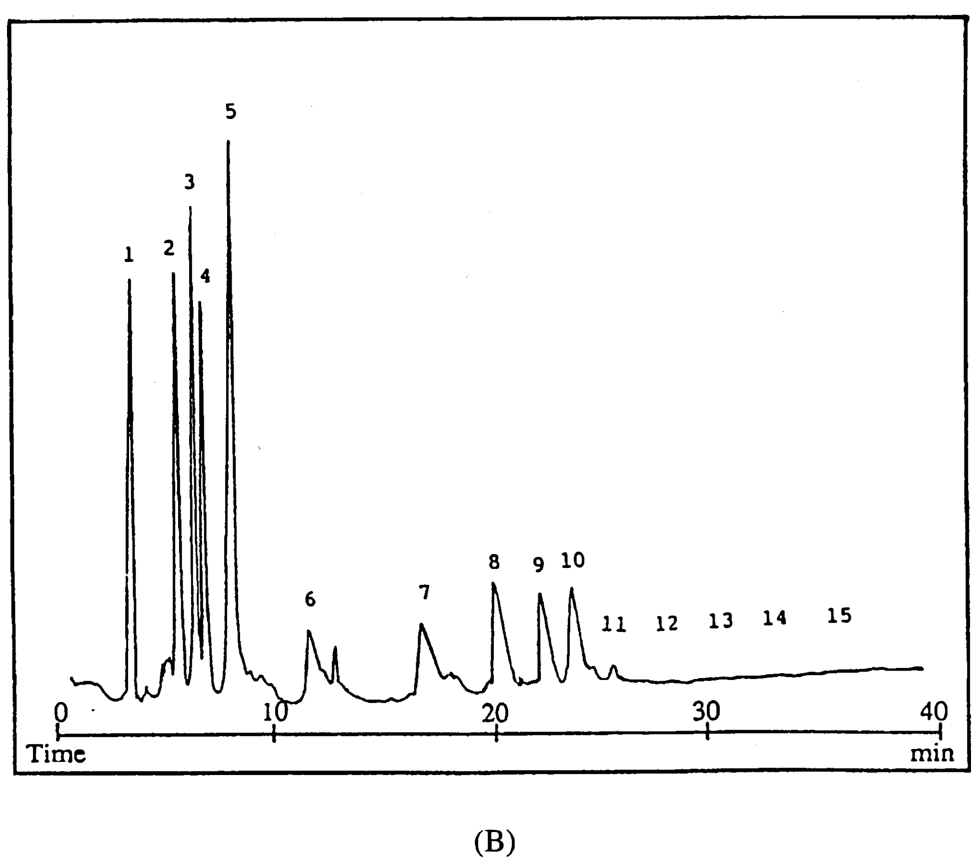
reflect the partial separation of different alkyl chain lengths (Holt *et al.*, 1986) which could be explained by partial degradation of the nonyl chain during bioflocculation process.

From Figures 5.11.5 and 5.11.6, it is clear that a total of approximately 50% of long derivative surfactant (6 to 15 EO units) were reduced, particularly the oligomers of 12 to 15 ethoxylate groups which were not present after the bioflocculation process. At the same time, an approximately 50% increase of shorter derivative surfactants with EO units between 1 to 5 units was evident. These results imply that, at the end of the bioflocculation process, the majority of surfactant in the liquor contained only 1 to 5 ethoxylate groups. The loss of EO units during the flocculation process resulted in the reduction of surfactant properties, therefore destabilising the grease emulsion.

Partial (primary) degradation of surfactant (NPEO) in the aquatic environment and sewage treatment has been reported (Ahel & Giger, 1985a; Brunner *et al.*, 1988; Giger *et al.*, 1987; Marcomini *et al.*, 1990; White, 1993). Although the degradation of NPEO observed in this study was not to completion, the shortening of the hydrophilic EO chain resulted in primary biodegradation (reducing surface activity properties), therefore decreasing the ability of NPEO to emulsify grease particles (Swisher, 1987). This is postulated to be one of the factors contributing to the coagulation and subsequent flocculation of grease emulsion during the anaerobic bioflocculation process.



(A)



(B)

Figure 5.11.4 Normal-phase high-performance liquid chromatograms of non-ionic surfactant NPEO in WSE; (A) before bioflocculation process; and (B) after 5 days bioflocculation process. Peak numbers refer to numbers of ethoxy unit.

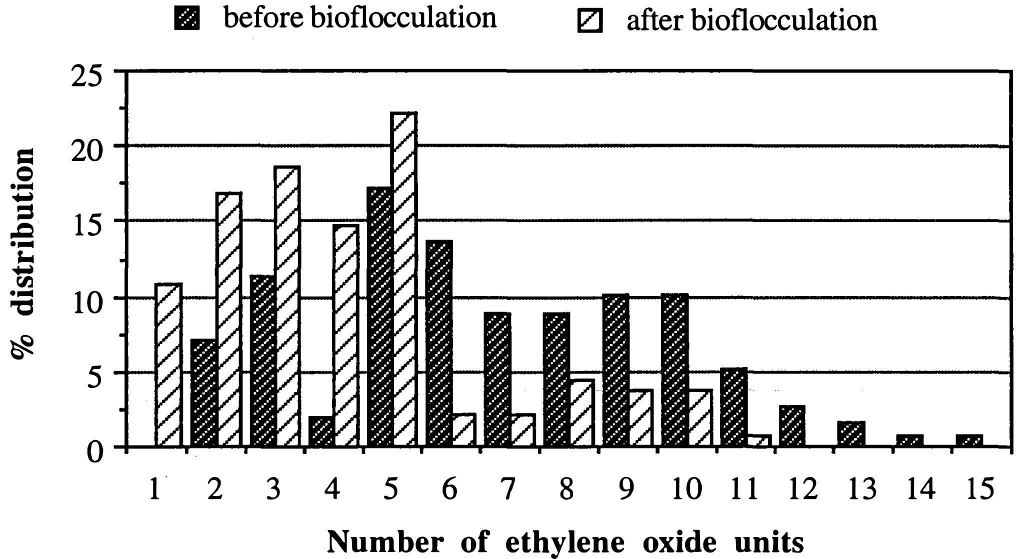


Figure 5.11.5 The distribution of surfactant with different ethoxylate groups before and after anaerobic bioflocculation (expressed as percentage distribution)

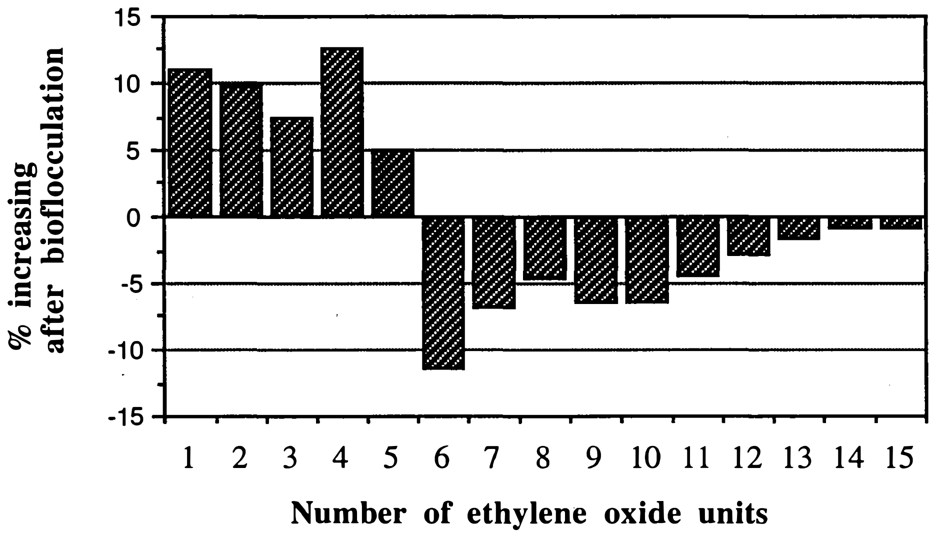


Figure 5.11.6 The change in distribution of the ethoxylate groups before and after anaerobic bioflocculation

5.12 The addition of nutrients to enhance the bioflocculation ability of WSE

The results of a previous experiment (see section 5.9) indicate that the bioflocculation of wool grease emulsion varied greatly depending on the type of WSE. From general microscopy, it was observed that during the bioflocculation process the difficult-to-flocculate WSE contained only a small number of microbes when compared to the easy-to-flocculate WSE, even though they were inoculated with the same amount of inoculum. This suggested that the difficult-to-flocculate WSE either contained inhibitory compounds or lacked some essential substrate required for the growth of microbes.

In order for the biochemical process of metabolism to take place, bacteria require adequate sources of energy. These comprise usually an organic substrate (electron donor) and an electron acceptor (usually oxygen). In the absence of oxygen, alternative electron acceptors are used in anaerobic respiration including nitrate (NO_3^-), sulphate (SO_4^{2-}), carbon dioxide (CO_2) or other organic compounds. Besides energy sources, to be able to synthesise biomass, bacteria also require a carbon source and other essential substances such as nitrogen, phosphorous and sulfur (Brock & Madigan, 1988).

This experiment was designed to investigate whether poor bioflocculation in difficult-to-flocculate WSE was due to the lack of essential substrates in the WSE. Selected nutrients (easily degradable substances and electron acceptors) were added to the difficult-to-flocculate WSE (sample D in section 5.9). The bioflocculation efficiency of these samples was then monitored compared to the WSE without nutrient addition.

Experimental set up

The experiment used 70 ml test-tubes as reactors (10 reactor per trial). The proportion of WSE and sludge supernatant was 75:25. Nutrients were added as shown in Figure 5.12.1. After the reactors were capped and had their head space replaced by N_2, they were gently mixed for 30 min and then incubated at 30 °C.

Results and discussion

The supplement of nitrate, ammonia, and phosphate did not stimulate the bioflocculation of wool grease emulsion from WSE (Figure 5.12.1). The addition of glucose slightly enhanced the flocculation of grease. From general microscopy, the number of bacteria in this trial was also found to be higher than other trials.

Sulphate seemed to considerably increase biological grease flocculation. However, the control trial (using distilled water instead of sludge supernatant) also gave a similar result (Figure 5.12.2). This suggested that the additional potassium sulphate in WSE acts as a destabilising agent for the wool grease emulsion, resulting in the flocculation of WSE. Since potassium sulphate is an inorganic electrolyte, the adding of this electrolyte possibly caused the salt-out of the non-ionic surfactant (Anderson & Christoe, 1981), surrounding the wool grease droplets in WSE, and therefore reducing the stability of the emulsion.

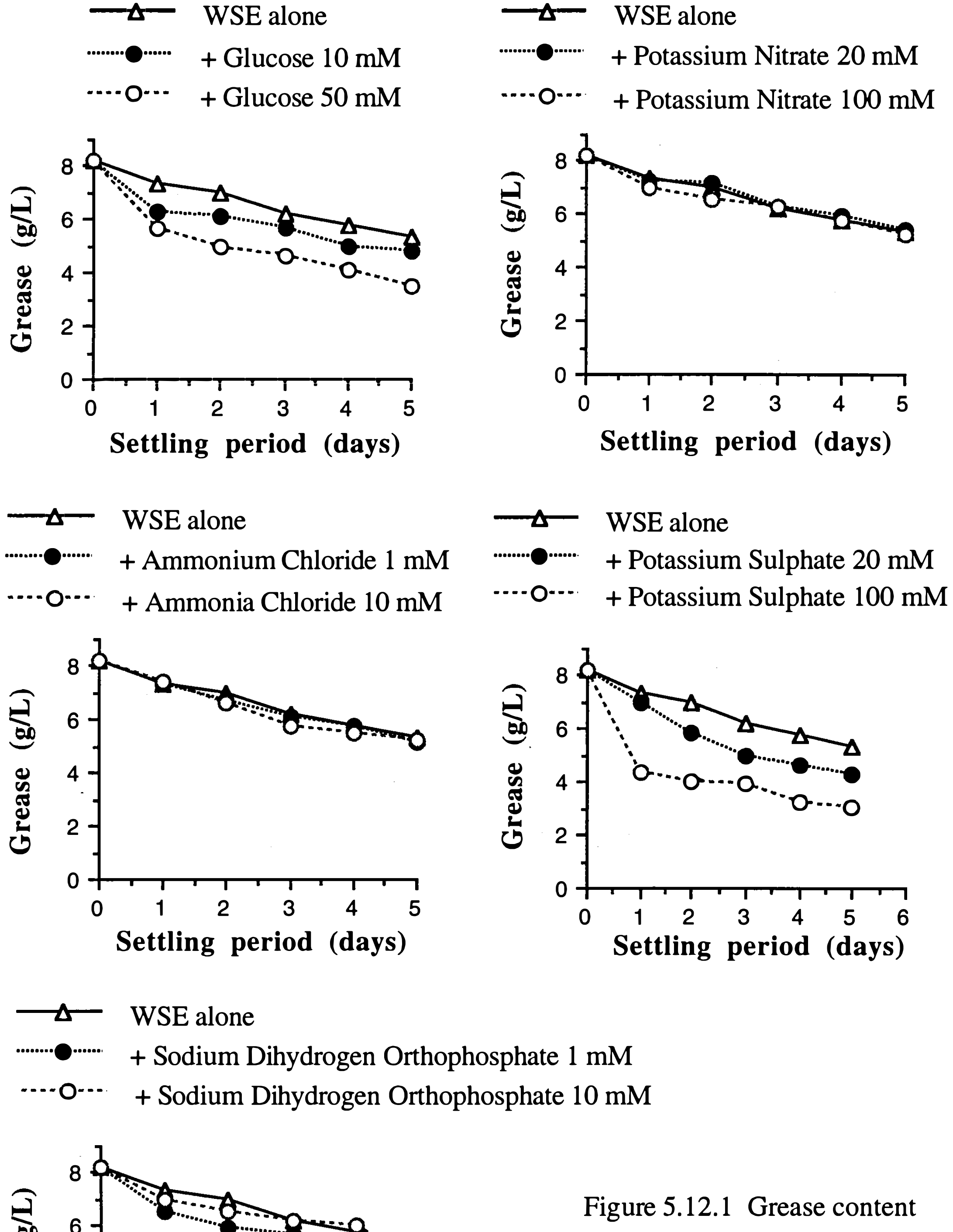


Figure 5.12.1 Grease content in the supernatant of difficult -to-flocculate WSE with and without nutrient supplements during bioflocculation process

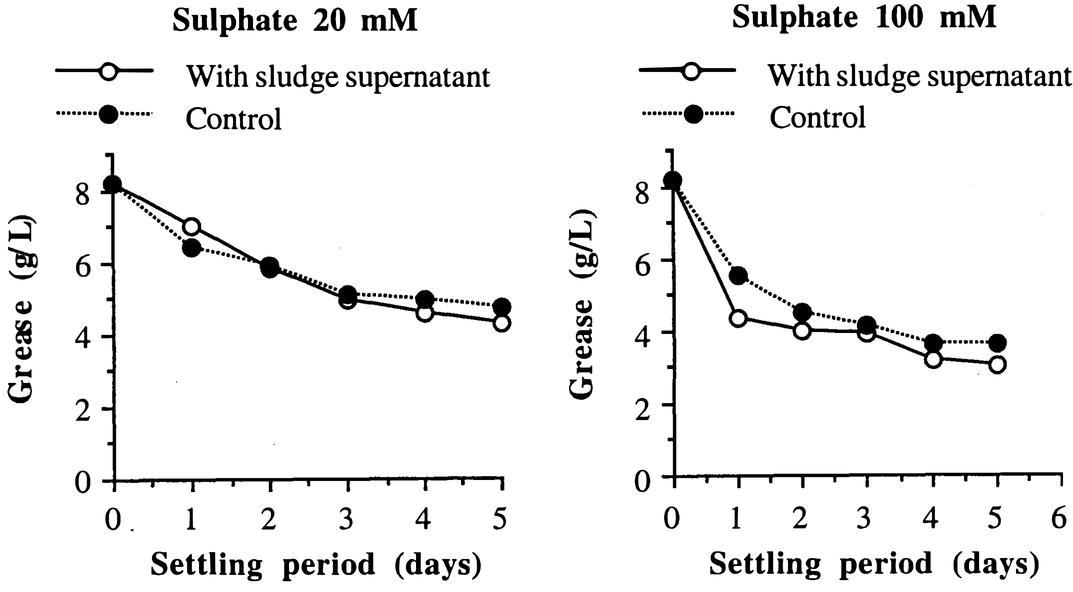


Figure 5.12.2 The flocculation efficiency of WSE with the sulphate supplement compared with the control

It is not possible to make a clear conclusion as to whether the reason some WSE samples were more difficult to bioflocculate is due to limiting bacterial growth caused by a lack of nutrients in the WSE. Although the addition of a carbon source slightly increased bioflocculation ability, it was believed to only be a minor factor contributing to the flocculation process. The quality of grease itself (oxidised and unoxidised), proteinaceous contaminates (Anderson, 1983), the size of emulsion droplets (Fukushima, 1984) have all been documented to influence the stability of wool grease emulsion (see section 2.3.4.4). These are postulated to contribute to the varying degrees of bioflocculation ability shown by different WSE samples.

Chapter 6 Investigation of bacteria involved in the anaerobic bioflocculation process of WSE

6.1 Introduction

A conclusion from results in the chapter 5 was that the anaerobic bioflocculation of wool grease emulsion was the result of the activity of anaerobic microbes. General microscopic observation and bacterial count (see section 3.4.3) had indicated that, during the process of bioflocculation, a large number (>10^8 cells/ml) of bacteria developed. This suggested that some selective bacteria from the anaerobic digestion sludge had the ability to survive and successfully grow in WSE. As a consequence of this bacterial growth, some microbial activities/mechanisms resulted in the destabilisation and subsequent flocculation of wool grease emulsion.

This chapter describes attempts to isolate and test the bacteria postulated to be responsible for causing the bioflocculation of wool grease emulsion in WSE. This was achieved by isolating as a pure culture the predominant bacteria (with populations of 10^6 cells/ml or greater), from WSE which had been bioflocculated for 3 days (see section 3.4.2 for details of the isolation procedure).

6.2 The isolation of bacteria from bioflocculated WSE

Table 6.2.1 presents the morphology and some characteristics of ten different bacterial strains that were isolated from the supernatant of bioflocculated WSE. All bacterial strains present stained Gram negative. Most of them were facultative microbes, that grow either in the presence or absence of oxygen. Only three strains were found to be strictly anaerobic microbes. It is notable that although the microbes were isolated from a process under anaerobic conditions, strain D behaved micro-aerophilically as they tended to accumulate near the border with the cover slip or at a certain distance from air bubbles under the cover slip.

Table 6.2.1 Characteristics of bacteria isolated from the supernatant of flocculated WSE.

	Strains *	Morphology	Length	Width	Gram reaction	Aerobic growth	Anaerobic growth	Growth on WSE **	Concentration in WSE***
A		rods in pairs or singly, non-motile, spore-forming	2-4 µm	1 µm	negative	-	+	-	10^8

* Phase-contrast microscopy with scale 10 µm = |———|

** can grow in WSE by direct inoculation

*** cells/ml

Strains *	Morphology	Length	Width	Gram reaction	Aerobic growth	Anaerobic growth	Growth on WSE **	Concentration in WSE***
B	rods in pairs, singly or chains, non-motile, spore-forming	2-3 μm	1 μm	negative	-	+	-	10^6
C	long rods in pairs or singly, but mainly in chains, motile(slow), non-spore forming	2-4 μm	1 μm	negative	+	+	-	10^8

Strains *	Morphology	Length	Width	Gram reaction	Aerobic growth	Anaerobic growth	Growth on WSE **	Concentration in WSE***
D	long rods in pairs or singly, motile(fast), non-spore forming	3-4 µm	1 µm	negative	+	+	+	10^8
E	short rods in pairs, singly or chains, non-motile, non-spore forming	1 µm	1-3 µm	negative	+	+	-	10^8

Strains *	Morphology	Length	Width	Gram reaction	Aerobic growth	Anaerobic growth	Growth on WSE **	Concentration in WSE***
F	short rods in pairs or singly, motile(slow), non-spore forming	1-2 µm	1 µm	negative	+	+	+	10^7
G	long rods singly, motile(slow), non-spore forming	2-5 µm	1 µm	negative	-	+	-	10^6

Strains *	Morphology	Length	Width	Gram reaction	Aerobic growth	Anaerobic growth	Growth on WSE **	Concentration in WSE***
H	long rods singly, motile(slow), non-spore forming	2-6 µm	1 µm	negative	+	+	-	10^8
I	rods in pairs or singly, motile(slow), non-spore forming	2-4 µm	1 µm	negative	+	+	-	10^7

Strains *	Morphology	Length	Width	Gram reaction	Aerobic growth	Anaerobic growth	Growth on WSE **	Concentration in WSE***
J	short rods singly or pairs, motile(fast), non-spore forming	1-2 µm	1 µm	negative	+	+	-	10^8

* Phase-contrast microscopy with scale 10 µm = |———|

** can grow in WSE by direct inoculation

*** cells/ml

6.3 Flocculation of wool grease by pure cultures

A first attempt at using these bacterial strains (Table 6.2.1) to flocculate raw WSE (easy-to-flocculate sample) involved inoculating bacteria directly from colonies on agar to fresh WSE in Hungate tubes. To ensure anaerobic conditions, nitrogen gas was used to displace air head space. All tubes were then mixed and incubated at 30 °C. After 24, 48, 72 and 96 hrs, they were monitored for bacterial growth using phase-contrast microscopy and plate counts.

It was found that, after 4 days of incubation, the purified strains isolated from (flocculated) WSE could not survive individually when they were returned as inoculates to WSE. The reason for this loss of microbial viability is unknown. Possibly, toxic substances in WSE such as detergent prohibited the growth of these bacteria (Isaac, 1991).

The second procedure involved inoculating these bacteria into diluted WSE (50% WSE and 50% distilled water), assuming that only toxic substances would be diluted. Only strains D and F were observed to survive and multiply (although not at high populations, approximately 10^4 cells/ml), however no flocculation of wool grease was observed.

All bacterial strains were therefore cultured in enriched liquid media, tryptic soy broth, for 24-48 hours. This stock culture was then used as inoculum to fresh WSE at the proportion of 25% inoculum and 75% WSE. Inoculating bacteria with the broth ensures a nutrient supply for the bacteria to grow in the fresh WSE. The number of bacteria (microscopy and plate count using tryptic soy plate- see section 3.4.3) and flocculation of the samples were monitored over four days (Table 6.3.1).

The four strains- A, E, G, and I, did not appear to be able to survive in WSE individually even after being cultured in the liquid media. Other strains could successfully grow in WSE after incubation of 24-72 hrs. Three of these strains (D, F, and J) reached high numbers (>10^7 cells/ml), after incubation of 24-72 hrs, and the flocculation of wool grease took place in these samples. However, the other strains (B, C, and H) which could survive and multiply in WSE, did not show any flocculation ability during the four day experiment. Note that the degree of flocculation achieved by the purified strains (only 20-30%) was far less than that obtained from the mixed culture (70-80%).

Table 6.3.1 Observation of bacteria viability and flocculation of wool grease in WSE which had been inoculated with pure strains.

Strain used	24 hrs incubation		48 hrs incubation		72 hrs incubation		96 hrs incubation	
	No. of bacteria*	floccula-tion**	No. of bacteria*	floccula-tion**	No. of bacteria *	floccula-tion**	No. of bacteria *	floccula-tion**
A	N	-	N	-	N	-	N	-
B	N	-	√	-	√	-	√√√	-
C	N	-	√	-	√	-	√√	-
D	√√	-	√√√	√	√√√	√	√√√	√√
E	N	-	N	-	N	-	N	-
F	N	-	N	-	√√	√	√√√	√√
G	N	-	N	-	N	-	N	-
H	√√	-	√√	-	√√√	√	√√√	-
I	N	-	N	-	N	-	N	-
J	√√	-	√√√	√	√√√	√√	√√√	√√

* Number of bacteria

N = nothing visible

√ = small number of bacteria ($<10^4$)

√√ = reasonable number of bacteria (10^4-10^7)

√√√ = high number of bacteria ($>10^7$)

** Flocculation

\- = no floc formation was observed

√ = small flocs were observed but did not settle to the bottom

√√ = flocculation was clearly observed

This experiment was repeated by inoculating these pure strains into a different WSE sample (Sample B in section 5.9). The results were somewhat inconsistent as shown

in Table 6.3.2. In this WSE sample, no clear flocculation was observed from any strain after 4 days incubation. Only small flocs were observed from strains A, D, and E. Strain F and J did not show any flocculation ability, while they did in the first trial of the experiment (Table 6.3.1).

Table 6.3.2 Observation of bacteria viability and flocculation of wool grease in WSE- sample B, which had been inoculated with pure strains.

Strain used	24 hrs incubation		48 hrs incubation		72 hrs incubation		96 hrs incubation	
	No. of bacteria*	floccula -tion**	No. of bacteria*	floccula-tion**	No. of bacteria*	floccula -tion**	No. of bacteria*	floccula -tion**
A	N	-	√	-	√√	√	√√√	√
B	N	-	N	-	N	-	N	-
C	N	-	N	-	√	-	√	-
D	√√	-	√√	-	√√√	-	√√√	√
E	N	-	√	-	√√	-	√√√	√
F	N	-	N	-	√√	-	√√√	-
G	N	-	N	-	N	-	N	-
H	√	-	√	-	√√	-	√√	-
I	N	-	N	-	N	-	N	-
J	N	-	N	-	√	-	√√	-

* and ** are the same as Table 6.3.1

From these two experiments, no clear conclusion can be drawn as to which bacterial strains were responsible for the flocculation of grease emulsion in WSE. However, the flocculation of wool grease was observed only when the bacteria could

successfully survive and multiply, while some bacteria could survive and reach high numbers without causing flocculation. This suggested that for the flocculation of wool grease to occur, growth of *specific* bacteria reaching suitably high populations in WSE is necessary. The reasons for the variation of bioflocculation between different WSE samples is still unclear.

Although the mechanisms of anaerobic bioflocculation cannot be fully explained from this investigation, the results show the ability of a number of single strains of bacteria to grow in WSE, resulting in the flocculation of wool grease emulsion in WSE. The process, however, took a longer time and resulted in less flocculation than that employing a mixed culture. The reduction of grease from the supernatant was only 30-35% of what would be obtained from the mixed culture, with the same WSE sample.

Chapter 7 Investigation of the feasibility of anaerobic bioflocculation treating WSE by semi-continuous operating system

7.1 Introduction

In the batch system (see chapter 5), the anaerobic bioflocculation process had shown an ability to destabilise and flocculate the wool grease emulsion in WSE. The extracts of previously bioflocculated WSE were effective in flocculating new WSE, with a similar level of performance to the original sludge. This indicates the possibility of developing this process to operate in a continuous manner. This chapter presents an investigation on the feasibility of employing the anaerobic bioflocculation process as a continuous system, which would provide a more economic and practical treatment system for the wool scouring industry.

In general, the effluents from a wool scouring process were found to vary greatly even when they were obtained from one wool scouring plant using the same scouring system. For example, the grease concentration of the effluent can be as low as less than 5 g/L or sometimes as high as over 20 g/L depending on the quality of raw wool being scoured (John Sheehan, personal communication). To avoid an extreme under or over loading of the system, an investigation was undertaken using the two ranges of concentrations of wool grease.

The first experiment involved the treatment of low grease (<10 g/L) WSE using a single-stage anaerobic bioflocculation process. The second experiment was designed to treat a high strength WSE, with grease a concentration higher than 10 g/L . This employed a two-stage bioflocculation process rather than a single-stage, since a single -stage system was unlikely to be able to remove grease to an appreciable level. In addition, the majority of grease was expected to flocculate and accumulate in the first stage. The rapid build up of flocculated grease in the sludge may reduce the

efficiency of the process. The second stage would allow the remaining grease to be treated in a less greasy biomass.

The experimental set up and operation of the single-stage and two-stage anaerobic bioflocculation was as described in section 3.3.2 and 3.3.3 respectively. From batch experiments, it was found that short gentle mixing and longer settling periods (see section 5.3) were required. The system used in these experiments was therefore modified to operate as a semi-continuous system as shown in Figure 3.3.2.3. This should allow the WSE to come in contact with the biomass and then the destabilised grease emulsion to form flocs and settle.

7.2 Performance of single-stage anaerobic bioflocculation process treating low grease WSE

To compare the efficiency of bioflocculation process under psychrophilic and mesophilic optimum temperature, two parallel systems were set up and operated at 20 °C and 37 °C respectively.

Throughout 93 days of continuous operation, no operational difficulties were experienced with the feeding or mixing pumps. There were no major blockages detected in the system. However, during the first four weeks of operation a few slight obstructions occurred in the U-shape liquid trap (Figure 3.3.2.2) in the trial operated at 37 °C. This was due to sludge frothing and washout from the reactor.

It is worthwhile noting that an attempt to operate the system in the same manner as a sludge blanket had been initiated. This was thought to be able to reduce the energy for mixing by diffusing the feed through the sludge bed. However, the process suffered from several problems:

- gas produced from the process was not enough to generate mixing in the reactor between new feed and biomass,

- settling grease which accumulated in the reactor combined with biomass to form a compact layer which did not allow the feed to diffuse through the blanket, indicated by the cracking of the blanket with the new feed running through the cracks without contacting the biomass.

7.2.1 Characteristics of anaerobic sludge and feed

Both systems were started up and operated with identical anaerobic digestion sludge and WSE. It can be seen that the anaerobic digestion sludge used in the experiment contained rather low grease content of only 1.17 g/L (Table 7.2.1.1), compared to WSE which had an average grease content of 7.75 g/L, with a range of 5.5 to 9.5 g/L (Table 7.2.1.2). The HRT of the processes was set to 3 days which resulted in fluctuations of the grease load of between 1.8 to 3.1 g/L/day.

Table 7.2.1.1 Characteristics of anaerobic sewage sludge used for the start up of the reactor.

	Mean ± Std. Dev.
Dry solids (TS) (g/L)	23.65 ± 0.35
VSS (g/L)	17.18 ± 0.46
Grease (g/L)	1.17 ± 0.014
Biomass (g/L)	16.00 ± 0.44
% Grease in dry solids	4.95 ± 0.014

Table 7.2.1.2 Characteristics of WSE used in single-stage bioflocculation process.

Parameter	Mean	Std. Dev.	Minimum	Maximum
Grease (g/L)	7.75	1.08	5.46	9.51
COD (g/L)	28.68	5.16	19.08	34.40
TS (g/L)	24.94	5.81	14.35	32.92
SS (g/L)	13.91	4.27	5.85	5.85
pH	7.95	0.28	7.46	8.63
VFA				
-Acetic acid (mM/L)	14.35	5.32	5.10	27.14
-Propionic acid (mM/L)	8.16	5.51	1.88	16.75
-Butyric acid (mM/L)	0.15	0.20	0.00	0.96

7.2.2 The efficiency of the systems

The removal of grease in both processes, at 20 °C and 37 °C, fluctuated considerably during the first half of the experiment, but stabilised over the latter half of the experiment (Figure 7.2.2.1). The average grease reduction was 40% and 44% from the process operated at 20 °C and 37 °C respectively.

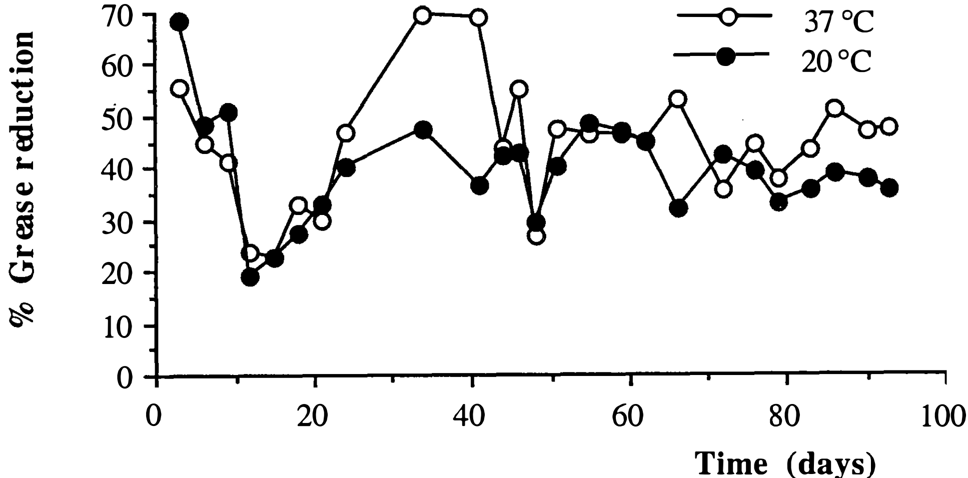


Figure 7.2.2.1 The efficiency of grease removal from single-stage anaerobic bioflocculation operated at 20 °C and 37 °C

As grease is the major source of COD in WSE, the pattern of % COD reduction of the processes as shown in Figure 7.2.2.2 is similar to that of % grease reduction as described previously. The average COD reduction from process operated at 20 °C and 37 °C was 37% and 43% respectively.

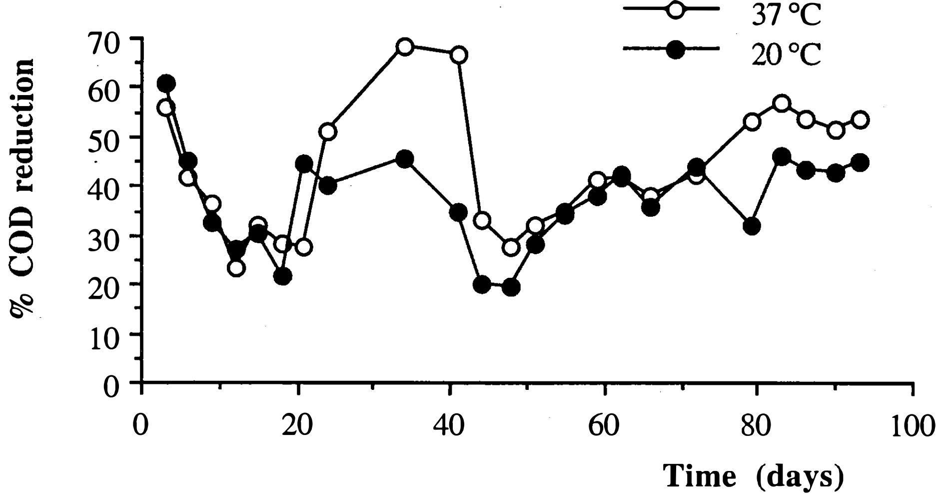


Figure 7.2.2.2 The efficiency of COD removal from single-stage anaerobic bioflocculation operated at 20 °C and 37 °C

There was no difference in terms of process efficiency between the process operating at 20 °C and 37 °C. Biomass in the process at 20 °C was much higher toward the end of the experimental period (Figure 7.2.2.3), due to sludge washout in the system operated at 37 °C. By contrast, sludge built up in the process operated at 20 °C. The efficiency of both processes however was still the same, or even slightly higher in the process operated at 37 °C.

Why? Why?

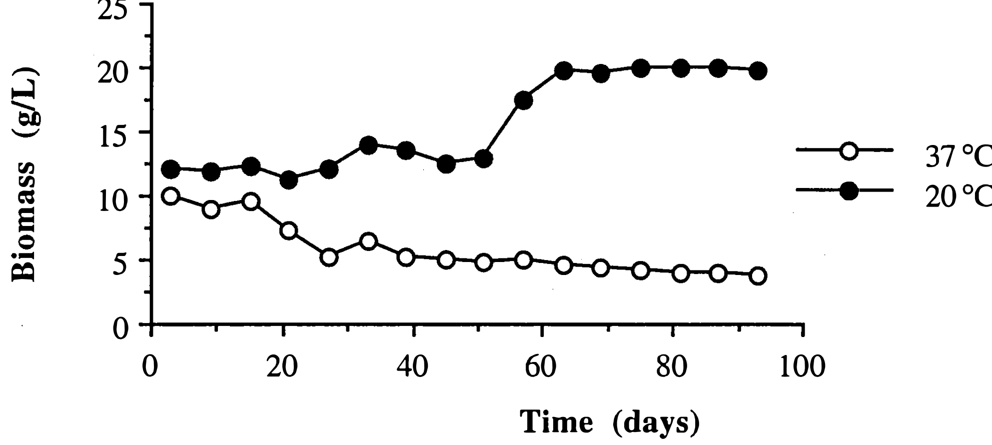


Figure 7.2.2.3 Biomass (calculated as volatile suspended solids minus grease) in the anaerobic bioflocculation reactors during the period of experiment

Figure 7.2.2.4 shows the specific grease reduction (presented per gram of biomass in the reactor per day) of both systems. It can be seen that the specific grease reduction of the process operated at 37 °C (average 300 mg grease/g biomass/day) was considerably higher than the process operated at 20 °C (average 50 mg grease/ g biomass/day). Since the accumulation of biomass in the system at 20 °C was not associated with the process efficiency, the microbes which successfully grew in this system were possibly not the species responsible for bioflocculation.

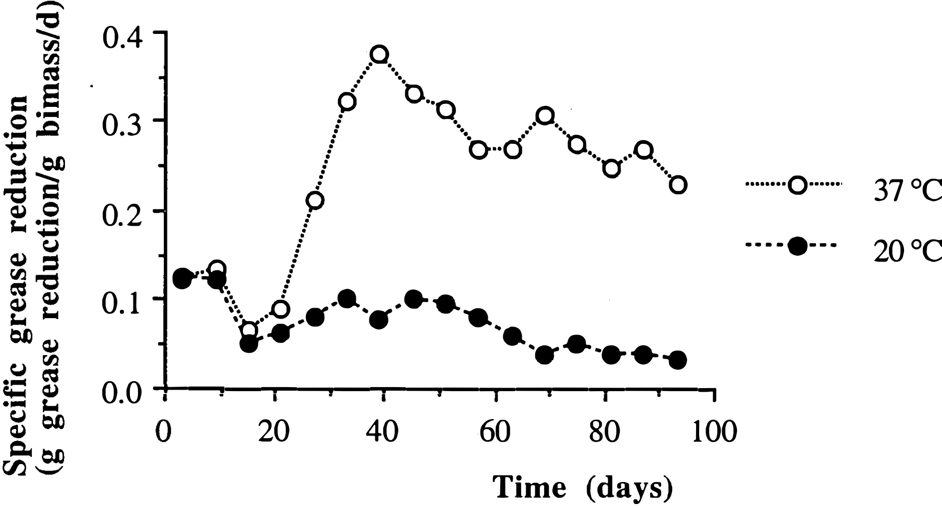


Figure 7.2.2.4 Specific grease reduction (presented as grease removal from the supernatant per gram of biomass per day) of single stage anaerobic bioflocculation operated at 20 °C and 37 °C

From the results of this experiment, the efficiency in terms of grease (44%) and COD (43%) removal from a semi-continuous anaerobic bioflocculation process was not as high as expected. In the batch system, an efficiency of over 60% grease removal could be obtained (see chapter 5). However, with a short HRT of 3 days the efficiency obtained from the process would still seem to be more favourable than in a conventional anaerobic digestion process, where to obtain the same level of grease reduction would require a longer HRT of 10-15 days (Genon *et al,* 1984; Whitaker & Stewart, 1985).

7.2.3 Biogas production and VFA reduction

In this experiment, even though anaerobic bacteria were maintained in the process, and the operating conditions provided for a biomethanation process (the trial at 37

°C), only small amounts of methane production (average 350 ml biogas/L reactor/day during the first 3 weeks) were observed at the beginning of the experiment, and almost no methane was produced when the process reached steady state (Figures 7.2.3.1). This indicated that using WSE as feed did not create conditions suitable for methanogenesis.

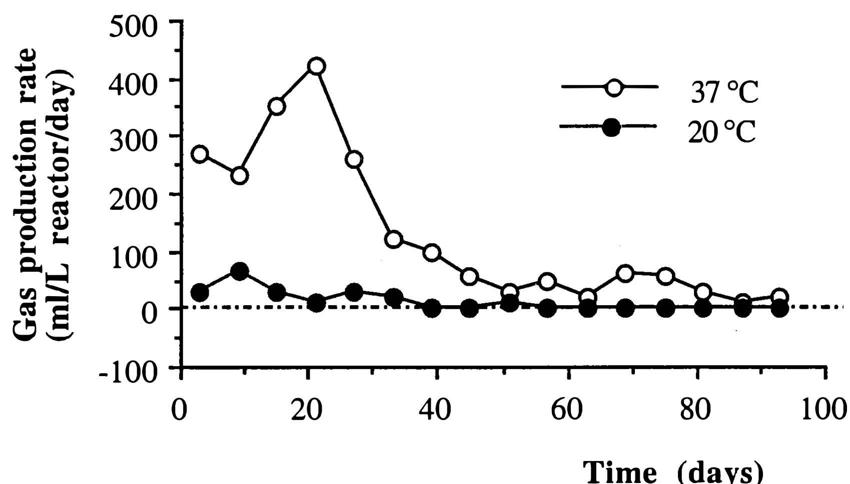


Figure 7.2.3.1 Gas production from the single-stage anaerobic bioflocculation process operated at 20 °C compared to 37 °C

Theoretically, at the average COD loading rate of 9.56 g/L reactor/day (Table 7.2.1.2), a reduction of 43% COD (4.11 g COD) should result in 1 570 ml of CH_4 produced per litre per day, based on 1 g COD producing 382 ml CH_4 (at 20 °C), or 2 242 ml of biogas per day (70% methane concentration was measured).

Under anaerobic conditions a net COD removal is only possible by the production of reduced gases such as CH_4. Therefore the removal of grease and COD from the process was not the result of complete biodegradation but rather bioflocculation and

adsorption of organics, particularly grease into the sludge phase. In the continuous systems, due to the sludge washout from the reactors, a reduction of biomass in the system occurred during the first 4 weeks (Figure 7.2.2.3). Thus a correct mass balance (grease and COD) could not be established. However, from batch experiments it was known that wool grease was not degraded during the anaerobic bioflocculation process (Table 5.2.3 and Figure 5.2.4).

It must be noted that, although all processes were started with anaerobic digestion sludge as the inoculum, the original gas production of the sludge decreased during the course of the experiment (Figure 7.2.3.1) indicating that using WSE as feed did not create conditions suitable for methanogenesis. The reason for a lack of methane production was not clear, as the solids residence time in the reactor was longer than 15 days and suitable methanogenic substrates (acetate, propionate) were present (Figure 7.2.3.2). Since there was no evidence of volatile fatty acid accumulation (Figure 7.2.3.2) and the pH of the processes were quite constant (Figure 7.2.3.3), the suppression of methane gas production was not due to organic overloading, which would result in VFA accumulation and a drop in pH. Organic overloading is likely to occur only with easily fermentable substrates (e.g. carbohydrates). It can be speculated that the methane producing bacteria were inhibited by substances in WSE such as the surfactant (Isaac, 1991).

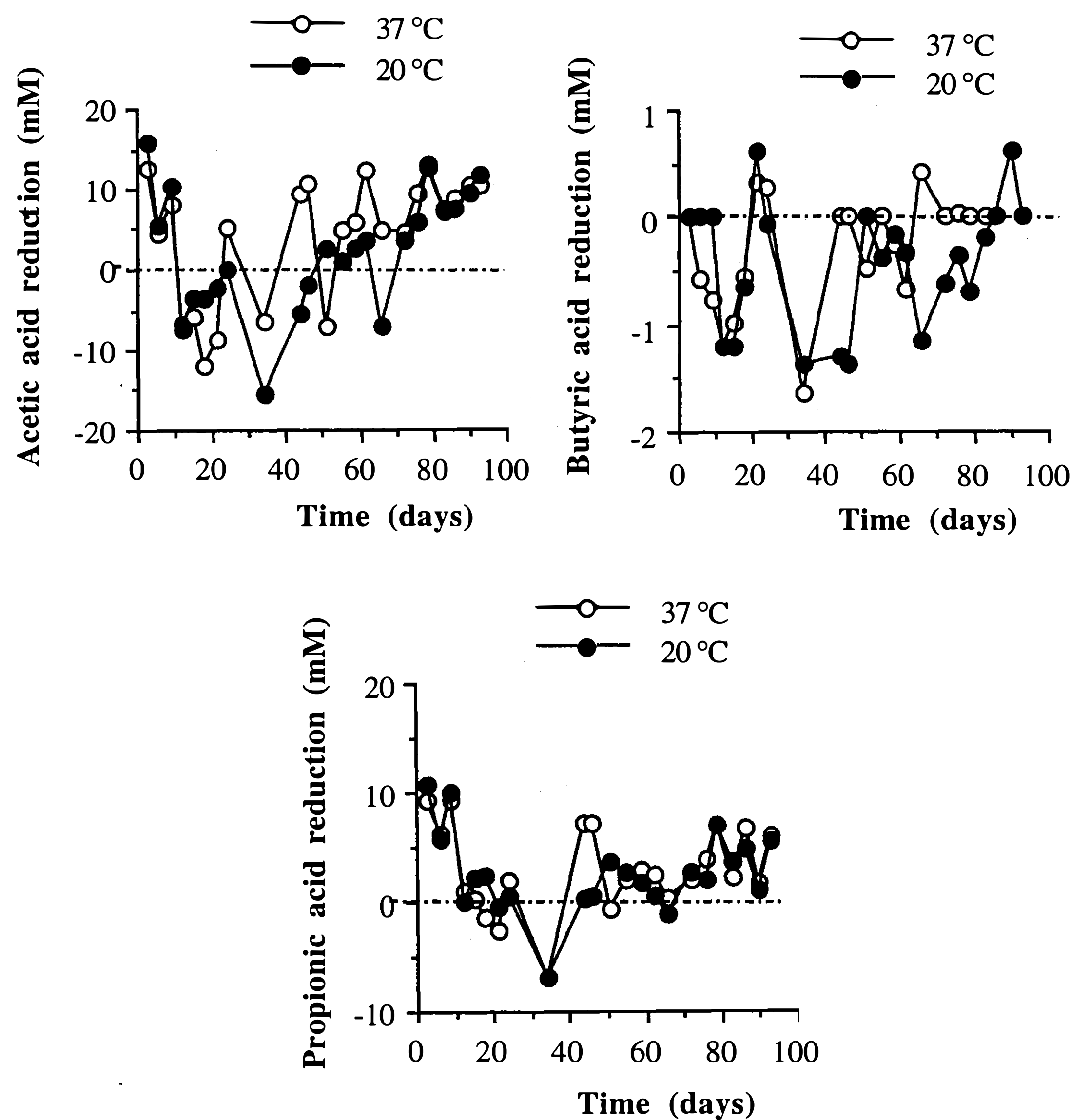


Figure 7.2.3.2 VFA reduction in the single-stage anaerobic bioflocculation process (calculated from VFA of the feed minus VFA in the effluent from the process, the negative value indicated VFA accumulation in the system)

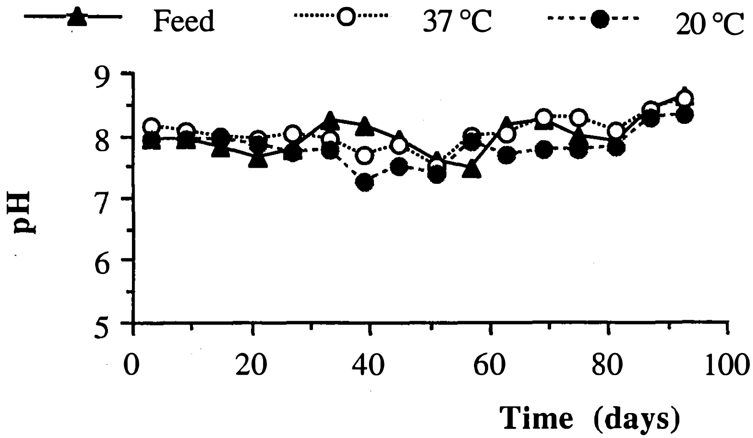


Figure 7.2.3.3 pH of treated WSE from anaerobic bioflocculation process compared with the feed

Since the anaerobic suspended bacteria did not produce any methane gas, their energy must be derived from the fermentation of some components within the WSE. It is unlikely that highly reduced compounds such as grease represent a good fermentation substrate. Probably, the suint fraction of WSE contains easily fermentable compounds allowing the flocculating bacteria to develop and as a consequence, destabilise wool grease emulsion. Further evidence of this is the increase in grease content inside the reactors from 4.95% (grease in dry solids) in the original sludge to 55.1% and 61.3% (reactor at 37 °C and 20 °C respectively) by day 95 of the experiment (Figure 7.2.3.4). This observation confirms the finding from the batch experiment (see section 5.2) that the anaerobic treatment did not cause biodegradation of wool grease, but rather flocculated the wool grease from the liquid into the sludge phase.

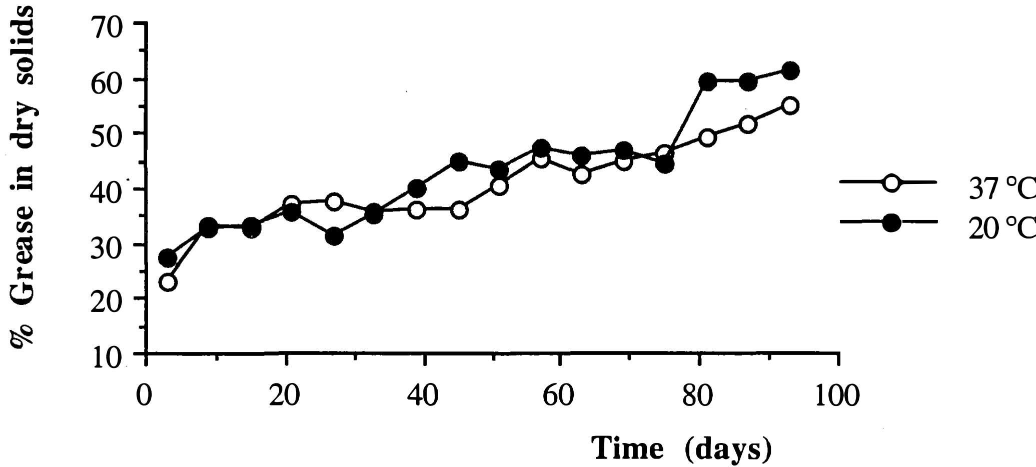


Figure 7.2.3.4 Accumulation of grease in the reactors (presented as percentage grease content in dry solids)

It can be noted that the accumulation of grease in the sludge (Figure 7.2.3.4) did not appear to reduce the efficiency of the process during the period of the experiment (Figure 7.2.2.1). However, the system may reach a point where the sludge becomes saturated with grease. The greasy sludge, therefore, will need to be withdrawn and treated separately.

7.2.4 Aerobic post-treatment of the effluent from anaerobic bioflocculation system

An aerobic treatment process, as described in section 3.3.4, was employed to purify the effluent from the anaerobic bioflocculation process. Since only about 40% of grease was removed by the bioflocculation system, the effluent used for feeding the aerobic process still contained high amounts of grease, averaging 4.6 g/L. By setting the HRT at 3 days, the grease load for the aerobic process was 1.5 g/L reactor/day.

The effluent from the anaerobic bioflocculation process was found to be successfully treated by an aerobic post treatment process without nutrient supplement (Figure 7.2.4.1). The efficiency of the process was very constant, with an average of 94% grease reduction. With the combined processes of anaerobic bioflocculation and aerobic treatment, approximately 96% of grease in WSE was removed at an HRT of 6 days; 3 days in the bioflocculation reactor and 3 days in the aerobic reactor (Figure 7.2.4.1).

In spite of continued aeration of the mixing chamber, the oxygen concentration in the settling chamber was found to be zero at all times. The black colour and the distinct odour of H_2S obtained indicated that anaerobic conditions existed here. However, the efficiency of the process was not affected.

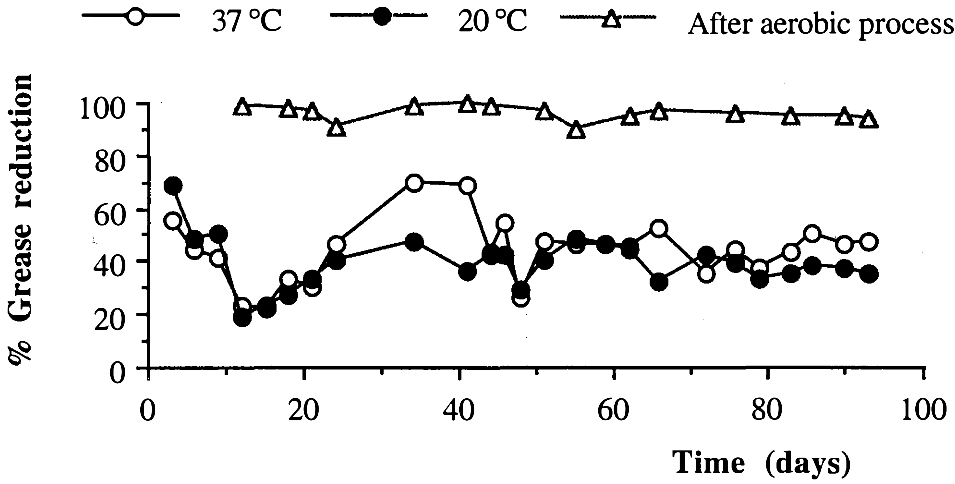


Figure 7.2.4.1 % Grease reduction of single-stage anaerobic bioflocculation alone and the total grease reduction with the additional aerobic process

7.3 Performance of two-stage anaerobic bioflocculation process treating high grease WSE

This experiment was designed to treat WSE containing high grease concentrations. The process employed a two-stage anaerobic bioflocculation as described in section 3.3.3. The WSE samples used as feed in the experiment were selected from samples with grease content of more than 10 g/L.

Since the aim of this experiment was to investigate the feasibility of employing the anaerobic bioflocculation process to treat high strength WSE, rather than to develop an understanding of the process mechanism, only the efficiency of the process in terms of grease and COD was monitored.

Throughout the operation period of 110 days, no problems were encountered with the feeding pumps. However, toward the end of the experiment some operational difficulties were experienced during mixing. As described in section 3.3.2, intermittent mixing was used in the experiment. During the settling period, grease was found to accumulate in the sludge, forming a compact layer of greasy sludge at the base of reactor. The compact greasy sludge sometimes blocked the sludge circulating outlet, resulting in impaired mixing between the sludge with fresh WSE. The problem was, however, resolved by recirculating the supernatant back to the base of the reactor to break the compact sludge, for 1 to 2 min at the start of the mixing interval, and found to be an effective way to remove all of the obstruction.

7.3.1 Characteristics of anaerobic sludge and feed

The characteristics of the anaerobic digestion sludge used for the start up of this process were similar to that used in the single stage (Table 7.3.1.1 and 7.2.1.1), while the WSE sample used in this experiment contained much higher grease

concentrations. At the start of the experiment grease obtained from the scouring plant was as high as 34 g/L and then gradually dropped to 13 g/L at the end of the experiment (Figure 7.3.1.1).

Table 7.3.1.1 Characteristics of anaerobic sewage sludge used for start up of a two-stage anaerobic bioflocculation.

	Mean $\pm$ Std. Dev.
Dry solids (TS) (g/L)	25.15 $\pm$ 0.30
VSS (g/L)	18.90 $\pm$ 0.36
Grease (g/L)	1.21 $\pm$ 0.011
Biomass (g/L)	17.69 $\pm$ 0.47
% Grease in dry solids	4.81 $\pm$ 0.014

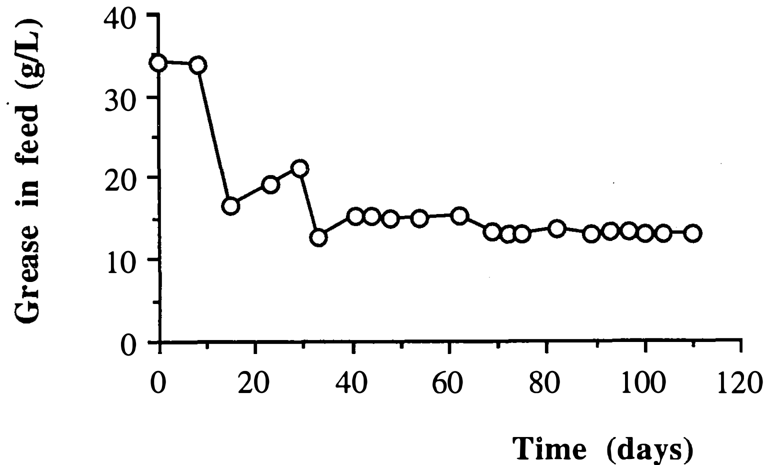


Figure 7.3.1.1 Grease concentration in WSE samples used as feed to the first stage of a two-stage reactor (Figure 3.3.3.1) during the period of experiment

By gradually reducing the hydraulic residence time (HRT) for stage 1 from 9 to 2 days, the grease loading rate of the system increased proportionally (Figure 7.3.1.2). From day 41, HRT was maintained constant through to the end of the experiment.

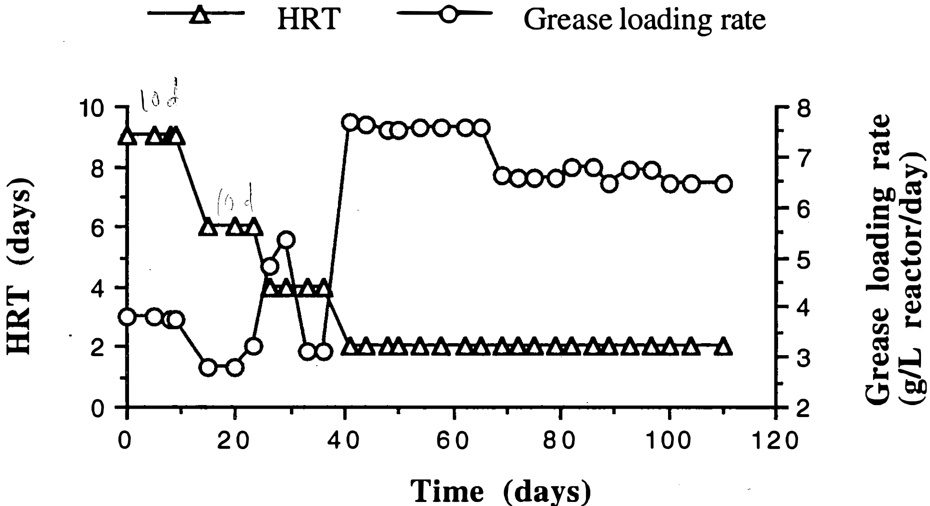


Figure 7.3.1.2 Hydraulic residence time and grease loading rate of the first stage anaerobic bioflocculation during the period of experiment

7.3.2 The efficiency of the systems

The efficiency of grease removal of the first stage (Figure 7.3.2.1) was found to be more stable and considerably higher than that obtained in the single-stage process used to treat low grease WSE in section 7.2. An efficiency as high as 80% was obtained with a long HRT of 6 to 9 days, with an average loading of 3.5 g grease/L/day. When the HRT was maintained at only 2 days (day 41 to day 110) and the average loading rate was doubled to 7 g grease/L/day, the average grease removal was still as high as 60% (about 40% grease reduction was obtained in the process treating low grease WSE).

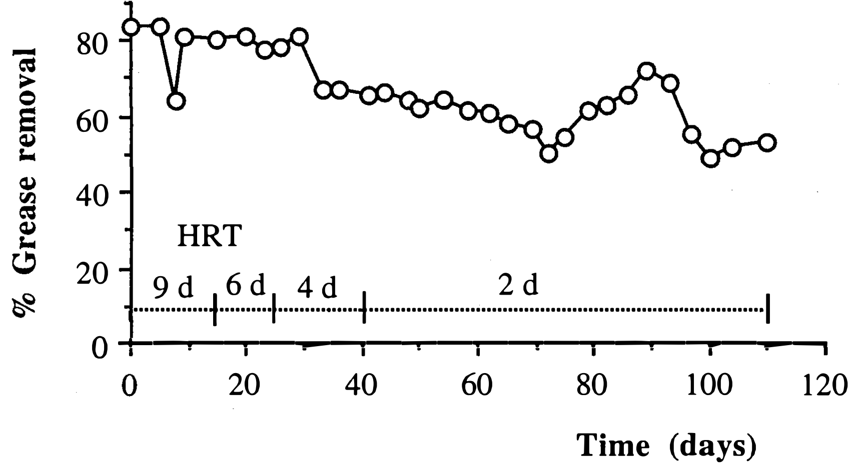


Figure 7.3.2.1 The efficiency of grease removal by the first stage of the two-stage anaerobic bioflocculation

It is interesting to compare these results with those achieved by Rodmell & Wilkie (1983) in a conventional stirred anaerobic system. In a pilot reactor treating high strength WSE (average grease concentration of 14 g/L), a long HRT of 20 days (loading rate of 0.7 g grease/L/day) was required to obtain 52% grease removal. The efficiency was markedly lower than that obtained in the bioflocculation system. To achieve a similar result, a considerably shorter HRT of only 2 days (with a loading rate of 7 g grease/L/day) was required. This study therefore showed a significant increase in grease removal in anaerobic bioflocculation over the anaerobic digestion process. With the reduction of HRT from 20 days to 2 days, the size of reactor would be considerably reduced. Together with the reduction in mixing required (see section 3.3.2), the capital and running costs of a bioflocculation process would be significantly lower than those of conventional anaerobic digestion. However, a drawback of bioflocculation process is the sludge produced is more greasy and rather

bulky than that from anaerobic digestion, and therefore requires more treatment before disposal.

In the second stage of bioflocculation where some grease content in the feed (effluent from the first stage) had been removed (dropping to an average of 5.2 g/L) the efficiency of the process fluctuated noticeably (Figure 7.3.2.2). The average efficiency of this stage (48.2% grease reduction) was considerably lower than obtained in the first stage. The more stable grease was more difficult to remove by flocculation (see section 2.3.4.3) . It is possible that the less stable (less oxidised) grease emulsion had been removed in the first stage. The feed for the second stage contained a more stable (more oxidised) grease emulsion, which therefore resulted in lower process efficiency.

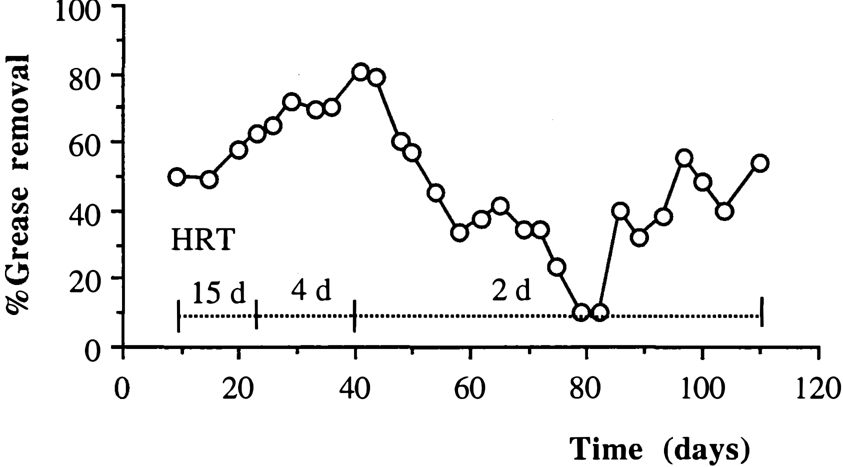


Figure 7.3.2.2 The efficiency of grease removal by the second stage of two-stage anaerobic bioflocculation

It is worthwhile noting that this reduction and fluctuation in process efficiency experienced in the second stage bioflocculation is somewhat similar to that obtained in the single stage process (see section 7.2) used to treated low grease WSE. Since one parameter in common for both processes is the lower grease concentration in the feed, this may suggest that the bioflocculation was more efficient with high-grease-containing WSE. This observation was similar to the finding obtained by Cord-Ruwisch *et al.* (1990), which indicated that, in an aerobic process treating WSE, at increased loading rate, COD removal by aerobic bioflocculation (sedimentation of grease with biomass) was higher and became more important than that by oxidation.

Although the reason of this phenomenon is not yet clear, a hypothesis is that the destabilisation of wool grease emulsion in concentrated WSE is easier due to one or more of:

(1) the presence of substrate which promotes growth of bacteria/microorganisms involved in the bioflocculation;

(2) the change of density of the aqueous phase. The stability of contaminants in the wool scouring liquor was due to the density of the combined particles of dirts, surfactant, suint, and grease being close to the density of the continuous phase (Wood *et al.,* 1979). When contaminants were increased the density of combined particles became higher, which as a consequence are easier to destabilise;

(3) the salting-out of the surfactant by suint salt (potassium salt of organic acids) (Anderson & Christoe, 1981).

The efficiency of bioflocculation in treating low-grease-containing WSE was similar to that obtained from a semi-continuous digester investigated by Cail *et al.* (1986). The concentration of feed used in their experiment averaged 7.0 g grease/L (average 7.7 g/L in this study). To obtain 47% grease reduction, the process required an HRT of 2.8 days with a loading rate of 2.75 g grease/L/day. In this study, a 44-48% grease reduction was achieved with a HRT of 2-3 days (loading rate approximately 3

g grease/L/day). The semi-continuous digester required an extra 3-10 mg polyelectrolytes/L reactor/day to maintain the biomass in the system. This would make it more expensive to operate than the bioflocculation process. By contrast, although the bioflocculation process seems to be less expensive to operate, the efficiency was, however, less stable and still requires more investigation before the process can be optimised.

Although anaerobic bioflocculation treatment of the low grease WSE did not reduce the pollution load markedly (about 40% COD removal), it was found to substantially increase the efficiency of further chemical flocculation by reducing considerably chemical flocculant requirements (when compared to the same WSE without previous bioflocculation) (Cord-Ruwisch, personal communication). This is confirmed by the findings of section 5.9 (Table 5.9.2), where the biological destabilisation of wool grease had occurred in the difficult-to-flocculate WSE, as in the easy-to-flocculate WSE. It was not, however, to the extent required to cause flocculation during the period of the experiment. Pilot scale trials (2 m^3 reactor) employing anaerobic bioflocculation assisted by chemical flocculation post-treatment are currently under investigation.

In conclusion, by employing a two-stage anaerobic bioflocculation system to treat high-grease-containing WSE, a high grease removal of average 80.5% (64% to 90%) was obtained with a combined HRT of 4 days, 2 days in each stage (Figure 7.3.2.3). The average efficiency of COD removal was 77.3% (60-86%).

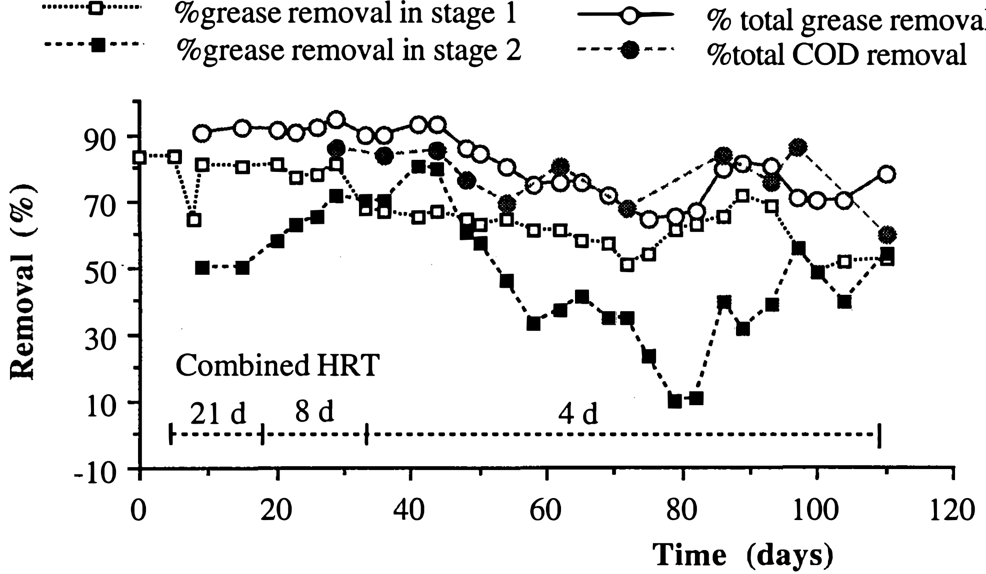


Figure 7.3.2.3 The efficiency of grease and COD removal using the two stage anaerobic bioflocculation treating high-grease-concentration WSE

Although the process removed up to 90% grease from the original sample the final effluent still contained more than 1 500 mg/L of grease which requires further purification before it can be released to the sewer or a receiving environment. A further stage of aerobic treatment (see section 3.3.4) was attached to the anaerobic bioflocculation stage and resulted in the continuous significant removal of the grease (>97%) to less than 100 mg/L (HRT 3 days). The total BOD_5 removal of the total treatment train was therefore greater than 99%.

Chapter 8 Conclusions and Recommendations

8.1 Conclusions

This study has proven that successful biological grease removal from WSE does not necessarily require degradation (as in the aerobic and anaerobic digestion process) but can be due to the destabilisation of the wool grease emulsion resulting in grease flocculation. Microbes from anaerobic sludge of a municipal wastewater treatment plant were able to grow in WSE which resulted in partial flocculation of grease in WSE. This process was termed an **Anaerobic Bioflocculation Process**. Since bioflocculation was found to occur much faster than biodegradation, such a process could serve as a low cost pretreatment of the WSE prior to a classical activated sludge type treatment. The mechanisms of anaerobic bioflocculation could therefore play a role in the development of a future treatment process for WSE.

In fact, the process of flocculating wool grease by anaerobic microbes also occurs naturally in anaerobic lagoons treating WSE (see section 4.5). However, contaminant removal from the supernatant was thought to be the consequence of biodegradation. The flocculation of grease emulsion in the lagoon had therefore been neglected. Under a controlled operation, the efficiency of the anaerobic bioflocculation process as investigated in the present study was found to be much higher than that of a lagoon system. To obtain 93% BOD_5 reduction, the combined anaerobic and aerobic lagooning system required a long HRT of 42 days (see section 2.4.4.1), while only 7 days were required by a combined anaerobic bioflocculation and aerobic digestion process (see chapter 7).

The growth of aerobic bacteria in WSE is also known to cause flocculation (Cord-Ruwisch *et al.*, 1990; Roth *et al.,* 1989). However, due to an extremely high oxygen

demand and foaming problems the classical aerobic treatment has significant disadvantages. Anaerobic digestion of WSE has been shown to be a difficult and slow process (Isaac & Cord-Ruwisch, 1991). Thus the removal of the bulk of the grease (about 70%) by technically simple anaerobic bioflocculation process appears to be an economic option. Moreover, bioflocculation of grease (COD) was found to play a greater role at higher organic loadings. A treatment based on bioflocculation may be a more economical way to remove emulsified wool grease from the effluent water phase than a treatment aiming at achieving the highest possible oxidation. This would generate a higher grease content sludge but would utilise less air, probably require less nutrient supplementation, and require a smaller reactor vessel than for more complete COD oxidations. In contrast to chemical flocculation or aerobic bioflocculation, the anaerobic bioflocculation does not need any further additives, such as flocculant or oxygen.

The major problem associated with the bioflocculation process was caused by the greasy sludge (50% grease in dry solids) produced from the system. Since the water content of the sludge was still high (85-90%), a sludge dewatering system is required before disposal. Alternatively, this sludge may be treated by anaerobic digestion (at prolonged HRT) to oxidise the wool grease with the biomass then recycled to the bioflocculation system. Another possibility is the recovery of saleable wool grease (lanolin) from the sludge (McCracken & Chaikin, 1978a), which may require an expensive extraction system. A further investigation of the sludge handling process is therefore essential.

8.2 Proposed treatment process for WSE

The performance of the culture was found to vary with grease concentration and the amount of free detergent (due to overdosing during wool scouring). At a constant hydraulic loading rate, the grease removal efficiency increased with increasing

grease and/or decreasing free detergent content. This indicates that close integration of the wool scouring technique and effluent treatment is necessary. It can be suggested from this study that:

(1) the type and dose of detergent used in the scouring process can impact on the efficiency of the effluent treatment process. The close monitoring of the detergent dose is recommended, with excess surfactant avoided;

(2) the effluent from wool scouring plants should be separated into high grease effluent from scouring and low grease effluent (rinse water) as in Figure 8.2.1. The effluent from the scouring bowl which contains high level of contaminants could be treated by an anaerobic bioflocculation process. The effluent from this system and the rinse liquor could be combined, and then treated aerobically. The greasy sludge from the bioflocculation system, as well as activated sludge, can be treated by anaerobic digestion. The non-greasy sludge (biomass) can be returned to the bioflocculation system. The excess sludge would be easily disposed.

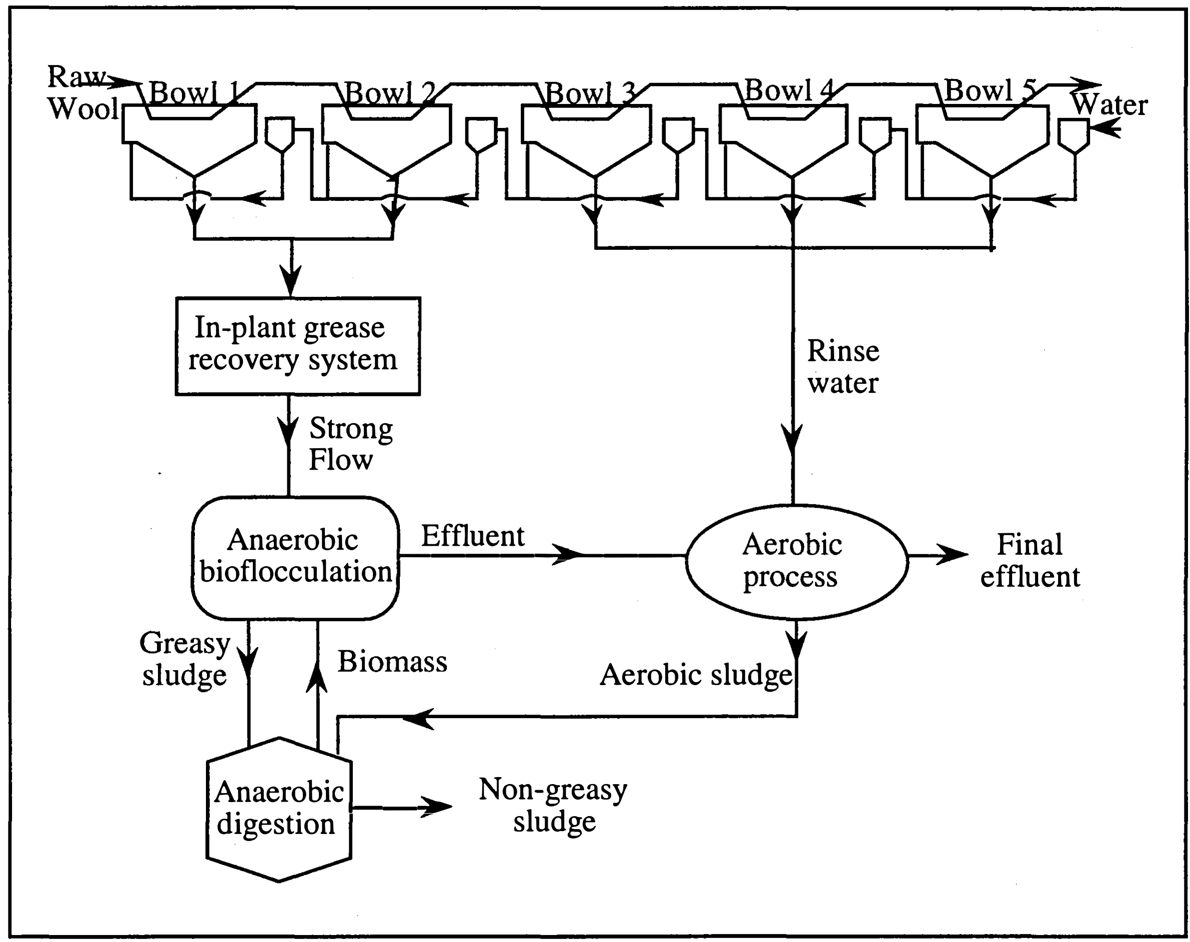


Figure 8.2.1 Proposed in-plant modification and effluent treatment process for wool scouring plant

8.3 Recommendations for further study

Although this study has shown the feasibility of employing the anaerobic bioflocculation process as a pretreatment process to remove wool grease emulsion from WSE, prior to classical aerobic or anaerobic process, knowledge of the process mechanisms is still limited. The successful application of this process within the industry therefore requires further studies, particularly the detailed process mechanisms. Without a clear picture of the fundamental mechanisms, the optimisation of the process is not possible.

Several aspects are recommended for further investigation:

1. This investigation provides evidence of partial biodegradation of non-ionic surfactant which is postulated to be one factor causing flocculation of wool grease emulsion in WSE during anaerobic bioflocculation process. Other factors, namely bio-polymers and enzymes, which may contribute to the process, require further investigation.

2. It was clearly shown that the characteristics of WSE itself play a major role in the success of the process. Further investigation of the parameters influencing destabilisation of wool grease emulsion, and therefore bioflocculation ability, is recommended. Knowledge of these parameters could assist industry to achieve constant production of easy-to-flocculate WSE, optimised for these treatment processes.

3. There is a requirement for investigation into the feasibility of methods for the disposal and/or further treatment of the greasy sludge produced by this process, as sludge from the process may contain as high as 50% wool grease in dry solids (Figure 7.2.3.4). A feasibility study on the recovery of wool grease from this greasy sludge may increase benefit for the scourer as well as reduce the sludge disposal costs.

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Appendix 1 The effect of autoclaved WSE on the efficiency of anaerobic bioflocculation

In chapter 5 the investigation of bioflocculation mechanisms using batch experiments required WSE with consistent characteristics throughout the period of experiment. One large batch sample of WSE (200 L) was taken. Autoclaving was chosen to be the preservation procedure by sterilising the sample, therefore inhibiting any further biological activity during storage. This experiment was performed to examine whether autoclaving had an effect on bioflocculation ability of the sample, and therefore the possibility of using this procedure to preserve WSE sample.

A 1.1 Materials and Methods

Five litres of WSE sample was taken from the local wool scourer. Half of the sample was then autoclaved immediately at 132 °C for 15 min.. The other half of the sample was kept at 4 °C. These two samples were then compared for the anaerobic bioflocculation ability by using 70 ml test-tubes as reactors. The WSE samples were mixed with anaerobic sludge, at the proportion of 75% WSE and 25% sludge.

Air in the head space was displaced by nitrogen gas, all reactors were then sealed and gently mixed for 30 min. before being incubated at 30 °C. After 15 min. and daily for 8 days, two reactors from each trial were taken, the supernatant of the liquor were analysed for grease content.

A 1.2 Results and discussion

Figure A 1.2.1 shows the comparison of bioflocculation ability of the same WSE sample with and without autoclaving. It can be seen that the pattern of bioflocculation of these two trials had no significant difference.

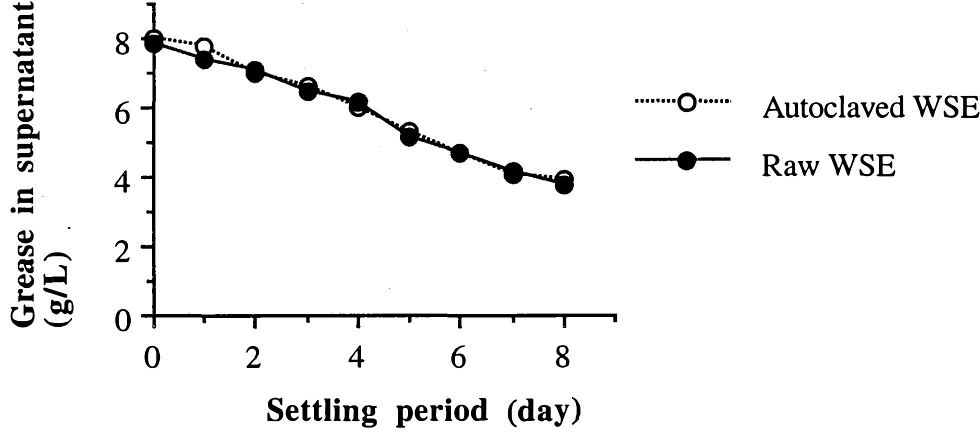

Figure A 1.2.1 Grease content in the supernatant of raw and autoclaved WSE during the anaerobic bioflocculation process

From this experiment, it can be concluded that autoclaving can be used as an acceptable method to preserve WSE sample for further experiments.

Appendix 2 Viable counts of WSE during anaerobic bioflocculation process

The aim of this experiment was to investigate different methods for counting the numbers of bacteria in WSE under anaerobic bioflocculation treatment. The most accurate method would then be selected to used in the further experiments.

A 2.1 Materials and methods

A 2.1.1 Liquid Count

A sample of WSE which had undergone anaerobic bioflocculated for 3 days was diluted in 10 fold dilution to 10^{-9} using tryptic soy broth as media. Duplicates of aerobic and anaerobic were made of each dilution series. Aerobic dilutions were made in screw caps test-tubes and anaerobic dilutions in Hungate tubes that were degassed with nitrogen gas immediately after autoclaving.

A 2.1.2 Plate counts

The same WSE sample of section A 2.1.1 was diluted in 10 fold dilution to 10^{-9} using sterile WSE. Samples of 100 µl of each series dilution were put onto plates of WSE agar (see section 3.3.1.1) and then spread via a sterile drigalki spatula. Each WSE dilution series was plated in duplicates and one complete set of duplicates incubated aerobically and the other incubated anaerobically using an anaerobic jar (see section 3.3.1.2).

All dilution series of broths and plates were incubated at 30 °C. Broths were checked after 24 and 48 hrs. The growth of bacteria was measured by an increase in optical density. This was performed by reading the absorbence of the sample (using spectrophotometer at 600 nm) after inoculation compared to the reading after 24 and 48

hrs. The results were confirmed by samples viewed under the microscope. The highest dilution step at which bacteria were found was then used to determine the number of bacteria.

Aerobic plates were checked after 24 hrs and anaerobic plates were checked after 96 hrs. The number of bacterial colonies were determined using a colony counter at a magnification of 10x.

A 2.2 Results and discussion

Anaerobic count were hindered due to the bacteria swarming over the agar surface and not forming discrete, distinct colonies. From the results in Table A 2.2.1, the numbers of bacteria counted in the broths were about 10 fold and 1 000 fold lower than plate counts for aerobic and anaerobic bacteria respectively. Aerobic WSE plate counts were considered to be the more sensitive method utilised.

Table A 2.2.1 Bacterial growth on tryptic soy broth and WSE plate, registered as either bacteria per ml of inoculant, positive or negative.

Dilution	Broth (bacteria/ml)		Plate (bacteria/ml)	
	Aerobic	Anaerobic	Aerobic	Anaerobic
10^{-3}	+	+	+	+
10^{-4}	+	+	+	+
10^{-5}	+	10^{-5}	+	+
10^{-6}	+	-	1.8×10^{-6}	+
10^{-7}	10^{-7}	-	2.6×10^{-7}	+
10^{-8}	-	-	4.0×10^{-8}	+
10^{-9}	-	-	-	-

Appendix 3 Raw data

A 3.1 Investigation of bioflocculation mechanisms by batch experiment

A 3.1.1 Confirmation of bioflocculation as a biological process

Table A 3.1.1.1 Removal of emulsified grease by flocculation in the supernatant incubated with active and sterilised sludge.

Mixing period (days)	Grease in the supernatant			
	Sterilised system		Non-sterilised system	
	Alone	With 24 hrs settling	Alone	With 24 hrs settling
0	4.96	4.66	4.06	3.85
1	5.01	4.64	3.95	2.33
2	5.03	4.40	3.96	2.33
3	4.53	4.41	2.93	1.96
4	4.20	3.99	2.45	1.69
5	4.26	3.94	2.43	1.73

Table A 3.1.1.2 Gas production during bioflocculation process using active and sterilised sludge.

Mixing period (days)	Sterilised system		Non-sterilised system	
	Biogas production (ml gas/L liquor)	Cumulative gas production (ml gas/L liquor)	Biogas production (ml gas/L liquor)	Cumulative gas production (ml gas/L liquor)
0	0	0	0	0
1	0	0	35	35
2	0	0	36	71
3	0	0	22	93
4	0	0	28	121
5	0	0	10	131

A 3.1.2 The effect of mixing and settling periods on grease removal

Table A 3.1.2.1 Grease content in the supernatant after mixing with anaerobic digestion sludge compared with control using distilled water instead of sludge.

Mixing period	With anaerobic sludge	Control
0	6.18	6.18
15 min	5.16	6.02
30 min	4.90	5.95
1 day	4.28	5.83
2 days	3.10	5.85
3 days	2.81	5.86
4 days	2.69	5.89
5 days	2.55	5.88

Table A 3.1.2.2 Grease content in the supernatant after mixing with anaerobic digestion sludge and then following by settling .

Settling period	Mixing period						
(days)	15 min	30 min	1 day	2 days	3 days	4 days	5 days
0	5.16	4.90	4.28	3.10	2.81	2.69	2.55
1	4.68	3.14	2.14	1.50	1.04	2.51	2.35
2	3.71	2.33	1.70	1.09	0.81	1.59	1.43
3	3.11	2.15	1.59	1.09	0.74	1.35	0.71
4	2.68	1.70	1.28	1.01	0.61	0.58	0.51
5	2.46	1.50	1.25	0.89	0.56	0.54	0.43

A 3.1.3 The effect of temperature on grease removal in anaerobic bioflocculation process

Table A 3.1.3.1 Grease content in the supernatant of WSE at different temperatures.

Settling period	Incubated temperature				
(days)	10 °C	20 °C	30 °C	40 °C	50 °C
0	5.53	5.53	5.53	5.53	5.53
1	5.55	5.53	2.35	2.00	2.20
2	5.55	5.02	1.50	1.60	1.70
3	5.40	4.80	1.25	1.30	1.25
4	5.55	4.25	1.10	1.25	1.11

Table A 3.1.3.2 Flocculated grease volume of WSE (based on % of total liquor) after anaerobic bioflocculation at different temperature.

Settling period	Incubated temperature				
(days)	10 °C	20 °C	30 °C	40 °C	50 °C
0	100.00	100.00	100.00	100.00	100.00
1	100.00	100.00	50.00	40.00	30.00
2	100.00	100.00	40.00	36.00	20.00
3	100.00	100.00	35.00	28.00	15.00
4	100.00	100.00	30.00	25.00	15.00

A 3.1.4 The role of fractionated anaerobic sludge (bulk anaerobic sludge, suspended microbes and sludge liquor) on the bioflocculation process

Table A 3.1.4.1 The effect of total sludge and sludge fractions on anaerobic grease flocculation in WSE of the trial using 50% inoculum.

Settling period (days)	Grease concentration in the supernatant (g/L)			
	Control	Total sludge	Solid phase	Liquid phase
0	5.56	5.56	5.56	5.56
1	5.45	4.02	5.34	5.19
2	5.56	2.77	5.17	4.51
3	5.30	2.11	5.12	4.04
4	5.34	1.91	5.11	3.03

Table A 3.1.4.2 The effect of total sludge and sludge fractions on anaerobic grease flocculation in WSE of the trial using 25% inoculum.

Settling period (days)	Grease concentration in the supernatant (g/L)			
	Control	Total sludge	Solid phase	Liquid phase
0	8.33	8.33	8.33	8.33
1	8.36	7.14	8.29	8.04
2	8.54	5.03	8.03	6.60
3	8.44	4.16	7.14	4.99
4	8.31	3.77	6.41	4.76

Table A 3.1.4.3 The effect of suspended anaerobic microbes and sludge liquor on grease bioflocculation in WSE (25% inoculum).

Settling period (days)	Grease concentration in the supernatant (g/L)		
	Control	Suspended microbes	Sludge liquor
0	8.23	8.23	8.23
1	7.98	7.21	6.20
2	7.98	7.19	5.34
3	7.96	7.15	5.03
4	7.94	7.10	4.40
5	7.90	7.00	4.22
6	7.87	6.90	4.13

Table A 3.1.4.4 Emulsified grease content in the supernatant compared to total grease (emulsified grease plus flocculated grease) in the mixed liquor during bioflocculation process.

Settling period (days)	Grease content (g/L)	
	Supernatant	Total
0	8.33	8.33
1	7.14	8.32
2	5.03	8.30
3	4.16	8.31
4	3.77	8.32

A 3.1.5 Efficacy of treated effluent extracts in flocculating raw WSE

Table A 3.1.5.1 The efficiency of grease bioflocculation in WSE using bioflocculation supernatant and flocculated grease compared to the original sludge supernatant.

Settling period (days)	Grease in the supernatant (g/L)			
	Control	Sludge supernatant	Bioflocculation supernatant	Flocculated grease
0	8.25	8.25	8.54	11.12
1	8.25	7.37	7.09	6.89
2	8.14	5.95	5.78	4.53
3	8.19	4.90	4.65	4.10
4	7.93	4.11	3.99	4.10

A 3.1.6 Bioflocculation ability of wool scouring effluent using supernatant of different sludge

Table A 3.1.6.1 Grease content in the supernatant of the trial using different sources of sludge supernatant.

	Grease in the supernatant (g/L)			
Settling period (days)	Control	Source of sludge supernatant		
		Sewage treatment plant	1st anaerobic lagoon	2nd anaerobic lagoon
0	9.00	9.43	10.28	9.86
1	8.85	6.55	7.68	7.53
2	8.70	5.25	7.25	6.75
3	8.65	4.60	5.85	5.38
4	8.50	4.10	5.63	5.38

A 3.1.7 Bioflocculation ability in different wool scouring effluents

Table A 3.1.7.1 Grease bioflocculation ability of different WSEs.

Settling period (days)	Grease content in the supernatant (g/L)							
	Sample A		Sample B		Sample C		Sample D	
	Control	With sludge	Control	With sludge	Control	With sludge	Control	With sludge
0	8.29	8.38	4.47	4.56	8.44	8.53	7.74	8.03
1	8.30	3.78	4.49	4.54	7.66	5.84	7.71	7.74
2	7.82	3.14	4.45	4.13	6.95	5.28	7.55	7.14
3	7.79	2.94	4.41	3.29	6.65	4.55	7.50	6.74
4	7.71	2.76	4.51	3.19	6.49	4.33	7.46	6.30
5	7.70	2.55	4.40	2.79	6.40	4.23	7.30	5.44
6	7.65	2.30	4.48	2.70	6.04	3.79	7.23	5.16
7	7.65	2.01	4.56	2.57	6.06	3.47	7.24	5.09

A 3.1.8 Biodegradation of non-ionic surfactant during anaerobic bioflocculation process

Table A 3.1.8.1 Grease and surfactant concentration in the supernatant compared to that in the mixed liquor (supernatant plus flocculated grease).

Settling period (days)	Grease (g/L)		Surfactant (mg/L)	
	Mixed liquor	Supernatant	Mixed liquor	Supernatant
0	7.34	7.34	232.00	228.00
1	7.30	5.81	229.00	123.62
2	7.32	4.79	231.00	79.99
3	7.25	3.91	220.00	86.45
4	7.16	3.83	205.00	76.75
5	7.15	3.51	210.00	50.90

A 3.2 Investigation of the feasibility of the feasibility of anaerobic bioflocculation treating WSE by semi-continuous operating system

A 3.2.1 Performance of single-stage anaerobic bioflocculation process treating low grease WSE

Table A 3.2.1.1 The efficiency of grease removal.

Experimental	Grease in feed	Grease load	% Grease reduction	
period (day)	(g/L)	(g/L reactor/d)	37 °C	20 °C
3	6.72	2.24	55.80	68.40
6	8.22	2.74	44.60	48.40
9	8.59	2.86	41.10	50.70
12	8.41	2.80	23.60	19.00
15	8.39	2.80	22.70	22.80
18	7.14	2.38	33.00	27.40
21	6.37	2.12	30.00	32.90
24	7.28	2.43	46.70	40.40
34	8.93	2.98	69.30	47.40
41	8.48	2.83	69.10	36.80
44	8.78	2.93	43.80	42.40
46	8.83	2.94	55.00	42.60
48	8.92	2.97	26.60	29.40
51	9.51	3.17	47.40	39.90
55	8.60	2.87	46.10	48.30
59	8.40	2.80	46.30	46.70
62	8.10	2.70	45.00	44.90
66	7.51	2.50	53.00	32.10
72	7.70	2.57	35.60	42.00
76	7.57	2.52	44.50	39.10
79	7.01	2.34	37.40	32.90
83	6.79	2.26	43.30	35.50
86	6.20	2.07	50.80	38.70
90	5.88	1.96	46.60	37.80
93	5.46	1.82	47.10	35.50

Table A 3.2.1.2 The efficiency of COD removal.

Experimental period (day)	COD in feed Grease	COD load (g/L reactor/d)	% COD reduction	
			37 °C	20 °C
3	32.13	10.71	56.10	60.70
6	32.90	10.97	41.90	45.00
9	34.40	11.47	36.60	32.30
12	33.80	11.27	23.40	26.90
15	33.60	11.20	32.10	30.60
18	27.40	9.13	28.10	21.50
21	33.90	11.30	27.70	44.50
24	34.10	11.37	51.00	40.10
34	27.30	9.10	68.60	45.80
41	28.40	9.47	66.90	34.50
44	28.70	9.57	33.10	19.90
48	27.90	9.30	27.90	19.70
51	29.30	9.77	32.10	28.00
55	30.80	10.27	34.70	34.40
59	31.60	10.53	41.10	37.90
62	32.30	10.77	41.80	42.41
66	33.70	11.23	37.98	35.90
72	24.90	8.30	42.20	44.20
79	22.70	7.57	53.30	32.10
83	21.80	7.27	56.90	46.30
86	19.60	6.53	53.83	43.16
90	19.08	6.36	51.50	43.00
93	19.24	6.41	53.90	45.30

Table A 3.2.1.3 Biomass in the reactors during the period of experiment.

Experimental period (days)	Biomass (g/L)	
	37 °C	20 °C
3	10.01	12.03
9	8.91	11.78
15	9.54	12.38
21	7.20	11.22
27	5.30	12.02
33	6.40	14.00
39	5.20	13.60
45	4.90	12.50
51	4.80	13.00
57	4.90	17.58
63	4.50	19.70
69	4.35	19.60
75	4.10	19.90
81	3.97	20.10
87	3.90	20.00
93	3.71	19.80

Table A 3.2.1.4 Gas production.

Experimental period	Gas production (ml/L reactor)	
(days)	37 °C	20 °C
3	270	30
9	234	66
15	350	28
21	420	10
27	260	30
33	120	20
39	100	0
45	55	0
51	30	10
57	50	0
63	20	0
69	60	0
75	55	0
81	30	0
87	10	0
93	20	0

Table A 3.2.1.5 Acetic acid in the feed and effluent from anaerobic bioflocculation process.

Experimental period	Acetic acid in feed	Acetic acid in the effluent (mM/L)	
(day)	(mM/L)	37 °C	20 °C
3	21.46	8.76	5.57
6	22.22	17.68	16.67
9	27.14	19.17	16.91
12	18.45	25.24	25.95
15	21.67	27.50	25.24
18	16.79	28.57	20.48
21	15.01	23.56	17.25
24	17.81	12.62	17.81
34	5.10	11.52	20.62
44	10.61	1.41	16.17
46	10.75	0.00	12.67
51	7.88	15.06	5.20
55	16.21	11.52	15.16
59	10.90	5.10	8.45
62	14.35	1.94	10.66
66	14.38	9.50	21.35
72	10.09	5.72	6.47
76	12.15	2.86	6.43
79	14.80	2.19	1.98
83	9.25	2.06	1.80
86	9.42	0.55	1.94
90	10.68	0.34	1.18
93	12.86	2.69	1.35

Table A 3.2.1.6 Propionic acid in the feed and effluent from anaerobic bioflocculation process.

Experimental period (day)	Propionic acid in feed (mM/L)	Propionic acid in the effluent (mM/L)	
		37 °C	20 °C
3	13.94	4.72	3.26
6	14.16	8.05	8.33
9	16.75	7.40	6.85
12	10.83	9.90	10.74
15	11.48	11.20	9.26
18	8.70	10.18	6.29
21	5.20	7.78	5.58
24	6.92	4.96	6.49
34	2.62	9.58	9.52
44	7.72	0.55	7.47
46	7.23	0.00	6.73
51	7.63	8.31	4.04
55	8.71	6.73	6.05
59	6.89	3.92	5.22
62	6.55	4.07	6.08
66	7.15	6.92	8.41
72	8.22	6.25	5.60
76	7.51	3.69	5.63
79	9.90	3.11	2.98
83	4.40	2.30	0.84
86	6.92	0.26	2.20
90	1.88	0.26	0.97
93	6.41	0.45	0.90

Table A 3.2.1.7 Butyric acid in the feed and effluent from anaerobic bioflocculation process.

Experimental period	Butyric acid in feed	Butyric acid in the effluent (mM/L)	
(day)	(mM/L)	37 °C	20 °C
3	0.00	0.00	0.00
6	0.00	0.57	0.00
9	0.00	0.76	0.00
12	0.00	1.20	1.20
15	0.00	0.98	1.20
18	0.55	1.09	1.20
21	0.96	0.63	0.35
24	0.57	0.30	0.63
34	0.00	1.64	1.38
44	0.00	0.00	1.31
46	0.00	0.00	1.38
51	0.00	0.48	0.00
55	0.00	0.00	0.37
59	0.00	0.26	0.16
62	0.00	0.66	0.34
66	0.42	0.00	1.57
72	0.00	0.00	0.62
76	0.26	0.22	0.62
79	0.00	0.00	0.70
83	0.00	0.00	0.18
86	0.00	0.00	0.00
90	0.62	0.00	0.00
93	0.00	0.00	0.00

Table A 3.2.1.8 pH in the feed and effluent from anaerobic bioflocculation process.

Experimental period (days)	pH in feed	pH in the effluent	
		35 °C	20 °C
3	7.95	8.13	7.95
9	7.94	8.05	7.92
15	7.82	7.98	7.94
21	7.65	7.95	7.84
27	7.77	8.02	7.72
33	8.23	7.94	7.76
39	8.14	7.66	7.26
45	7.94	7.87	7.53
51	7.60	7.52	7.38
57	7.48	7.98	7.91
63	8.13	8.02	7.69
69	8.24	8.26	7.76
75	7.98	8.26	7.76
81	7.88	8.07	7.82
87	8.42	8.41	8.26
93	8.63	8.56	8.33

Table A 3.2.1.8 Grease in dry solids.

Experimental period	% grease in dry solids	
(days)	37 °C	20 °C
3	23.05	27.59
9	33.25	32.96
15	32.71	33.46
21	36.86	35.65
27	37.47	31.11
33	35.60	35.40
39	36.20	40.20
45	36.01	44.60
51	40.60	43.20
57	45.10	47.41
63	42.60	45.97
69	44.80	46.90
75	46.34	44.32
81	49.02	59.21
87	51.60	59.26
93	55.10	61.30

Table A 3.2.1.9 The efficiency of aerobic post-treatment.

Experimental period (days)	Grease in feed (g/L)	Grease in the effluent (g/L)	% Grease reduction*	% total grease reduction**
12	4.60	0.09	98.00	98.93
18	6.76	0.14	97.90	98.04
21	6.80	0.20	97.10	96.86
24	6.92	0.65	90.60	91.07
34	4.95	0.12	98.40	98.66
41.	3.59	0.04	98.90	99.53
44	4.00	0.05	98.70	99.43
51	4.46	0.28	93.70	97.06
55	4.10	0.91	87.00	89.42
62	4.76	0.39	91.80	95.19
66	3.69	0.23	93.80	96.94
76	3.87	0.31	92.00	95.90
83	3.91	0.34	91.30	94.99
90	3.56	0.29	91.80	95.07
93	3.49	0.31	91.00	94.32

* % grease reduction of aerobic process alone

** % grease reduction of anaerobic bioflocculation plus aerobic process

A 3.2.2 Performance of two-stage anaerobic bioflocculation process treating high grease WSE

Table A 3.2.2.1 First stage anaerobic bioflocculation process.

Experimental period (day)	Grease in feed (g/L)	HRT (day)	Grease load (g/L/d)	% Grease reduction
0	34.00	9	3.78	83.50
5		9	3.78	83.40
8	33.70	9	3.74	64.10
9		9	3.74	81.20
15	16.70	6	2.78	80.00
20		6	2.78	80.60
23	19.30	6	3.22	77.10
26		4	3.22	77.70
29	21.30	4	5.33	80.90
33	12.53	4	3.13	67.04
36		4	3.13	66.60
41	15.40	2	7.70	65.20
44	15.21	2	7.61	66.20
48	15.00	2	7.50	64.00
50		2	7.50	62.20
54	15.10	2	7.55	64.20
58		2	7.55	61.20
62	15.20	2	7.60	60.60
65		2	7.60	57.60
69	13.25	2	6.63	56.60
72	13.10	2	6.55	50.20
75	13.10	2	6.55	54.10
79		2	6.55	61.00
82	13.52	2	6.76	62.40
86		2	6.76	65.10
89	12.90	2	6.45	71.60
93	13.40	2	6.70	68.20
97	13.40	2	6.70	55.08
100	12.90	2	6.45	48.50
104	12.90	2	6.45	51.60
110	12.90	2	6.45	52.40

Table A 3.2.2.2 Second stage anaerobic bioflocculation process.

Experimental period (day)	Grease in feed (g/L)	HRT (day)	Grease load (g/L/d)	% Grease reduction
0				
5				
8				
9	6.34	15	0.42	49.70
15	3.34	15	0.22	49.60
20	3.24	15	0.22	57.90
23	4.42	15	0.29	62.60
26	4.30	4	1.08	64.80
29	4.07	4	1.02	71.70
33	4.13	4	1.03	69.70
36	4.19	4	1.05	70.10
41	5.36	2	2.68	80.20
44	5.14	2	2.57	79.10
48	5.40	2	2.70	60.10
50	5.67	2	2.84	57.30
54	5.41	2	2.71	45.40
58	5.86	2	2.93	33.30
62	5.99	2	3.00	37.30
65	6.44	2	3.22	41.10
69	5.75	2	2.88	34.60
72	6.52	2	3.26	34.50
75	6.01	2	3.01	23.30
79	5.11	2	2.56	9.90
82	5.08	2	2.54	10.40
86	4.72	2	2.36	39.80
89	3.66	2	1.83	31.70
93	4.26	2	2.13	38.60
97	6.02	2	3.01	55.10
100	6.64	2	3.32	48.50
104	6.24	2	3.12	39.50
110	6.14	2	3.07	53.80

Table A 3.2.2.2 Total grease and COD reduction of Two-stage anaerobic bioflocculation process.

Experimental period (day)	HRT (days)	% Grease reduction	% COD reduction
0			
5			
8			
9	21	90.30	
15	21	91.80	
20	21	91.00	
23	21	90.70	
26	8	91.90	
29	8	94.60	85.40
33	8	90.00	
36	8	90.00	83.60
41	4	93.10	
44	4	92.90	85.00
48	4	85.70	76.10
50	4	83.90	
54	4	80.40	68.80
58	4	74.10	
62	4	75.30	80.00
65	4	75.00	
69	4	71.70	
72	4	67.10	67.70
75	4	64.10	
79	4	64.90	
82	4	66.30	
86	4	79.00	83.40
89	4	80.60	
93	4	80.40	75.10
97	4	70.20	85.90
100	4	70.00	
104	4	70.10	
110	4	78.02	59.10