MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 General

All glasswares used were rinsed with chromic acid, washed with tap water and rinsed with distilled water prior to use. The plastic sampling containers used were rinsed with concentrated hydrochloric acid, tap water and with distilled water prior to use.

Distilled water was used for the preparation of reagents and media unless otherwise stated. All chemicals used were of analytical grade and were obtained from MERCK (West Germany) and all media used were from DIFCO (USA) unless otherwise stated.

3.2 Sample Collection And Preservation.

Samples of detergent wastewater were obtained from a detergent plant in Petaling Jaya, Selangor, Malaysia. Composite samples were collected in plastic bins over a weekly period for use in the experiments. They were collected before and after physical-chemical treatment of the detergent wastewater and were stored at $4^{o}C$ before use.

3.3 Physical And Chemical Determination

All chemical and physical determinations of the detergent wastewater was according to the Standard Methods for the Examination of Water and Wastewater(APHA 1980) unless otherwise stated.

3.4 Physical examination

3.4.1 Mixed liquor suspended solids.

The total non-filtrable residue was the residue obtained on a Whatman glass-microfibre filter after filtration of a well mixed sample. The residue was dried at 103°C to 105°C and cooled in a dessicator before being weighed. The weighed residue was then reported as the mixed liquor suspended solids (MLSS) of the activated sludge system in mg/l.

3.4.2 Mixed liquor volatile suspended solids

The non-filtrable residue obtained after filtering through the Whatman glass microfibre filter was ignited for 20 minutes at 550°C, transferred to a

dessicator and then weighed. The weighed residue was reported as mixed liquor volatile suspended solids (MLVSS) in mg/l.

3.4.3 Oxygen transfer in distilled-deionized water and in physical-chemical treated detergent wastewater.

Oxygen transfer was determined in a one-litre Quickfit (UK) culture vessel containing the sample at 29°C. The vessel was mounted on a Whatman microprocessor controlled hot plate/stirrer Model 430 (UK). A porous diffuser of 1mm pore size was used to deliver air or oxygen to the container. The test water prepared from distilled-deionized water had an ionic strength of 0.01μ. The test water was deoxygenated by stripping with nitrogen gas. The gas rates were monitored by a New Brunswick Scientific Co. (U S A) rotameter having a range of 0 - 8 l/min. The rotameter was calibrated by a soap film technique (Gurol and Singer 1982). Re-aeration was carried out with air by using a EM-6200 aquarium air pump (Japan) and pure oxygen at various flow rates. Complete mixing was obtained with the gas flow as well as by stirring at 150 rpm with a magnetic flea of 1 in

length. Dissolved oxygen measurements were made with a dissolved oxygen meter Model 4000, Syland Scienctific (West Germany).

Calculation

Oxygen transfer in clean water and the detergent wastewater sample as defined earlier in section is:

$$dC/dt = k_L a \, (C_s - C)$$

The $k_L a$ values were obtained using a differential method of analysis. In this method dC/dt is approximated by $\Delta C/\Delta t$ and the $\Delta C/\Delta t$ is plotted versus C_{av} and the slope obtained will be $k_L a$.

3.4.4 Sludge volume index

The sludge volume index (SV1) is the volume in ml occupied by 1 gm of a suspension after 30 min settling. The settled sludge volume was determined using a one-litre graduated measuring cylinder and the settled sludge volume was recorded after 30 min settling.

3.4.5 pH value

pH determination was made using a Suntek Digital pH/mv Meter, Model SP-35 (U S A). The glass pH electrode was calibrated with pH standard solutions before use and the normal limits of accuracy reported for this electrode were $\pm$ 0.1 pH unit.

3.5 Chemical Examination

3.5.1 Nitrogen

Nitrogen in the wastewater was determined as Total Kjeldahl Nitrogen. The ammonia liberated after distillation of the digested sample was determined using the phenate method. A calibration curve as shown in Fig 3.1 was obtained for the NH_3-N in the range of 0.1 to 5 µg. The results of the nitrogen in the sample was then determined using this calibration curve.

3.5.2 Phosphorous

Total phosphorous was determined after passing the sample through a 0.45 µm membrane filter. The sample was digested with an admixture of sulphuric

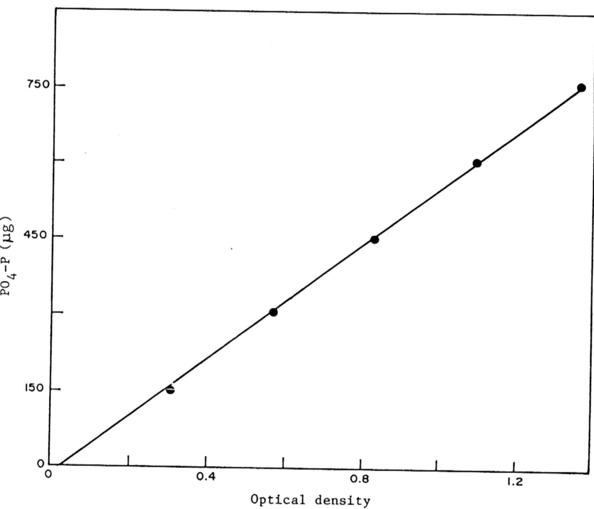


Fig 3.2 : Calibration curve for phosphate.

acid and nitric acid and the ortho-phosphate released was determined colorimetrically using the Vanadomolybdophosphoric Acid Colorimetric method. A calibration curve as shown in Fig 3.2 was prepared and during each batch of samples tested, one standard was used to countercheck the calibration curve. The phosphorous present was reported as PO_4-P(mg/l).

3.5.3 Dissolved oxygen

Dissolved oxygen measurements were carried out using a dissolved oxygen meter Model 4000, Syland Scientific (West Germany). The membrane electrode was calibrated against the azide modification of the Iodometeric Method for determination of dissolved oxygen. The results were reported as parts per million (ppm or mg/l) dissolved oxygen in sample.

3.5.4 Biochemical oxygen demand

BOD measurements were made on samples before and after incubation at 20°C for 5 days. The dissolved oxygen measurements were made using a dissolved oxygen meter Model 4000, Syland Scientific (West Germany). Seeding was carried out using 2 mg/l of a population of microorganisms from the acclimatized culture

to the detergent wastewater. The BOD readings were determined after subtracting the seed dissolved oxygen uptake from the total dissolved oxygen uptake. The BOD readings were reported as BOD_5^{20} (mg/l).

3.5.5 Chemical oxygen demand

The COD is a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. In this work, the dichromate reflux method was used.

3.5.6 Methylene blue active substances

Slack's (1959) modification of the Longwell and Maniece method was used. The principle of the method involved the formation of a complex of the detergent with methylene blue in alkaline solution which was then extracted into chloroform. The chloroform extract was washed with an acid solution of methylene blue and compared colorimetrically with standards prepared from Manoxol OT.

The reagents are:

i. Alkaline phosphate solution

10 g anhydrous sodium dihydrogen phosphate NaH_2PO_4

was dissolved in water. The solution was adjusted to pH 10 with sodium hydroxide and made up to one litre.

ii. Neutral methylene blue solution

0.35 g of methylene blue was dissolved in water and made up to one litre.

iii. Acid methylene blue solution

0.35 g of methylene blue was initially dissolved in 500 ml of water. Then 6.5 ml sulphuric acid of SG 1.84 was added and the resultant solution made up to one litre with distilled water.

iv. Chloroform

v. Manoxol OT (sodium dioctyl sulphosuccinate stock solution

0.100 gm of manoxol OT was dissolved in water and made up to one litre.

vi. Manoxol OT working solution

10 ml of the stock solution was diluted to 100 ml with distilled water.

The procedure was as follows:

A sample containing 20 to 150 mg of the alkyl-benzene sulphonate was made up to 100 ml in a separating

funnel. Then 10 ml of alkaline phosphate solution, 5 ml neutral methylene blue solution and 50 ml of chloroform were added. Extraction of alkylbenzene sulphonate was carried out by shaking gently and evenly twice in one second for over a period of one minute. Then the mixtures were allowed to separate. The chloroform was run into a second separating funnel containing 5 ml acid methylene blue solution and 110 ml water. The contents were shaken in the same manner and the chloroform layer was run through a small funnel plugged with cotton wool which was moistened with chloroform. The first 5 ml of the filtrate was rejected and the rest used for optical density measurements at 650 nm using a uv-vis Varian 634 Series Spectrophotometer (USA).

The calibration graph was prepared by running the standards in a similar fashion through the above procedure. A calibration curve as shown in Fig 3.3 was plotted for Manoxol OT covering the range 10 to 200 µg Manoxol OT. The same procedure was used to determine the blank value using distilled water as the sample. The equivalent mg of Manoxol OT was then read from this calibration graph and expressed as mg Manoxol OT per litre of sample or mg/l MBAS.

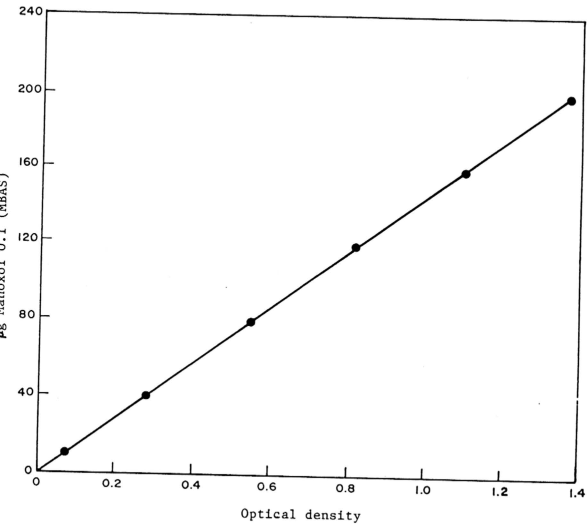


Fig 3. 3 : Calibration curve for MBAS

3.6 Microbiological Examination Of The Activated Sludge System.

3.6.1 Media

Plate agar media were prepared by pouring 20 ml of agar into petri dishes. The agar slants were prepared using 2 ml or 7 ml of agar media in Bijou bottles or McCartney bottles. All the prepared media were autoclaved at $121^{o}C$ at 15 psi for 15 min except for heat labile substances which were filter sterilized. All media used were standard preparations obtained from Difco laboratories (USA) except for the following:

i. Activated sludge extract agar

One litre of the mixed liquor from the activated sludge tank was autoclaved for 20 minutes at $121^{o}C$ and 15 psi. The resultant solution was filtered using a Whatman GF/A glass micro-fibre filter and stored at $4^{o}C$ for use in the preparations of activated sludge extract agar and slants. The constituents of the final activated sludge agar preparations were:

KH_2PO_4	8.50 mg	$MgSO_4.7H_2O$	22.50 mg
K_2HPO_4	21.75 mg	$CaCl_2$	27.50 mg
$Na_2HPO_4.7H_2O$	33.40 mg	$FeCl_3.6H_2O$	0.25 mg
NH_4Cl	1.70 mg	Agar	15.00 g
Activated sludge extract		1000 ml	
pH		7.0	

The ingredients were melted over a water bath at 70°C, and then autoclaved at 121°C at 15 psi for 20 minutes. They were then poured into sterile petri dishes or made into agar slants. The phosphate salts and ammonium chloride were filter sterilized using a Millipore filter of 0.22 µm and added into the cooled autoclaved mixture. In the activated sludge broth preparation, the agar was left out.

ii. Alkylbenzene sulphonate agar

Alkylbenzene sulphonate agar was prepared by using the alkylbenzene sulphonate surfactant raw material used in the detergent plant. The composition of the agar was as follows:

KH_2PO_4	8.50 mg	$MgSO_4.7H_2O$	22.50 mg
K_2HPO_4	21.75 mg	$CaCl_2$	27.50 mg
$Na_2HPO_4.7H_2O$	33.40 mg	$FeCl_3.6H_2O$	0.25 mg
NH_4Cl	1.70 mg	Agar	15.00 g
Tridecylbenzene sulphonate	200.00 mg MBAS		
Distilled water	1 litre		
pH	7.0		

iv. King's B medium

This medium was used to enhance the production of fluorescin by Pseudomonas species particularly _Pseudomonas fluorescens_. The constituents were:

Bacto protease peptone No.3	20.00 g
Glycerol	10.00 ml
$K_2HPO_4.7H_2O$	1.50 g
$MgSO_4.7H_2O$	1.50 g
Agar	12.00 g
Distilled water	1000.00 ml
pH	7.2

The medium was sterilized by autoclaving at 121°C and 15 psi for 15 mins. A 20 ml volume of the medium was poured into each sterile petri dish for use.

v. Cetrimide agar

This medium was used for selective isolation and identification of _Pseudomonas aeruginosa_. The cetrimide inhibited most other organisms and promoted both pyocyanin production and fluorescence under u-v light of cultures of _Pseudomonas aeruginosa_.

The constituents were:

Gelysate peptone	20.00 g
Magnesium chloride	1.40 g
Potassium sulphate	10.00 g
Cetrimide	0.30 g
Glycerol	10.00 ml
Agar	13.60 g
Distilled water	1000.00 ml

The medium was autoclaved at 121°C and 15 psi for 15 mins. 15 ml of the medium was poured into each sterile petri dish.

3.7 Determination Of Inoculum Type And Concentration For The Biodegradation Of Detergent Wastewater.

Two types of inocula, one from sewage and another from soil were obtained for the experiments on the determination of inoculum type and concentration to be used in the biodegradation of the detergent wastewater. The sewage inocula was obtained from an aerated lagoon of a sewage treatment plant in Kuala Lumpur whereas the soil inocula was collected from the river banks in the proximity of the detergent plant.

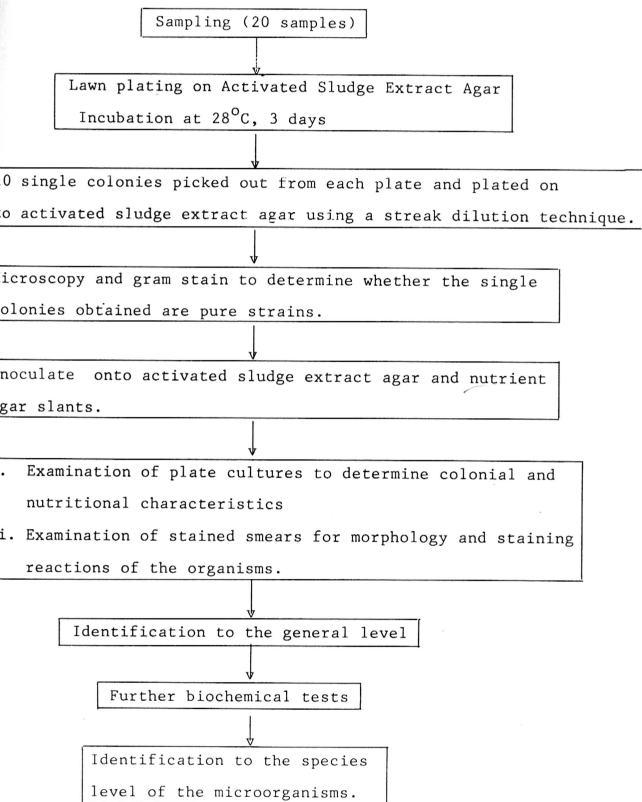
Both the inocula obtained were centrifuged at 3000 rpm for 5 min at 4°C using a Beckman centrifuge Model J21-B (USA). The supernatants were stored at 4°C and then were diluted to an optical density of 0.1 before being used in the experiments.

The experiments were conducted in 250 ml bottles and the sample volume of the raw and treated detergent wastewaters used was 100ml. Each bottle was aerated with the aid of an air line connected to several aquarium air pumps EM6200 (Japan). The air flow rate was 100 ml/min and it was monitored daily by using a New Brunswick rotameter (USA). To prevent losses due to evaporation, parafilm was used to seal the bottle openings. The HRT of the wastewater in the bottles was 5 days.

3.8 Isolation And Identification Of Microorganisms In The Activated Sludge Tank.

A schematic diagram for the isolation of micro-organisms in the activated sludge tank treating detergent wastewater is as shown in Fig 3.4.

ig 3.4: A schematic flow diagram for the isolation and identification of microorganisms.



3.9 Sampling

Samples were collected from the completely mixed activated sludge tank by using sterile McCartney bottles. The bottles were opened in the tank and the sample collected was at once aseptically plated onto a culture media. In this way, twenty samples were collected for the isolation and identification of the microorganisms.

3.10 Isolation

The microorganisms were lawn cultured on activated sludge extract agar and incubated at 28°C for two days. Twenty colonies from each of the plates were randomly selected and plated again onto activated sludge extract agar. The streak dilution technique was employed to obtain single colonies and the plates were incubated for 3 days at 28°C.

A portion of a single colony in the agar plate was picked off, smeared, stained by Gram stain and examined for morphology. If examination under the microscope indicated that the culture was pure, the remainder of the colony was plated onto activated sludge extract slants and nutrient agar slants. The

organisms in the nutrient agar slants were used as the stock culture for subsequent studies and for maintaining them in a viable state. This stock cultures were kept at $4^{o}C$.

3.11 Identification

The identification of the microorganisms were carried out according to Skerman's (1967) key to the identification of the genera of bacteria and Bergey's Manual of Determinative Bacteriology (Bergey 1974). The following three methods were mainly used in identifying the microorganism:

i. Examination of plate cultures to determine colonial and nutritional characteristics.

The terms used to describe the colony characteristcis were as follows:

Shape:	Circular, irregular or rhizoid colonies, "swarming growth"
Size:	Diameter (mm)
Elevation:	Flat, convex, umbonate
Surface:	Smooth or rough, dull or glistening
Edge:	Entire, undulate, crenated or rhizoid
Pigment:	Colour and diffusibility

Opacity:	Transparent, translucent or opaque
Structure:	Amorphous, granular or filamentous
Consistency:	Butyrous, sticky or friable
Emulsifiability:	Easy or difficult
Odour:	Absent, decided or identifiable
Effect on medium:	Haemolysis, clearing, pearly halo

ii. Examination of stained smears for morphology and staining reactions of the organisms

The terms used to describe the morphology and arrangement of mircoorganisms in stained smears are as follows:

Shape:	Cocci, rods, filaments, spirals, pleomorphic
Size:	Length and breadth, or diameter, expressed in micrometers.
Axis:	Straight or curved, spiral or twisted
Ends:	Rounded, square or pointed
Arrangement:	Singly, pairs, chains, clusters, palisade forms, bundles, etc.
Irregular forms:	Variations in size and shape, clubs, beaded or filamentous forms.

Staining:	Gram positive or gram negative, even, beaded bipolar, irregular or barred.
Reactions to special stains:	Capsule present or absent, acid fast, spore present or absent.
Special features:	Motility. flagella - monotrichous, lophotrichous or peritrichous. Spores. shape, size, location in or on the rod, whether spore distends the rod.

The various stains used for staining the micro-organisms are as follows:

i. Gram stain reaction

The gram stain procedure is as follows:-

a. A colony emulsified in distilled water was smeared on a clean, grease free slide.
b. The smear was then heat fixed to the glass slide. A 1% crystal violet solution and a few drops of sodium hydrogen carbonate were then added to the smear for 30 seconds.
c. The smear was then rinsed with distilled water before Burke's iodine solution was added to it for

a further 30 seconds.

d. The smear was then decolourized with 95% alcohol until no more stain came off.

e. This was followed by washing with distilled water before the smear was counter stained with safranin for 20 seconds.

f. The slide was finally rinsed with distilled water, dried and observed under the oil immersion lens at 10 x 100 magnification.

The results were judged as follows:

Gram positive - violet or purple
Gram negative - red

ii. Staining of spores

The staining of spores was carried out using a Carbol-Fuschsin Methylene Blue Technique. The procedure adopted was as follows:

a. A thin smear of the organism was prepared on a glass slide and heat fixed.

b. The smear was stained with strong*Carbol fuschin and placed over a steam bath for 5 minutes.

c. It was washed with distilled water, decolourized with 0.5% sulphuric acid for 1 to 2 minutes and rewashed with distilled water.

d. It was then counter stained with 1% methylene blue for 2 to 3 minutes, washed, dried and finally observed under a microscope at 10 x 100 magnification.

* Strong carbol fuchsin

Constituents

Basic fuchsin	1 gm
Phenol crystals	5 gm
Absolute alcohol	10 ml
Distilled water	1000 ml

Steam fuchsin with phenol, add alcohol and water. Filter through filter paper.

The results were judged as follows:

Spores - red

Vegetative cells - blue

iii. Ziehl - Neelsen stain (modified for acid and alcohol fast organism).

The procedure adopted to determine acid fast organisms was as follows:

a. The smear was heat fixed to the glass slide and stained with carbol fuchsin.
b. It was steamed for 10 minutes and then rinsed with distilled water.
c. An acid-alcohol solution containing 3 ml of 5% H_2SO_4 and 47 ml of 70% alcohol was used to decolourize the smear. The smear was then rinsed with distilled water at regular intervals over a period of 4 minutes.
d. The smear was washed with distilled water and then counter stained with 1% methylene blue.
e. It was again rinsed with distilled water and then dried over a bunsen flame before being observed under 10 x 100 magnification.

The results were judged as follows:

Acid fast organisms - red
Non-acid fast organisms - blue

iv. Flagella Stain

The silver impregnation stain (Blenden and Goldberg, 1965) was used as the flagella stain. The organisms were grown in liquid nutrient broth for 6 hours. The culture was then centrifuged at 3000 rpm for 5 minute, the supernatant decanted and the pellet carefully suspended in distilled water. The suspension was then reincubated for 5 minutes and checked for motility by the hanging drop method. A smear was then prepared by placing a drop of the suspension at the end of a clean tilted slide and allowing it to run down the slide. The smear was then allowed to dry in air. The procedure for staining was as follows:

a. The air dried smear was covered with reagent A for 4 minutes.
b. The smear was then rinsed with distilled water.
c. Reagent B was added to the smear for 30 seconds.
d. The smear was rinsed in distilled water, air dried and examined under oil immersion.

The constituents of the reagents are as follows:

Reagent A: 5.0 g tannic acid
1.5 g ferric chloride
2.0 ml 15% formalin
1.0 ml 1% sodium hydroxide
distilled water to 100 ml

Reagent B: 100 ml of 2% silver nitrate solution was prepared and to 90 ml of it, ammonium hydroxide was added dropwise until the heavy precipitate which formed just dissolved. Then 2% silver nitrate solution was added dropwise until a slight cloudiness persisted. This solution was used within 4 hours of preparation.

iii. Examination of nutritional and biochemical characteristics of the microorganisms.

The following nutritional and biochemical tests were carried out to identify the microorganisms MacFaddin 1976, Skerman 1967).

Arginine dihydrolase production

Carotenoid presence

Catalase test

Deamination of phenylalanine

Decarboxylases presence

Arginine decarboxylase

Lysine decarboxylase

Ornithine decarboxylase

Deoxyribonuclease production

Egg yolk reaction

Fermentation of carbohydrates

Adonitol
Aesculin
Arabinose
Dulcitol
Erythritol
Fructose
Galactose
Glycerol
Glucose
Xylose
Inositol
Inulin
Lactose
Maltose
Mannitol
Raffinose
Sorbitol
Starch
Sucrose
Trehalose

Growth in/at

Activated sludge extract agar
Adenine
Adipic acid
Agar
β-Alantine
Alkylbenzene sulphonate agar
anaerobic conditions
Anaerobic glucose broth
DL-Arginine
Aspartic acid
Azide (0.2%) at 55%

Cetrimide agar
Cellobiose
Cellulose
Citrate

Hypoxanthin
KCN (Potassium cyanide agar)
2-Ketogluconate
King's A medium
King's B medium

Motility medium

Nutrient agar and broth

Paraffin

pH 6

Saboraud dextrose agar

Sebacic acid

Sodium acetate

Sodium butyrate

Sodium chloride (7%)

Sodium malate

Sodium propionate

Sodium pyruvate

Sporulation medium for *Bacillus subtilis*

Succinate

Trehalose

Triple sugar iron agar

Tyrosinase

5°C

40°C

50°C

Urea

Valine

Hydrogen sulphide production

Hydrolysis of:

Aesculin

Agar

Allantoin

Benzidine

Casein

Cellulose

Chitin

3.12 Microscopy

An Olympus microscope (Japan) was used for the microscopy work. The size of the organisms were determined by using a stage micrometer and a micrometer eyepiece. The image of the stage micrometer placed in position on the microscope stage was focused/superimposed with the eyepiece scale. Then the number of divisions of the eyepiece scale corresponding to the stage scale was noted. The measurement of the eyepiece division was then calculated in µm. The size of the organisms were then determined using the micrometer eyepiece under 10 x 100 magnification and recorded in µm.

3.13 Respirometric Studies

A Gilson Differential Respirometer Model IGRP-14 (France) was used. The flasks were prepared by placing a small piece of pleated filter paper into the centre well containing 0.2 ml 10% KOH. The mixed microbial cultures and pure cultures used in the experiments were initially centrifuged using a Model J-21B Beckman centrifuge (USA) at 3000 rpm and $4^{o}C$ for 10 minutes. This was followed by washing thrice with Krebs-Ringer solution and finally suspending

it in the same solution. The microorganism concentration was finally adjusted to approximately 1000 mg/1 for each culture. Aliquots of 0.5 ml of this suspension were used as the inocula for the studies. The experiments were carried out at 28°C with constant shaking.

3.13.1 Preparation of pure and mixed cultures for respirometric studies.

Pure cultures were prepared by growing them on broth containing activated sludge extract which was supplemented with nitrogen and phosphate. In a 500 ml Bellco triple baffled flask, 150 ml of the broth was innoculated with the pure cultures. The flasks were incubated at 28°C for 72 hours in a Labline 2597-1 (USA) rotary shaker moving at 220 rpm. Mixed cultures were directly obtained from the activated sludge tanks.

3.13.2 Foaming

The foaming apparatus used to obtain the 3 portions of the wastewater for respirometric studies is as described below. The portions obtained are the before foaming, after foaming and the foam concentrate.

The apparatus consisted of a 1 L conical flask fitted with a rubber bung with openings for an air line and a glass foam column of 60cm height. At the receiving end of the column, a beaker was placed to collect the foam concentrate. Foaming of the 1L sample in the conical flask was initiated by passing air into it through fine bubble diffusers of 1 mm pore size. The air flow rate was maintained at 2 l/min for 5 hours using a New Brunswick rotameter (USA) connected to an EM6200 (Japan) aquarium air pump.

3.14 Effect Of Calcium Hydroxide And Aluminium Sulphate At A 2:1 Ratio On Oxygen Uptake By A Microbial Culture.

Various concentration of calcium hydroxide, $Ca(OH)_2$ and aluminium sulphate, $Al_2(SO_4)_3.14H_2O$ in a 2:1 ratio were prepared. The admixtures were then filtered using a Whatman glass microfibre filter. Tridecylbenzene sulphonate equivalent to 200 mg/l MBAS was added to the admixtures. Studies were carried out on the oxygen uptake of a microbial culture from the activated sludge tank to the prepared samples contained in the BOD bottles. The concentration of the microbial culture used was 2000 mg/l MLVSS. Each sample was initially aerated until a high dissolved oxygen content

was obtained. Subsequently a dissolved oxygen probe was attached to the BOD bottle and the dissolved oxygen reduction was monitored with a Model 4000, Syland Scientific (West Germany) dissolved oxygen meter. The observed dissolved oxygen readings versus time were plotted on an arithmetic graph paper and the gradient of the line of best fit was determined. The gradient represents the oxygen uptake rate measured in mg/l per minute.

3.15 Wastewater Comparison And Feed To The Activated Sludge Systems.

Due to the continuous variability exhibited by industrial wastes in general, it was necessary to establish the range and mean values of the polluting parameters of the detergent wastewater before and after chemical treatment.
This is shown in Table 3.1.

Table 3.1: Detergent wastewater characteristics before and after physical-chemical treatment.

Parameter (mg/l)	Raw Wastewater	Treated wastewater
	Range of values	
pH (units)	3 - 11.0	10.5 - 11.5
Tot. BOD	221 - 1,021	99 - 408
Tot. COD	3,480 - 9,540	1044 - 1908
MBAS	1,230 - 2,300	123 - 250
Phosphate	50 - 800	0 - 1
Amm. Nitrogen	0.2 - 2 ppm	0

3.16 The Laboratory Activated Sludge Units

The laboratory activated sludge unit used in the studies was a continuous stirred aeration tank with sludge recycle, shown in Fig 3.5 .

Each activated sludge unit consisted of a 25 l plastic tank with an effective volume of 10 l of wastewater. Each tank was connected to a 1 l settling cone

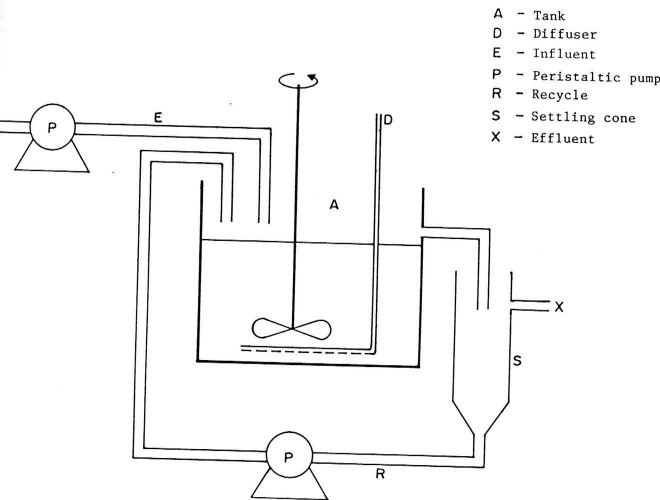


Fig 3. 5 : Laboratory activated sludge unit

for sludge settlement and recycle. The wastewater was continuously fed into the aeration tank from a nutrient reservoir using a Gilson Minipuls 2, (France) peristaltic pump. Complete mixing in the aeration tank was achieved with a mechanical paddle stirrer mounted on a GCE motor (UK) which was connected to a Delux slide (Japan) speed regulator. The stirrer speed was maintained at 100 rpm.

Aeration was achieved by using an aquarium air pump of type EM6200 (Japan) linked to aquarium stone diffusers. The air flow rate was regulated with a New Brunswick(USA) rotameter with a range of 0 - 8 l/min. The dissolved oxygen concentration in the aeration tank was maintained above 3 mg/l. The control of pH at 7 was made possible using a New Brunswick (USA) automatic pH controller. The clear treated supernatant then overflowed into an effluent collection tank.

Sludge recycling was performed by a Gilson Minipuls 2 (France) peristaltic pump. The sludge recycle ratio was determined and maintained to ensure a constant activated sludge concentration in the aeration tank. Sludge wasting was accomplished by wasting directly from the settling cone on a daily basis.

Completely mixed tanks without sludge recycle were also used in some experiments. They were essentially the same as the tanks described above. In some other experiments, three completely mixed tanks in series were used. They were also similar to the facilities described above and they had no sludge recycle lines. However, the total volume of wastewater in the three tanks was similar to the wastewater volume in a single completely mixed tank without or with sludge recycle.

3.17 Acclimatization Of A Microbial Culture To The Treatment Of Detergent Wastewater.

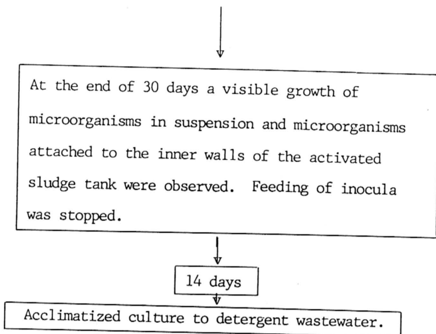
Acclimatization of the microorganisms to the detergent wastewater was carried out according to the following schedule:

Activated slude unit	
Effective volume	:10 L
Hydraulic retention time (HRT)	:10 days
Inocula to wastewater ratio	1:100 (v/v)

Inoculum was obtained from the aerated lagoons, Pantai Dalam Sewage Treatment Plant, Kuala Lumpur, Malaysia.

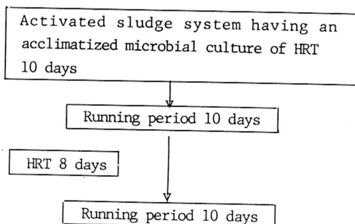
↓

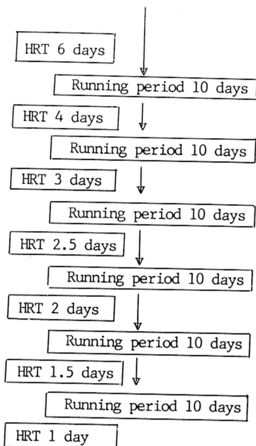
Running period 30 days



3.18 Optimization Of The Activated Sludge System Treating Detergent Wastewater To A HRT of 1 day

The optimization of the activated sludge system treating detergent wastewater to a 1 day HRT was carried out as shown:





As the HRT shortened, the interval between each successive HRT also shortened since, at short HRT, the chances of instability was higher.

3.19 Steady State Operation Of The Activated Sludge System Treating Detergent Wastewater At A 1 Day HRT.

The steady state parameters were established after successful completion of acclimatization and optimization of the activated sludge system to a 1 day HRT.

The criteria used in steady state operation determination was when the following parameters were at constant levels.

i. constant microbial mass concentration

ii. constant BOD reduction

iii. constant COD reduction

iv. constant MBAS reduction

These criteria, when satisfied, indicated steady state operation. The steady state operation was carried out at various SRT/θ_c to evaluate the biokinetic parameters. Kinetic measurements were initially made at the longest SRT and then at successively shorter SRT. In between each successive SRT, a time gap of 14 days was introduced till steady state operation was achieved. The kinetic measurements were then made over a period of 7 days at steady state operation.

The wastewater fed to the activated sludge units were pre-adjusted to pH 7 and supplemented with a nitrogen source in the form of urea and a phosphate source in the form of anhydrous potassium phosphate such that the C:N:P ratio of 100:6:1 was achieved. BOD was used as the indication to the amount of carbon

present in the wastewater. The nutrient adjustment was necessary to ensure that only the carbon component of the detergent wastewater acted as a factor limiting microbial growth.

3.20 Sampling And Analysis

The wastewater flow rate to the activated sludge tanks were determined daily and on each day, samples were withdrawn for analysis. The frequency at which the chemical analysis were performed is shown in Table 3.2.

Table 3.2: Frequency of chemical analysis of samples.

Parameters	Frequency per week
pH	5
Phosphate	5
Nitrogen	5
MLSS	5
MLVSS	5
COD (Tot./Sol.)	5
BOD (Tot./Sol.)	5
MBAS	5
Air flow rate	5
SVI	5
DO	5

3.21 Evaluation Of Biokinetic Coefficients

In the evaluation of the biokinetic coefficients, the basic steady state biokinetic model for completely mixed microbial systems with sludge recycle was used (Lawrence and McCarty, 1970).

The two relevant equations for the above model can be written as follows:

$$X = \frac{\theta_c Y(S_o - S)}{\theta(1 + k_d \theta_c)} \qquad 4.1$$

$$S = \frac{K_s(1 + k_d \theta_c)}{\theta_c(Yk - k_d) - 1} \qquad 4.2$$

From equations 4.1 and 4.2 we can get

$$\frac{S_o - S}{X\theta} = \frac{1}{Y}\left(\frac{1}{\theta_c}\right) + \frac{k_d}{Y} \qquad 4.3$$

$$\frac{\theta_c}{1 + k_d \theta_c} = \frac{K_s}{Yk}\left(\frac{1}{S}\right) + \frac{1}{Yk} \qquad 4.4$$

The values of the biokinetic coefficients Y, k_d, K_s and k were determined from respective plots of the

variables S_o, S_e, X, θ and θ_c. The values of Y and K_d were initially determined from the plot of equation 4.3. Then k and K_s were determined from the plot of equation 4.4, using the solved values Y and k_d from equation 4.3.

3.22 Determination Of Kinetic Model Parameters

3.22.1 Solids retention time (SRT/θ_c)

The SRT was determined from

$$\theta_c = \frac{\theta}{1 + R - RX_r/X}$$

where

θ_c	solids retention time (per day)
θ	hydraulic retention time (per day)
R	recycle ratio(Q_r/Q_o)
Q_r	recycle flow rate (l/day)
Q_o	flow rate into tank (l/day)
X_r	microorganism concentration in recycle (mg/l)
X	microorganism concentration in activated sludge tank (mg/l)

3.22.2 Hydraulic retention time (HRT/θ)

The HRT was determined from

$$\theta = \frac{V}{Q_o}$$

where

θ	hydraulic retention time (per day)
V	volume of activated sludge tank (1)
Q_o	flow rate into activated sludge tank (1/day)

.22.3 Microorganism concentration (X or X_r)

X or X_r was taken to be the mixed liquor volatile suspended solids (MLVSS) in mg/1.

.22.4 Substrate concentration (S_o or S)

S_o and S were the soluble BOD (mg/1) values determined in the samples.