SEMINAR ON THE MANAGEMENT OF HAZARDOUS CHEMICALS AND WASTES

PROGRAMME

27 March 1997 (Thursday)
Ballroom A
The Pan Pacific Glenmarie Resort
Shah Alam

08:30	Registration of Participants
09:00	Welcoming speech by Dr. Mohd. Ariffin bin Haji Aton, President / Chief Executive, SIRIM Berhad
09:15	Speech by Mr. Ryuzo Nishimaki Resident Representative, JICA Malaysia
09:30	Opening Speech by Y. B. Datuk Law Hieng Ding Minister of Science, Technology and the Environment, Malaysia
09:45	Refreshment

SESSION 1 Management and Monitoringof Hazardous Wastes
Chairman: Dr. Chong Chok Ngee
Vice President, Research & Development Services
SIRIM Berhad

10:10	Keynote 1: Recent Developments in the Management of Hazardous Wastes in Malaysia *Hajah Rosnani Ibarahim, Deputy Director General of DOE*
10:50	Keynote 2: Industrial Waste Management in Japan - Landfill Leachate Treatment *Professor Kenji Kida, Kumamoto University*
11:30	Treatment of Rubber Thread Manufacturing Wastewater *Ms Siti Shapura Mashood, SIRIM*
12:00	Use of Algae in the Monitoring and Bioremediation of Heavy Metal Wastes *Dr Phang Siew Mooi, University of Malaya*
12:30	Scheduled Waste in Malaysia *Mr. Zainol Rashid Zainuddin, Managing Director, Kualiti Alam Sdn. Bhd.*
13:00	Lunch

SESSION II Evaluation of Hazardous Chemicals
Chairman : Dr. Hiroshi Tadokoro
Chief Technical Advisor
SIRIM - JICA Hazardous Chemicals and Wastes Project

14:30 A Simple Model for Estimating the Distribution of Phenoxy Toluene in the Environment
Ms. Letchumi Thannimalay, SIRIM

15:00 Bioaccumulation of Diphenyl Derivatives in Carp and Tilapia,
Ms. Wan Mazlina Wan Hussein, SIRIM

15:30 Establishing the Biodegradation Test for Chemicals, Detergents and Wastewater Using Local Standard Sludge
Mr. Mansor Jamil, SIRIM

16:00 Toxicity Evaluation of Cadmium on the Aquatic Environment Using Local Fishes and Crustaceans
Mr. Izham Bakar, SIRIM

16:30 Closing Speech by Mr. Akira Yamazaki
Deputy Managing Director,
Mining and Industrial Development Cooperation Department, JICA
(Leader of Japanese Evaluation Team)

16:45 Refreshment

Industrial Waste Management in Japan -Landfill Leachate Treatment-

Dept. of Applied Chemistry and Biochemistry,
Faculty of Engineering, Kumamoto University
Kenji Kida

1. Introduction

Most industrial wastes in Japan are disposed of in landfills and are usually pretreated by processes such as incineration for reduction of volume. Owing to expansion of economic activities, the amounts of industrial wastes are increasing yearly and are consequently being disposed of by sea-dumping as well as landfilling. The amount of waste disposed of by sea-dumping reached 4,550,000 tons in 1991 and Japan has been the leading country for total amount of sea-dumping. However, due to the London Treaty which inhibits sea-dumping, this practice has been terminated in Japan since 1996. Therefore, recent research has focused on recycling and development of processes to decrease the amounts of wastes being produced. These activities have resulting in consortiums such as Clean Project which is dedicated to decreasing industrial waste volumes. However, on-going research has far from solved the problem and, in particular, effective processes for treatment and disposal of organic sludge from waste water treatment facilities and municipal sewage works have yet to be established. Presently, usually after dehydration, organic sludges are disposed of by landfilling and the ratio of organic matter to ammonium ion in leachate is getting higher. As a result, it is becoming increasingly difficult to treat leachate by conventional activated sludge followed by coagulation and sedimentation. Research addressing this problem has been initiated in our laboratory in an effort to develop biological treatment systems, with a focus on anaerobic processes, that will be effective for such leachates.

In this symposium, firstly the following items will be addressed and then results of current work related to leachate treatment will be shown:

a) The present state of industrial waste disposal in Japan
b) Treatment of leachate and related problems
c) Influence of mineral nutrients on enhanced performance of methane fermentation
d) Treatment of high-strength leachate by biological processes such as methane fermentation

2. The present state of industrial waste disposal in Japan

As shown in Fig. 1, the annual production of industrial waste in Japan increased until 1990 and then leveled off. Fig. 2 demonstrates that few places remain available for landfilling, though the years remaining for use of landfills has been increasing since 1990. Accordingly, the Japanese government and other concerned parties have made efforts to reduce industrial waste volumes by recycling as much as possible. Figs. 3, 4, and 5 are shown to exemplify that recycling percentages of paper, steel, aluminum, etc. are on the increase.

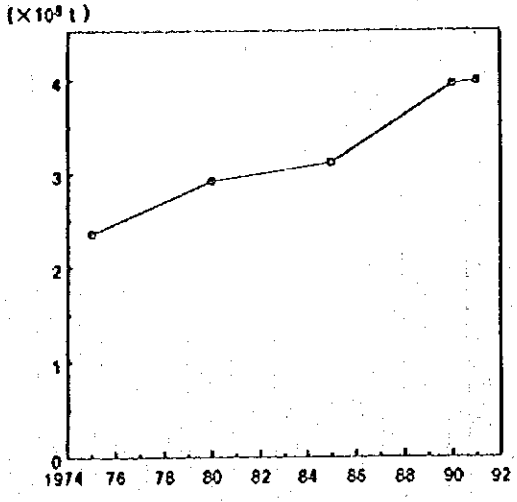


Fig. 1 Yearly increase of industrial waste production. (Annual production of industrial waste in Japan.)

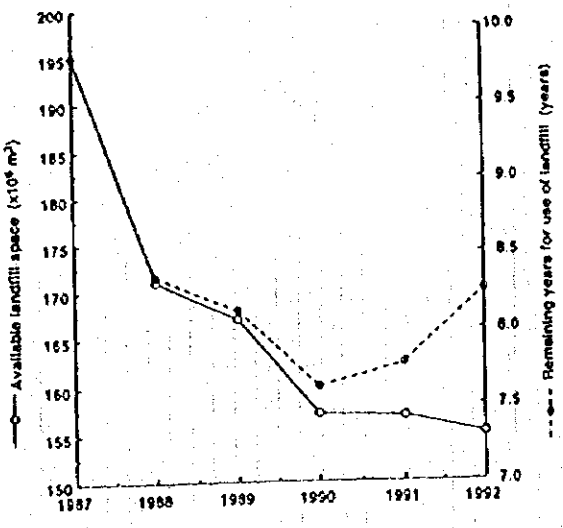


Fig. 2 Annual decrease in available landfill space and change in remaining years available for landfill use.

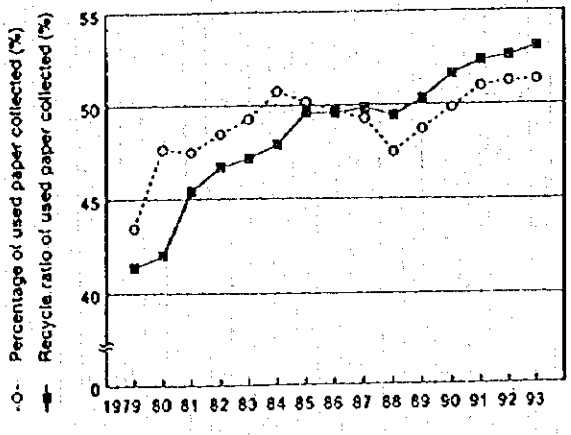


Fig. 3 Annual changes in recycle ratio of used paper collected and percentage of used paper collected.

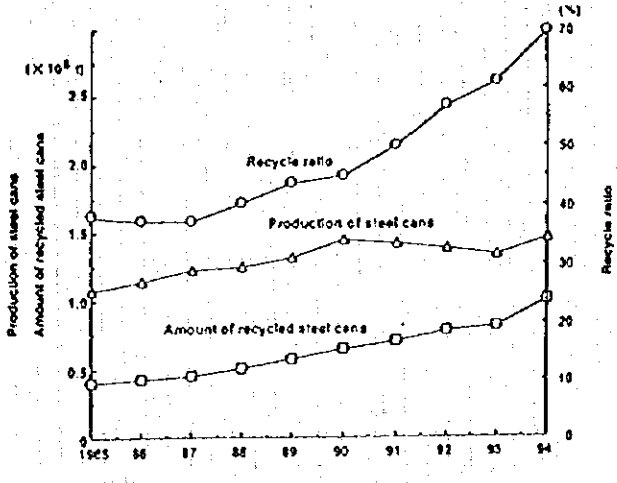


Fig. 4 Annual increase of ratio of recycled steel cans to production of new steel cans.

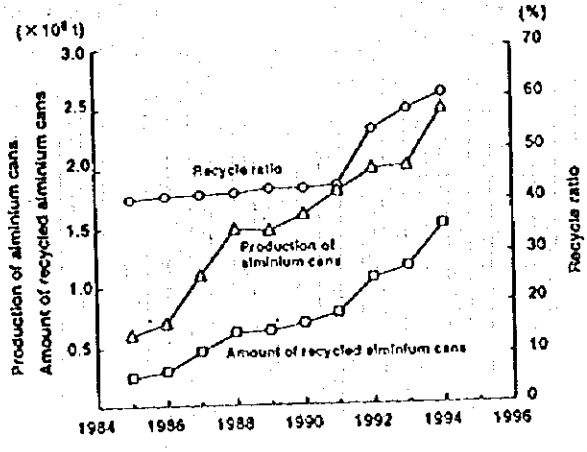


Fig. 5 Yearly increase of ratio of recycled aluminium cans to production of new aluminium cans.

Table 1 Classification of industrial waste.

Group 1 :
Construction debris, electric furnace residual, broken glass and pottery waste, waste plastic, metal waste etc.

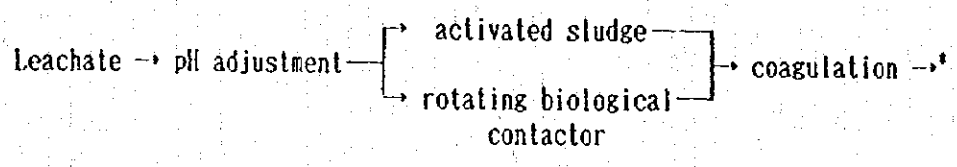
Group 2 :
Sewage sludge, surplus from aeration tank, ash, wood waste, used paper, animal waste, dead animal waste etc.

Group 3 :
Waste oil, acid waste, alcaline waste, infection hospital waste etc.

Industrial wastes are divided into three groups as shown in Table 1: Group 1 consist of mainly inorganic matter. Group 2 is organic matter. and Group 3 is acid waste and alcaline waste etc. Also, there are three types of landfills: inorganic waste landfills (pertaining to Group 1 . above). organic waste landfills (Group 2). and hazardous waste landfills (Group 3). Furthermore, there are two reasons why it is very difficult to get new spaces for landfills which is of particular concern due to the few remaining years for landfill use as shown in Fig. 2. One reason is the practice of illegal dumping and the other is opposition of local residents. It is imperative that industrial waste related problems in Japan be solved in order to maintain economic growth. Not only is severe punishment for illegal disposal required but establishment of more reliable treatment and disposal technologies are necessary. Educating the public to such issues may also play an important role.

3. Treatment of leachate and related problems

Organic wastewater with biochemical oxygen demand (BOD) concentrations of about 1,000 mg l^{-1}, chemical oxygen demand (COD) concentration of about 600 mg l^{-1} is eluted from organic waste landfills. This drainage, also called leachate, is generally treated by an activated sludge process followed by coagulation for removal of color yielding contaminants as follows:



The quality of effluent (mg l^{-1}) is generally: BOD, 20; SS, 20 and is allowed to be discharge into natural water bodies.

However, as noted above, sea-dumping of organic sludges was prohibited as of January 1996 and most of these sludges are now disposed of at organic waste landfills. This has resulted in an increase in the organic component of landfilled industrial waste. This has been complicated by the practice of restricting rain water input in an attempt to reduce leachate volumes. As a result, not only levels of organic matter but ammonium ion concentrations as well are increasing.

This high strength leachate, however, is still largely being treating by traditional systems as described above and thus it is very difficult to produce effluents suitable for discharge to natural water bodies. Prior to discharging into a natural receiving water, leachate treated by activated sludge and coagulation must also be treated by an activated carbon adsorption process. Complete costs for such a treatment train can be more than ¥ 10,000 per cubic meter of leachate. Furthermore, in areas with strict nitrogen regulations, ammonium ion must also be removed. Due to these problems, more effective treatment systems must be developed.

4. Influence of mineral nutrients on enhanced performance of methane ferm entation.

In general, high-strength wastewater has been treated by methane fermentation

followed by activated sludge to produce the effluent suitable for water course discharge. Accordingly, leachate with a high concentration of organic matter has to be firstly treated by methane fermentation instead of activated sludge.

In anaerobic treatment by methane fermentation organic matters with a high molecular weight are firstly hydrolyzed and then converted to aliphatic acid such as butyric, propionic and acetic acids as shown in Fig. 6. CO_2 and H_2 generated during anaerobic treatment are reduced to methane via Cl cycle and aliphatic acids are also reduced to methane via acetic acid.

When the distillery wastewater without SS was treated anaerobically, dilution of the distillery wastewater was found to be necessary. A maximum TOC volumetric loading rate of 10 g l^{-1} d^{-1} was achieved only in the case of five-fold dilution of the wastewater. This appeared to be due to the accumulation of propionic acid during the anaerobic treatment. The reaction that converts

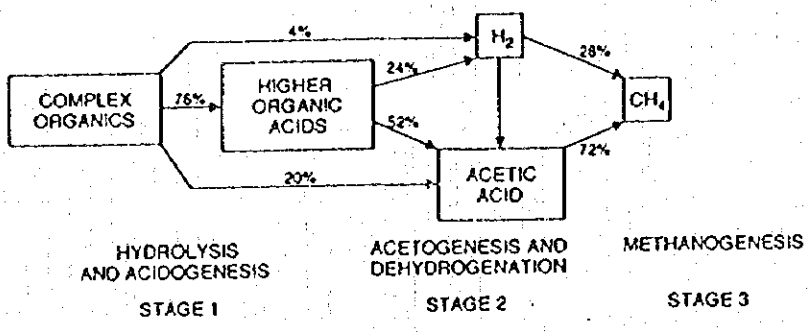


Fig. 6 The three stages of the methane fermentation.

propionic acid to acetic acid requires considerable energy, since $'G^{0'}$ is greater than zero ($'G^{0'}>0$) at a partial pressure of H_2 of more than 10^{-5} atm (McCarty, 1982). However, co-culture techniques with H_2-consumers, such as methanogens, may result in reaction rate improvement (Boone, 1980). It is well known that metal enzymes are involved in the pathway for the production of methane (Takashima, 1989). On the basis of this knowledge, we anaerobically treated five-fold-diluted distillery wastewater, to which the mineral nutrients Ni^{2+} and Co^{2+} were added, by the mesophilic AFBR (anaerobic fluidized bed reactor) process. A maximum TOC volumetric loading rate of 24 g l^{-1} d^{-1} with a TOC removal efficiency of 80% was achieved. This rate was five-fold higher than that without the addition of the mineral nutrients (Kida, 1993). However, the influence of Ni^{2+} and Co^{2+} on activities of microorganisms and their enzymes was not examined. In this work we attempt to clarify the influence of Ni^{2+} and Co^{2+} on the activities of microorganisms and their enzymes.

4.1 Materials and methods

(1) The composition of synthetic wastewater used in the study was as follows (g l^{-1}): sodium acetate, 5.46; acetic acid, 16.0; KH_2PO_4, 0.3; $KHCO_3$, 4.0; NH_4Cl, 1.0; NaCl, 0.6; $MgCl_2 \cdot 6H_2O$, 0.82; $CaCl_2 \cdot 2H_2O$, 0.08; cystein-HCl $\cdot H_2O$, 0.1; mineral solution without Ni^{2+} and Co^{2+}, 10 ml; vitamin solution without B12, 10 ml. Synthetic wastewater with added Ni^{2+} and Co^{2+} to concentrations of 0.5 and 0.2 mg l^{-1},

respectively.

(2) A continuous stirred tank reactor (CSTR), which was made glass and had a working volume of 1.7 l, was mixed thoroughly with a magnetic stirrer as shown in Fig. 7. The temperature during the continuous culture was maintained at 37℃ by immersion of the reactor in a thermostated water-bath, and the pH was maintained automatically at 7 by feeding a 1 N NaOH solution. 1.7 l portions of the mesophilic acclimated sludge which had been washed with the synthetic wastewater without Ni^{2+} and Co^{2+} under anaerobic conditions was placed in each CSTR. Synthetic wastewater with and without Ni^{2+} and Co^{2+} was then fed into the CSTR, respectively. The feeding rate was increased in steps from 17 to 1,020 ml d^{-1}.

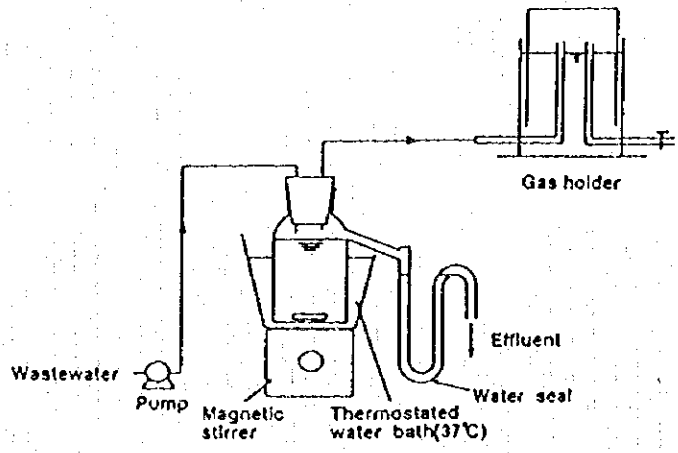


Fig. 7 Schematic diagram of an anaerobic treatment process using a completely stirred tank reacter.

(3) There are two metal enzymes, Methyltransferase and Methylreductase, which form coenzymes with tetra-pyrrole ring structures containing Ni^{2+} or Co^{2+}, respectively, by chelate bonds in the synthetic pathway of methane from acetic acid. At each dilution rate, quality of effluent and gas evolution rate were determined and a 50 ml aliquot of sample was taken from the CSTR to measure the activity of microorganisms and their enzymes. A 10 ml aliquot of sample was transferred to a vial, which contained 0.2 ml of 3.57 M sodium acetate solution and was connected to a volumetric pipette using vinyl tube, emmersed in a thermostated water-bath. Specific gas evolution rate was then measured during incubation with shaking at 37℃ and 100 rpm to determine microbial activity at each dilution rate. The remaining sample was used to determine the concentration of Ni^{2+} and Co^{2+} contained in the methyltransferase and methylreductase coenzymes, respectively. The sample was centrifuged and KCN was added to the ppt for measurement of the coenzyme content of methyltransferase. The coenzyme was extracted from the microorganisms by heating at 105℃ for 20 min. In contrast, the coenzyme of methylreductase was extracted from the microorganisms by heating at 120℃ for 20 min without addition of KCN. Their supernatants were clarified by centrifugation and then passed through an amberlite reverse phase column. The coenzymes were then eluted using methanol and analyzed by atomic absorption.

4.2 Results and discussion

The continuous cultivation was carried out, using synthetic wastewater with

and without addition of Ni^{2+} and Co^{2+}. By addition of Ni^{2+} and Co^{2+}, continuous cultivation was stable even at a high dilution rate of 0.6 d^{-1}. However, without the addition of Ni^{2+} and Co^{2+} the concentration of VFA increased rapidly concomitant with increasing the dilution rate to 0.05 d^{-1}. Using steady-state data at each dilution rate, TOC removal efficiency, gas production rate, and concentration of microorganisms (VSS) were determined (Fig. 8). In the case of non-addition of Ni^{2+} and Co^{2+}, it appeared that wash-out occurred at a dilution rate of 0.05 d^{-1} judging from decrease of TOC removal efficiency. In contrast, with addition of Ni^{2+} and Co^{2+}, continuous cultivation was stable over the range of 0.01 to 0.6 d^{-1}. There was a corresponding increase in gas evolution rate with an increase in dilution rate. The gas evolution rate reached 7,600 ml^{-1} l^{-1} d^{-1} at a dilution rate of 0.6 d^{-1}. TOC removal efficiency of greater than 95% was achieved over the range of dilution rates examined. The concentration of microorganisms increased slightly with an increase in dilution rate at dilution rates less than 0.3 d^{-1}. The cell yield was 7 to 14% with respect to TOC consumed. Without addition of Ni^{2+} and Co^{2+} to the synthetic wastewater these ions were not detected in the microorganisms. Conversely, the addition of Ni^{2+} and Co^{2+} caused an increased in the level of Ni^{2+} and Co^{2+} in the microorganisms as shown in Fig. 2. And their concentrations at dilution rates greater than 0.12 d^{-1} were constant, being 1.1 μmol Ni^{2+} (g VSS)$^{-1}$ and 0.7 μmol Co^{2+} (g VSS)$^{-1}$, respectively. It could be considered that the activity of methanogens with 1.1 μmol Ni^{2+} (g VSS)$^{-1}$ and 0.7 μmol Co^{2+} (g VSS)$^{-1}$ should be maximum, namely such methanogens have an activity of 1.

The specific gas evolution rate of microorganisms at each dilution rate was also depicted in Fig. 9. Without addition of Ni^{2+} and Co^{2+} to the synthetic wastewater the specific gas evolution rate was zero. In contrast, with addition of Ni^{2+} and Co^{2+} the specific gas evolution rate increased with increase in dilution rate. As apparent from these results, the specific gas evolution rate of the methanogens with an activity of 1 seems to be controlled by the dilution rate, namely the specific growth rate.

From the results at a dilution rate of 0.3 d^{-1}, a material balance of Ni^{2+} and Co^{2+} was performed. 20% of Ni^{2+} and Co^{2+} added were taken up in the microorganisms and about 80% remained in the liquid. Accordingly, the amount of Ni^{2+} and Co^{2+} addition to the synthetic wastewater might be decreased by a factor of four.

These results are summarized in Table 2.. It is apparent from this data that the addition of Ni^{2+} and Co^{2+} contributed to the increase of coenzymes and activities of microorganisms. It is also apparent why the high TOC loading rate could be achieved with the addition of Ni^{2+} and Co^{2+} when treating the five-fold diluted distillery wastewater in the AFBR (cf. Introduction). The concentration of microorganisms and the TOC removal rate at a dilution rate of 0.3 d^{-1} in the CSTR were 1.1 g l^{-1} and 2.4 g l^{-1} d^{-1}, respectively. Accordingly, the specific TOC removal rate is 2.15 g g^{-1} d^{-1}. In contrast, the concentration of microorganism and the maximum removal rate in the AFBR was 10 g l^{-1} and 20.6 g l^{-1} d^{-1}, respectively. Accordingly, the specific TOC removal rate was 2.06 g g^{-1} d^{-1}. There is good corroboration among these results.

In conclusion, the addition of Ni^{2+} and Co^{2+} to the synthetic wastewater increased the amount of coenzymes methyltransferase and methylreductase and resulted

in acceleration of methanogenic activity. As a result, a high organic matter removal rate could be achieved.

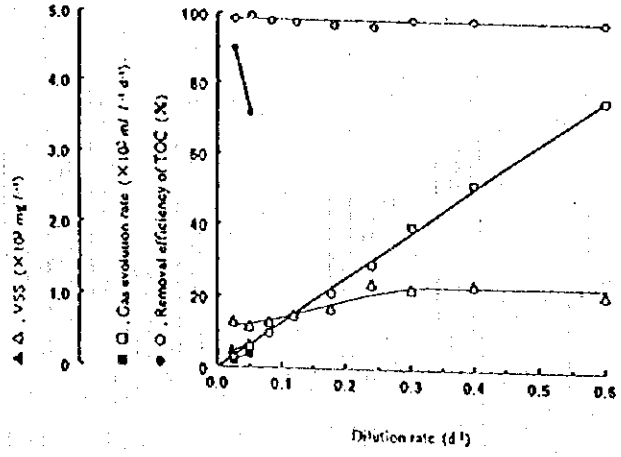


Fig. 8 Effect of dilution rate on TOC removal efficiency and gas evolution rate in continuous cultivation,using synthetic wastewater with and without addition of Ni^{2+} and Co^{2+}.Open symbols,addition; closed symbols,non-addition.

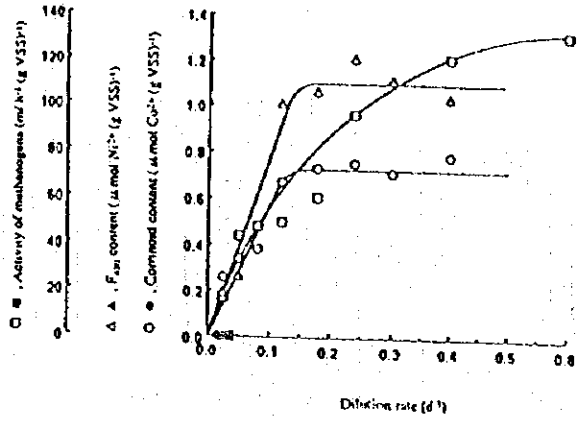


Fig. 9 Effects of mineral nutrients and dilution rate on amount of coenzymes in microorganisms and activity of methanogens. Open symbols,addition of Ni^{2+} and Co^{2+}; closed symbols,non-addition of Ni^{2+} and Co^{2+}.

Table 2 Evaluation of fermentation with addition of Ni^{2+} and Co^{2+}.

Continuous cultivation at a dilution rate of more than 0.32 d^{-1} using a CSTR.		
Amount of coenzyme		
corrinoid	(μmol Co^{2+} $(g\ VSS)^{-1}$)	0.7
F430	(μmol Ni^{2+} $(g\ VSS)^{-1}$)	1.1
Activity of methanogens	($ml\ h^{-1}$ $(g\ VSS)^{-1}$)	50 ~ 132
Comparison of performance between methane fermentation in CSTR and AFBR.		
	CSTR	AFBR
TOC loading rate ($g\ l^{-1}\ d^{-1}$)	2.4	2.4
TOC removal rate (%)	98.7	86
VSS ($g\ l^{-1}$)	3.1	10
Specific TOC removal efficiency ($g\ g^{-1}\ d^{-1}$)	2.13	2.06

These values in AFBR were obtained when five-fold diluted distillery wastewater with addition of Ni^{2+} and Co^{2+} was anaerobically treated (Kida, 1993).

5. Treatment of high-strength leachate by biological processes such as methane fermentation

With the termination of sea-dumping, most organic sludges are disposed of in landfills at present. The leachate from landfills in general has become high-strength organic wastewater with BOD concentrations of more than 10,000 mg l^{-1}. These high-strength leachates are still treated aerobically by activated sludge as mentioned above. However, the quality of effluent from an activated sludge system, even at a high HRT of more than 20 days, is not acceptable for discharge into natural water courses.

In our work the high-strength leachate was treated anaerobically by methane fermentation using an UAFP reactor and then treated aerobically by activated sludge using an aeration tank (hereafter called biological treatment system). Also the quality of effluent from a commercial-scale activated sludge process and the biological treatment system were compared. Afterward, coagulation tests were carried out to remove color materials remaining in the effluent from the system.

5.1 Materials and methods

(1) Table 3 shows compositions of leachates (A), (B) and (C) used in the treatment tests. Although the compositions of leachate collected at various times varied slightly, the BOD levels should be more than 10,000 mg l^{-1} judging from their CODcr values. The leachates had high ash contents of about 10,000 mg l^{-1}, in which K^+, Ca^{2+}, Na^+ and Cl^- were dominant. The concentration of NH_4^+ was also high, being more than 1,000 mg l^{-1}, but SO_4^{2+} and PO_4^{3-} were very low, and were not detected in leachate (A). Moreover, the pHs were very high (greater than 10). On the basis of these analysis, the pH was adjusted to 6.0 and then $MgCl_2 \cdot 6H_2O$, NaH_2PO_4 and L-cysteine HCl · H_2O were added to concentration of 160, 110, 54 and 100, respectively.

(2) Fig. 10 shows a schematic diagram of the single-phase anaerobic treatment system using an UAFP. The reactor, with a working volume of 0.78 l^{-1}, was made of glass and packed with a support medium for microbial adhesion. The support medium, which was supplied by Herding GmbH (Germany), was composed of a mixture of clay (particle diameter, 0.1-2.0 mm) and high density polyethylene Type 9M (diameter, 0.5-2.0 mm) in a ratio of 45 : 55, and the mixture was burnt at 100-230 ℃. The temperature of the reactor was maintained thermostatically at 37 ℃ by the circulation of thermostated water through a water jacket. The linear velocity against the surface area of the reactor was controlled at 5 cm min^{-1} by circulating the liquid in the reactor. the evolved gas was collected in a gas holder.

An 800 ml aliquot of the acclimated mesophilic sludge (VSS, 5, 110, mg l^{-1}) was introduced into the reactor. The liquid in the reactor was then circulated overnight at 37 ℃ by pump P-2. During acclimation, synthetic wastewater (TOC, 7,600mg l^{-1}) was fed into the reactor at a TOC volumetric loading rate of 1 g l^{-1} d^{-1}.

After a 25-d acclimation period with the synthetic wastewater, the leachate with addition of nutrients was fed into the bottom of the reactor at the same TOC loading rate (HRT, 6.8 days). The effect of TOC loading rate on performance during anaerobic treatment was investigated by adjusting the feeding rate.

(3) Activated sludge treatment.

Fig. 11 shows a schematic diagram of the aerobic activated sludge treatment system. The aeration vessel, made of acrylic resin, had a partition wall separating the 0.7 *l* settling zone of 0.7 l^{-1} from the aeration zone with a working volume of 2 *l*. The temperature was controlled at 20℃ via a bimetal sensor. Aeration was introduced through a ball filter installed in the bottom end of the reactor from a blower (P-2) which also served to mix the liquor in the reactor.

An 2,800 ml aliquot of activated sludge, which was provided by a sewage treatment works, was introduced into the reactor and synthetic sewage (TOC, 860 mg l^{-1}) was then fed into the reactor at a feeding rate of 0.2 l d^{-1}. After 44-d of operation, the anaerobically treated leachate was fed into the reactor instead of the synthetic sewage.

(4) The leachate and effluents from anaerobic and aerobic processes were diluted 7.1 times with distilled water. Next, 1 ml of each diluted sample was placed on a Saphacryl S-300 column (20x1,000 mm). The eluted fractions were collected with a fraction collector (SF-2120, ADVANTEC, Tokyo), and the TOC concentration of each fraction was measured. The molecular markers of standards were as follows: bovine

serum albumin (68,000 Da), trypsin (23,000 Da), cytochrone C (12,000 Da), insulin B (3,500 Da).

Table 3 Composition of Leachate

(Unit : mg l^{-1})

Period	Leachate(A) 7/Oct/'95-23/Jan/'97	Leachate(B) 23/Jan-18/Feb	Leachate(C) 18/Feb-
TOC	8430	6195	7335
IC	0	0	0
COD$_{Cr}$	6035	6010	6275
BOD	-	11372	-
Protein	3619	-	-
Lactic Acid	0	0	45
Formic Acid	0	293	954
Acetic Acid	5736	3785	4165
Propionic Acid	1039	651	762
Butyric Acid	1749	1461	1423
i-Valeric Acid	0	440	511
n-Valeric acid	0	498	519
Total VFA	8564	6736	7973
TS	21248	19400	21154
TVS	10388	9930	10454
Ash	11193	9960	10234
SS	661	810	422
VSS	-	431	214
Ash	-	379	208
K^+	906	-	957
Ca^{2+}	1755	-	1388
Na^+	1498	-	2312
Mg^{2+}	N.D.	-	N.D.
NH_4^+	1960	1538	1183
Cu^{2+}	N.D.	N.D.	-
NO_2^-	0.6	8	-
NO_3^-	0	8	0
SO_4^{2-}	0	520	435
PO_4^{3-}	0	61	42
Cl^-	1853	1436	3011
pH (-)	10.6	10.1	10.1
Conductivity (mS/cm)	11.6	13.0	13.2

N.D : non detected - : not determined

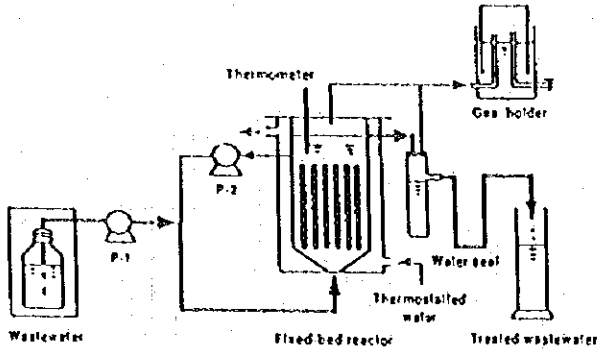


Fig. 10 Schematic diagram of anaerobic treatment system using an upflow anaerobic filter process (UAFP) reactor.

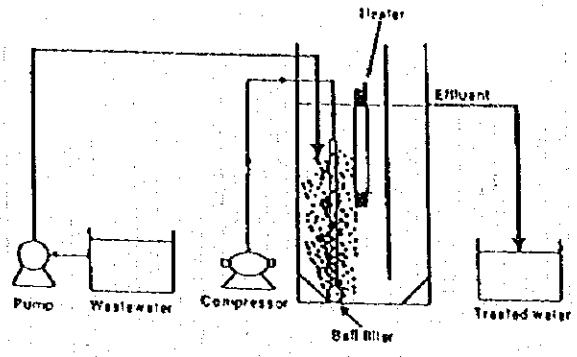


Fig. 11 Schematic diagram of aerobic treatment system using an aeration tank consists of an aeration zone settling zone.

(5) The effluent from the biological treatment system was used in coagulation test. $FeCl_2 \cdot 6H_2O$ at concentrations ranging from 200-500 mg l^{-1}, was added separately into 200 ml of the effluent at pH 6. The liquid was first stirred at 120 rpm for 5 min and then at 30 rpm for 20 min without interruption. After centrifugation, TOC and color intensity in the supernatant were measured. The effect of pH was also studied by varying the pH from 6 to 9 at a $FeCl_2 \cdot 6H_2O$ concentration of 300 mg l^{-1}.

5.2 Results and Discussion

(1) Anaerobic treatment of leachate using the UAFP reactor

Fig. 12 shows the variation in effluent quality and gas production over the period of the anaerobic treatment test using the UAFP reactor. When a TOC removal efficiency of 90 % was achieved for the treatment of synthetic wastewater, the leachate with addition of nutrients was fed into the reactor in place of the synthetic wastewater. At a TOC loading rate of 2 g l^{-1} d^{-1} the TOC removal efficiency decreased to 70 %, but the gas production rate was increasing and the VFA concentration in the effluent was about 1,000 mg l^{-1}. The TOC loading rate was then increased step wisely with an increase in feeding rate. The TOC removal efficiency

and the VFA concentration in the effluent were more or less constant at a TOC loading rate of less than 6 g l^{-1} d^{-1} while the gas production rate increased with an increase in TOC loading rate. The TOC loading rate was then increased to 8 g l^{-1} d^{-1}. The VFA concentration increased to about 1,300 mg l^{-1} and the TOC removal efficiency decreased to about 63%, but their values were constant at this TOC loading rate which corresponded to an HRT of 0.9 days. It was found that the high-strength leachate was efficiently treated under anaerobic conditions at a short HRT by addition of nutrients to the leachate. Using steady-state data at each TOC loading rate, the effect of TOC loading rate on TOC removal efficiency, gas production rates and effluent TOC concentration were determined (Fig. 13). There was a corresponding increase in gas production rate with an increase in TOC loading, and the gas production yield was 1.2 l (g TOC)$^{-1}$ with respect to TOC consumed. This value was 64% of the theoretical value (1.87 l (g TOC consumed)$^{-1}$), but the methane content was high, being 77%. The TOC concentration in the effluent was almost constant 2,000 mg l^{-1} at a TOC loading rate of less than 6 g l^{-1} d^{-1}. This suggests that there were probably undegradable compounds in the leachate. It was also observed that crystals were formed around the bottom of the reactor during anaerobic treatment. The crystals seems to be derived from Ca^{2+} judging from the decrease of minerals before and after treatment.

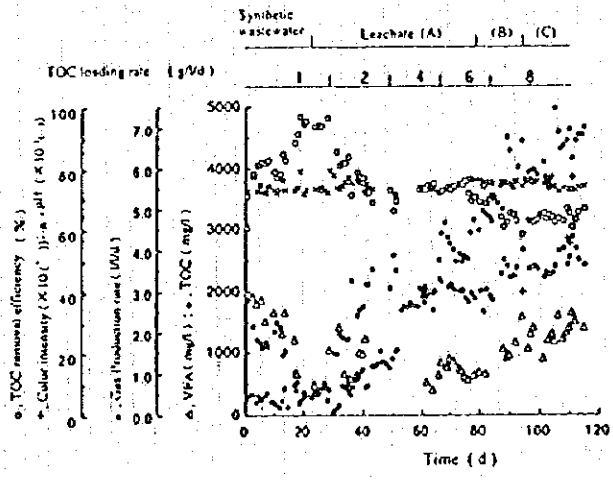


Fig. 12 Effluent quality and gas production during anaerobic treatment of leachate by the UAFP

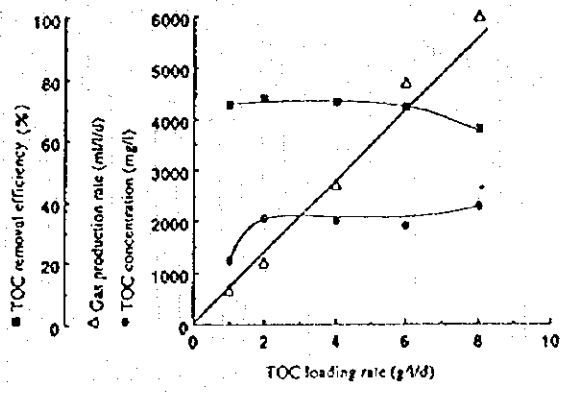


Fig. 13 Effect of TOC loading rate on TOC removal efficiency and gas production rate during anaerobic treatment

(2) Aerobic treatment of the anaerobically treated leachate by activated sludge.

An aerobic treatment test was carried out, using the anaerobically treated leachate which had been previously stored. Its compositions were as follows (mg l^{-1}): TOC, 2,116; CODcr, 3,249; BOD, 1,206; NH_4^+, 3,800; PO_4^{3-}, ND. Although PO_4^{3-} was added to the leachate when it was treated in the UAFP, PO_4^{3-} was not detected in the anaerobically treated leachate. After an addition of 46.7 mg l^{-1} of NaH_2PO_4 to the anaerobically treated leachate to provide the ratio of BOD : P of 100 : 1, the leachate was used for the aerobic treatment test.

Fig. 14 shows variation of effluent quality and MLSS over the period of the aerobic treatment test with synthetic sewage (1-46 days operation) and anaerobically treated wastewater (after 46 days operation). During the aerobic treatment of

synthetic sewage activated sludge was sometimes withdrawn to maintain the MLSS concentration at 2,000-3,000 mg l^{-1}. The concentration of MLSS decreased as soon as the anaerobically treated leachate was introduced in place of the synthetic sewage, but the MLSS subsequently increased. However, the concentration of MLSS dropped sharply again. Activated sludge acclimated in our laboratory was then added to the aeration tank to maintain the MLSS concentration of 2,000-3,000 mg g^{-1} after 68-d operation. Afterward the decrease of MLSS was observed again, but the quality of effluent was constant with a TOC concentration of about 1,300 mg l^{-1} and BOD was below detection. As apparent from the quality of the effluent, there are probably some recalcitrant compounds in the leachate.

(3) Change of TOC components by biological treatment

Fig. 15 shows elution curves of gel filtration of the original leachate, the anaerobically treated leachate, and the anaerobically-aerobically treated leachate. The leachate has two kinds of peaks with a high molecular weight (av., 4,500 Da; peak A) and a low molecular weight (av., 1,300 Da; peak B). As apparent from the figure, not only compounds in peak B but also most of the compounds in peak A were degraded by anaerobic treatment. Among the compounds remained in the anaerobically treated leachate, low molecular compounds rather than high molecular compounds were found to be easily degraded by activated sludge.

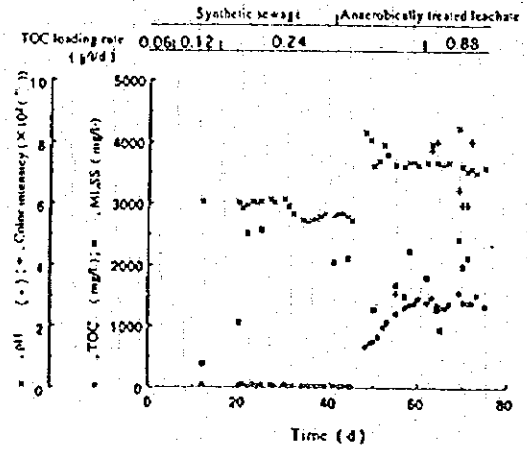


Fig. 14 Effluent quality and MLSS levels of anaerobically pretreated leachate during aerobic activated sludge treatment.

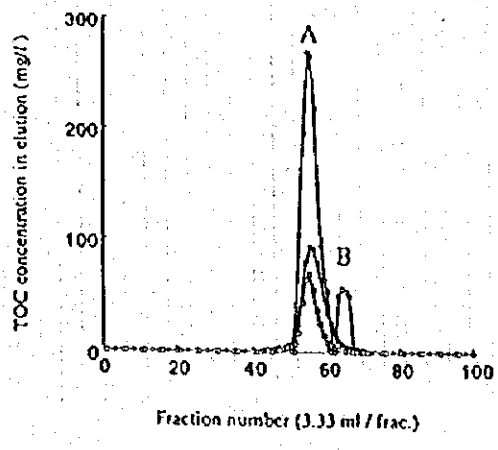


Fig. 15 Changes of elution pattern of leachate and biologically treated leachate. Symbols : -o-, leachate ; -•-, anaerobically treated leachate ; -○-, anaerobically and aerobically treated leachate.

(4) Removal of color yielding materials remaining in the leachate treated by the biological treatment system

Fig. 16 shows the effect of the amount of $FeCl_2 \cdot 6H_2O$ addition on removal efficiency of color at a pH of 6. The color intensity and the TOC concentration decreased gradually with an increase in addition of $FeCl_2 \cdot 6H_2O$. As the amount of sludge generated increased rapidly at a $FeCl_2 \cdot 6H_2O$ concentration of 500 mg l^{-1}, the amount of $FeCl_2 \cdot 6H_2O$ determined to be most suitable was 300 mg l^{-1}. Fig. 17 shows the effects of pH. The color intensity and TOC concentration were lowest at a pH 7. However, it was found the thorough removal of the residual TOC components and color

yielding materials was very difficult by coagulation after biological treatment.

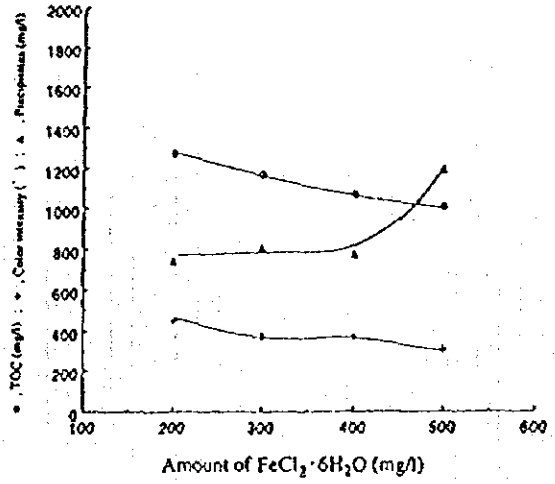


Fig. 16 Effect of amount of $FeCl_2 \cdot 6H_2O$ added on removal efficiency of color materials and TOC at a pH of 6.

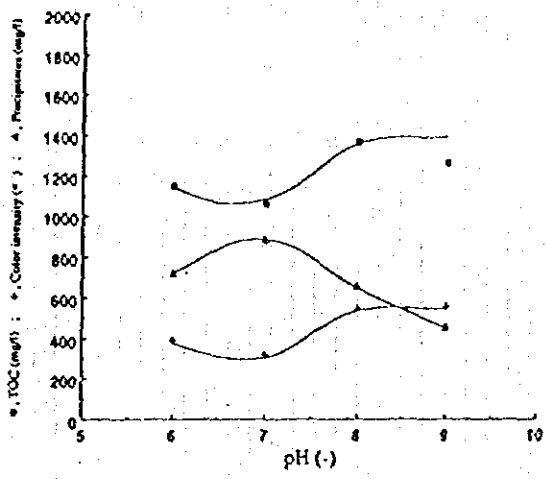


Fig. 17 Effect of pH on removal efficiency of color and TOC with 300 mg l^{-1} of $FeCl_2 \cdot 6H_2O$.

(5) Evaluation of performance between biological treatment and traditional systems

Table 4 shows the treatment conditions and effluent qualities of both systems.

Table 4 Evaluation of performance between biological and conventional systems.

Biological treatment system

Addition of nutrients (PO_4^{3-}, Mg^{2+}, Ca^{2+}, S)

Conditions		leachate	pH adjustment	UAFP	Activated sludge	Coagulation	Effluent
HRT	(days)			0.9	2.6		
pH	(—)	10.2	6.0	7.2	7.2	7.0	
Temp	(℃)			37	30		

Quality of effluent						
TOC	(mg l^{-1})	7,335	3,303	1,416	1,062	
Color intensity (°)		654	566	630	314	→ 580
COD$_{Cr}$	(mg l^{-1})	6,275	3,249	2,400	2,120	
BOD	(mg l^{-1})		1,306			

Conventional system

Addition of PO_4^{3-}

Conditions		leachate	Conditioning tank	pH adjustment	Activated sludge	Coagulation	Effluent
HRT	(days)		7.5		23.0		
pH	(—)	9.6	6.9		7.5	8.4	
Temp	(℃)						

Quality of effluent						
TOC	(mg l^{-1})	6,245		1,494	1,260	
Color intensity (°)		602		1,033	718	
COD$_{Mn}$	(mg l^{-1})	2,425	1,714	1,500	1,400	
BOD	(mg l^{-1})	8,500	2,700	20		

The biological treatment system requires only 0.9 days for anaerobic and 2.6 days for aerobic treatment. In contrast, the commercial-scale traditional system requires 23 days for aerobic treatment alone. With respect to the quality of effluent, the TOC

concentration decreased to 1,416 mg l^{-1} by aerobic treatment following anaerobic treatment in the biological treatment system. In contrast, the TOC concentration in the effluent from the commercial-scale activated sludge was almost the same value even though the leachate was aerobically treated at a HRT of 23 days. As mentioned above, the leachate will certainly contain recalcitrant materials. By coagulation with $FeCl_3$, about 25% of residual TOC components and 54% of color materials were removed, respectively, thus most of the TOC components were still remaining. An efficient process for treatment of high-strength leachate will have to be developed to produce an effluent suitable for discharge to water course.

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TREATMENT OF RUBBER-THREAD MANUFACTURING WASTEWATER --- ANAEROBIC DIGESTION COUPLED WITH CHEMICAL COAGULATION

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ABSTRACT

Wastewater from a rubber-thread manufacturing industry containing about 200 to 300 mg/L of zinc was first coagulated with sodium sulphide ennehydrate at Zn^{2+} : Na_2S molar ratio of 1:1 to remove more than 99% of zinc ions. By pH adjustment, 99% of zinc removal was achieved when the pH was adjusted to 9.0 and 10.0. When this coagulated wastewater was treated in the upflow anaerobic filter process (UAFP) reactor, the TOC removal efficiency was constant at about 90% even at a volumetric loading rate of 8 gTOC/L/d which corresponded to a hydraulic retention time of 0.63 d. The gas evolution rate increased with the increase in TOC loading rate resulting in a gas yield of 1.25 L/gTOC consumed at 8 gTOC/L/d of loading rate. The amount of methane generated was about 70% of the total amount of gas evolved.

INTRODUCTION

Total export of rubber products in Malaysia increased by 12.8 percent to reach US 1 billion with the latex-based products sub-sector contributing 66.0 percent of the total according to 1994 statistics. However only a small percentage of the said figure was contributed by rubber thread. Due to the small number of rubber-thread manufacturers in Malaysia, little attention has been paid to the management of wastewater generated by them. The raw wastewater, although organic in nature, contains a significant amount of zinc ions, which renders it a hazardous waste. The heavy metal needs to be effectively removed prior to biological treatment, which otherwise would be seriously affected. In addition, it is important to select the most suitable and cost-effective coagulant to avoid the generation of excessive chemical sludge which is designated as a scheduled waste in Malaysia. In this study, sodium sulphide was employed to coagulate the zinc ions prior to biological treatment.

MATERIALS AND METHODS

Chemical Coagulation for the Removal of Zinc Ions

Jar Tester Method

A series of sample containing 400-mL rubber-thread manufacturing wastewater (RTMW) were prepared in 500-mL beakers. The pH was adjusted to 5.0 by the addition of 1N NaOH. Sodium hydrogen carbonate ($NaHCO_3$; Wako Pure Chem. Ind. Ltd) was added appropriately into each sample to give a final concentration of 30mg/L. The samples were then placed in the jar tester and agitated at 120 rpm for 2 minutes. Then appropriate amount of Na_2S was added and agitation was continued for another 5 minutes at the same speed. The agitation speed was then decreased to 30 rpm to produce a mild agitation for another 15 minutes. At the end of mild agitation the samples were left unstirred for 20 minutes.

Determination of Optimum Dosage of Coagulant

Following the jar-tester method described above, the concentration of coagulant was varied in each sample containing RTMW. The concentrations of Na_2S added were 1.0, 1.5, 2.0 and 2.5 moles corresponding to the zinc concentration in the wastewater. The amount of residual zinc ions in the supernatant was measured with an atomic absorption spectrophotometer (Shimadzu AA 640-13S). The amount of suspended solids (SS) formed was also measured. In determining the SS, the supernatant was carefully decanted and the bottom layer which contained mostly solids was kept for the measurement. The calculation of SS however was made based on the total volume of the samples, which was 400 mL.

Determination of Optimum pH for the Zinc Coagulation

Following the jar-tester method, the pH was varied in each sample containing RTMW. In determining the optimum pH for coagulation of zinc, the pH was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 9.0 and 10.0. In order to maintain the pH, sodium hydrogen carbonate was not added in the samples of which the pH was adjusted to below pH 7. Into all of these samples, sodium sulphide was added at a molar concentration of 1.5 times of the zinc concentration in the wastewater. The amount of residual zinc ions and SS were determined as described above.

Removal of Zinc Via pH Adjustment

The same procedure as above was carried out except that sodium sulphide was not added to the samples.

Analytical Methods

All parameters, with the exception of SS, were analyzed for the supernatant after centrifugation at 10,000 rpm for 10 min. The SS value was obtained by drying the pellet (centrifugation at 10,600 x g for 10 min) in a Gooch crucible overnight at 105°C, cooled in a dessicator, and weighed (1). The COD value was obtained by open Reflux Digestion method (2).

Soluble total organic carbon (TOC) was analyzed with a TOC autoanalyzer (TOC -; Rosmount, USA) (2). Total volatile fatty acids (VFA) were analyzed by a post-label method, in which the absorbance of volatile compound reacts with a solution of bromothymol blue, at 450 nm (870 - UV/VIS detector, Japan Spectroscopic Co. Ltd., Tokyo) after separation of VFA from the wastewater by high performance liquid chromatograph (HPLC 860-CO, Japan Spectroscopic Co. Ltd., Tokyo) on a column designed for the analysis of VFA (Shim-pack SCR-101 H; Shimadzu, Kyoto).

The cations and anions were analyzed by using an ion chromatograph (High-pressure Chromatograph Module CRB; DIONEX Corp., California, USA with a Conductivity - Detector - 3 (CDM-3) which employs an Anion Self-Regenerating Suppressor. ASRS-I 4 mm (P/N 043189) and a Cation Self-Regenerating Suppressor CSRS-I 4 mm (P/N 043190) after separation on a column designed for the cation and anion analysis (for cations: IonPac CG12 (P/N 44002) and IonPac CS12 4mm (P/N 44001), for anions : IonPac AG12A 4mm (P/N 46035) and IonPac AS12A 4mm (P/N 46034); DIONEX Corp., CA, USA)

The determination of zinc was carried out by aspirating the sample into an air-acetylene flame and the amount of light absorbed by the atomized element in the flame as measured by a detector in the atomic absorption spectrophotometer (Shimadzu AA 640-13S) (2).

Protein analysis was perfomed following the Lowry-Folin Method in which the measurement of proteins was presented with the Folin Phenol reagent after alkaline copper treatment and the colour was measured with UV-VIS 160A Shimadzu (3).

The Biological Treatment System

An upflow anaerobic filter process (UAFP) reactor (Shouwa Engineering Co., Tokyo) was used for the anaerobic treatment. The support medium was composed of clay (particle Ø, 0.1 ~ mm) mixed with high density polyethylene (HDPE) Type 99M (Ø, 0.52~ 2 mm) at 45:55 proportion and the mixture burnt at 100-230°C. The UAFP reactor was made of glass with a working volume of 4.9 L. The reactor temperature was maintained at 35°C by the circulation of thermostated water through a water jacket. The gas evolved was led into a wet gas holder and analyzed daily. The schematic diagram of the anaerobic treatment system is shown in Fig. 1.

The seeding sludge was obtained from an acclimated reactor (CSTR type) which was fed with synthetic wastewater containing (g/L) : glucose, 35; corn steep liquour, 35; K_2HPO_4, 3; KH_2PO_4, 2; $(NH_4)CO_3.H_2O$, 5; $FeCl_3.6H_2O$,1; pH 6.3, and maintained at an organic loading rate of 1 to 2 gTOC/L/d by a draw-and-fill method. In this study, trace elements were added at concentrations of 1.4 mg/L and 0.4 mg/L per 10000 mgTOC/L using $NiCl_2.6H_2O$ and $CoCl_2.6H_20$ respectively. The reactor was continously fed with coagulated RTMW where zinc ions had been removed by using sodium sulphide as coagulant. The concentration of the coagulant added was in the molar ratio of 1:1 corresponding to the zinc concentration in the raw wastewater. The coagulated wastewater was preserved in a refrigerator and was fed from the bottom of the UAFP reactor by a peristaltic pump and the effluent was allowed to overflow from the top of the reactor into a waste bottle.

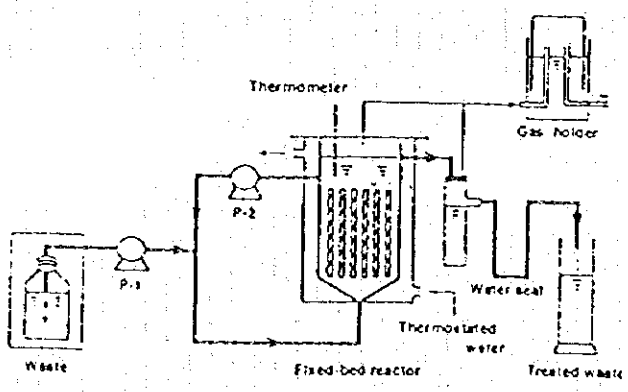

Fig. 1. Schematic diagram of anaerobic treatment system using an upflow anaerobic filter process (UAFP) reactor

RESULTS AND DISCUSSION

Characterisation of Raw Wastewater (RTMW)

The composition of the raw RTMW was found to vary at each batch of collection. As shown in Table 1, the TOC concentration was in the range of 2000 to 5000 mg/L. The COD concentration was found to be about two and a half times as much as the TOC concentration. The VFA concentration ranged from 6000 to 10000 mg/L. With respect to the VFA concentration, the TOC concentration was about 40 to 45% as much. It is also important to note that the raw wastewater contained about 250 mg/L of zinc which corresponded to about 8% of the TOC concentration. The amount of SS was found to be about 5% of the TOC concentration. The levels of cations and anions were, however, found to be inconsistent in each batch of the raw wastewater with a fairly high level of chloride ions compared to the others.

The pH was, however, observed to be fairly consistent at about 4.0 in all of the three collected samples.

Table 1. Composition of raw rubber-thread manufacturing wastewater

Parameter [1]	Sample 1	Sample 2	Sample 3
TOC	2467	4200	5200
IC	0	86	29
VFA	5910	9452	9860
SS	142	238	262
COD	NM	10402	12905
Cation: NH_4^+	146	166	98
Mg^{2+}	0.5	NM[2]	NM[2]
Ca^{2+}	7.2	5.4	4.2
Zn^{2+}	244	340	160
Anion: Cl^-	341.9	390.2	240.4
NO_3^-	1.4	8.2	2.0
PO_4^{3-}	69.7	58.4	33.6
SO_4^{2-}	13.3	14.6	8.2
pH (-)	4.12	3.98	4.30

(1) All parameters, except pH, are expressed in mg/L.

(2) NM = not measured

Removal of Zinc Ions

Optimum dosage of coagulant

Zinc ions were removed by coagulation via the Jar-tester method. Sodium sulphide ennehydrate ($Na_2S.9H_2O$, 96%; Nacalai Teque) was used as the coagulant. The stoichiometric equation of the zinc coagulation with sodium sulphide is as follows:

$$Zn^{2+}_{(aq)} + 2Na_2S_{(s)} + 2H_2O_{(l)} \rightarrow ZnS_{(s)} + 4Na^{+}_{(aq)} + 2OH^{-}_{(aq)} + H_2S_{(g)} \quad (1)$$

Based on equation(1) the optimum amount of coagulant to be used was investigated and the results are as shown in Fig. 2.

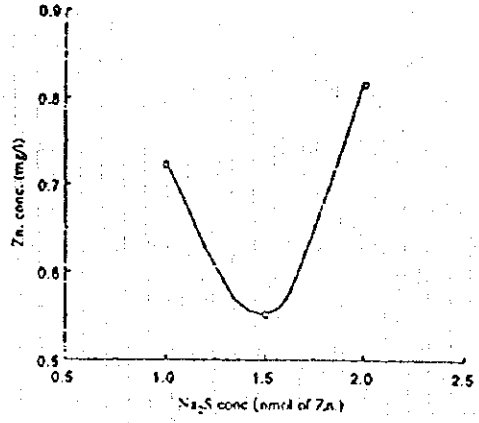

Fig.2 Amount of residual Zn^{2+} in the filtrate after coagulation with sodium sulphide at n moles of Zn^{2+} conc. in the raw rubber-thread manufacturing wastewater

As depicted in Fig. 2, the removal of zinc were 99.7%, 99.8% and 99.6% with the addition of 1.0, 1.5 and 2.0 moles as much as the zinc concentration in the raw respectively. Therefore it can be concluded that a nearly complete removal of zinc could be achieved even at coagulation of 1:1 molar ratio of Zn^{2+} : Na_2S.

Optimum pH for the coagulation of zinc ions

In the coagulation process, it is also important to determine the optimum pH which favours the removal of zinc with the addition of optimum amount of the coagulant. Beside using the coagulant, zinc ions can also be precipitated as zinc hydroxide, $Zn(OH)_2$, at pH 5.5 and above. Because of this, the optimum pH at which zinc could be precipitated by adjusting the pH from 5.0 to 10.0 with 1N NaOH was investigated simultaneously. The results are summarized in Fig. 3.

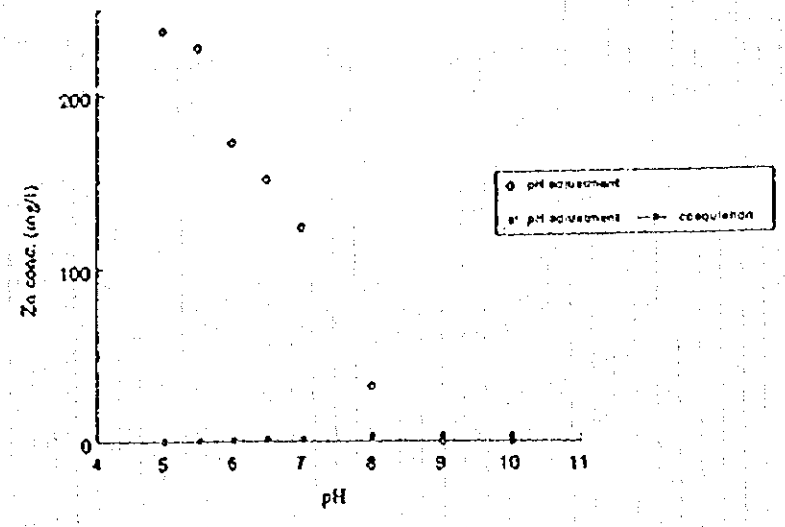

Fig. 3. Residual Zn^{2+} in the raw rubber-thread manufacturing wastewater after coagulation (Na_2S was added) and after pH adjustment (without Na_2S)

It was found that zinc was completely removed when coagulation was carried out at pH between 5.0 and 10.0. However, without the addition of coagulant, 30 to 50% of zinc removal was achieved when pH was adjusted from 6.0 to 7.0. At pH 8, about 80% of zinc was removed and at pH 9.0 and 10.0, more than 99% of zinc removal was recorded. These findings suggest that coagulation at pH 5 would be significant if more than 50% of zinc ions present in the raw wastewater were to be removed completely. This condition was adopted in preparing the coagulated raw RTMW for the anaerobic treatment process.

The amount of dried suspended matter obtained from the coagulation process was found to be about 500 mg/L at all pH variations as shown in Fig. 4. Even though the SS obtained during pH adjustment was about half of that obtained in the coagulation process at below pH 7 it would still be desirable to adopt sodium sulphide as the coagulant since the price of commercial-grade sodium sulfide is about one-third of that of caustic soda. Additionally, the amount of solid waste production was also found to be about half of that normally generated in the conventional reactor such as CSTR and ponding system where lime is commonly used as the coagulant.

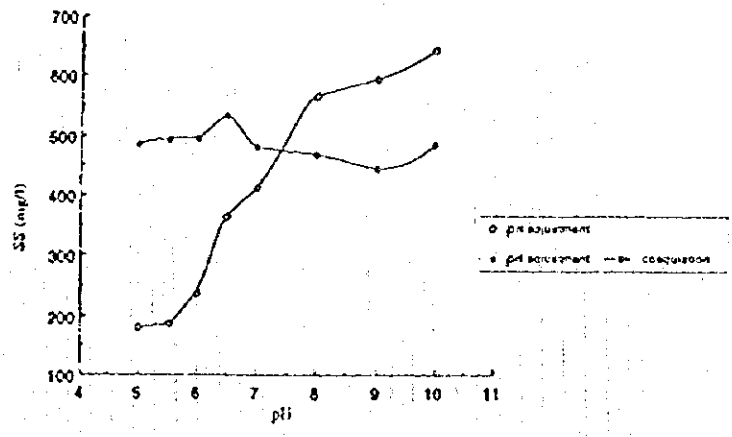

Fig.4. The amount of suspended solids obtained during coagulation and pH adjustment

Anaerobic Treatment Using UAFP

After acclimation of the system with ten-fold diluted synthetic wastewater (TOC 18800 mg/L), coagulated wastewater was fed into the UAFP reactor at an initial TOC loading rate of 0.5 g/l/d . The loading rate was increased stepwise to 8 gTOC/lL/d which was the maximum loading rate reported in this paper. Fig.5 shows the amount of gas evolved and the pH during the anaerobic treatment process. At a low volumetric loading rate the pH was found to be between 7.0 and 8.0. However at a high volumetric loading rate the pH was fairly constant at about 7.5. The gas evolution rate increased with increase in the TOC loading rate. The gas yield increased drastically from 0.52 to 1.25 L/g of TOC consumed at TOC loading rates from 1.0 up to about 8.0gTOC/L/d. The gas yield obtained at a high TOC loading rate corresponded to about 67% of the theoretical value. At a low loading rate the amount of methane gas generated was about 80 % whereas at a high loading rate it was between 65 to 72 % of the total amount of gas evolved.

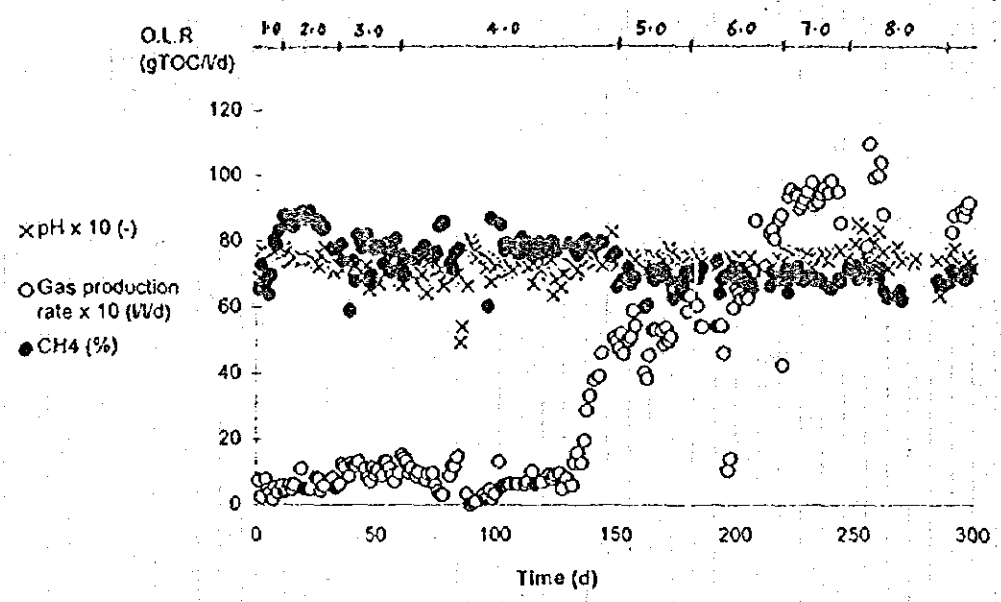

Fig.5. Gas production rate and pH during the anaerobic treatment of rubber-thread manufacturing wastewater using UAFP

Fig.6 shows the quality of the anaerobically treated wastewater. The TOC concentration increased with increase in the TOC loading rate. At a low TOC loading rate it was found to be about 200 mg/L and at high loading rate it was about 500 mg/L. The later corresponded to about 1333 mg/L of COD theoretical value since the organic carbon source from the raw wastewater was mainly acetic acid. As shown in Fig.6 the COD concentration at a high volumetric loading rate was about 1500 mg/L and this correlated well with that of TOC. The TOC removal efficiency was constant at about 90% even at a loading rate of 8 gTOC/L/d. This corresponded to a hydraulic retention time of 0.63 day. The amount of inorganic carbon increased with the increase of the TOC loading rate due to the effect brought about by the accumulation of carbonates. The addition of mineral nutrients for enhancing anaerobic treatment has been discussed by a number of researchers. Takashima and Speece (4) reported the effects of the addition of Ni^{2+} and Co^{2+} ; Yang *et al.* (5) reported the incorporation of Ni^{2+} into carbon monooxide dehydrogenase in Acetogen while Kasakura and Kawase (6) reported the influence of mineral nutrients such as Ni^{2+} and Co^{2+} on the anaerobic treatment of sent beer. The consumption of Ni^{2+} and Co^{2+} is essential for the activity of metal enzymes such as B_{12}-CH_3 and F_{430} which are active in the pathway for the conversion of acetate to methane (7) and the C_1 cycle for the reduction of CO_2 to methane (8), thereby resulting in the acceleration of methane fermentation.

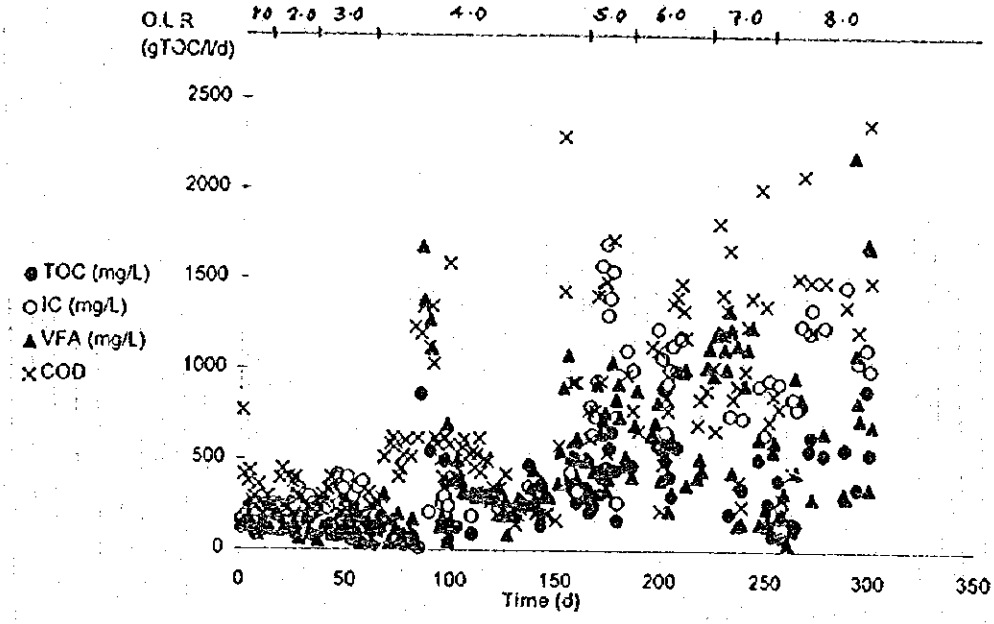

Fig.6. Time course of the effluent quality during the anaerobic treatment of raw rubber-thread manufacturing wastewater

In order to comply with the regulatory standards for effluent discharge from rubber processing in Malaysia, the anaerobically treated wastewater needs be subjected to an aerobic process such as the activated sludge system. This is being investigated in our laboratory with the aim to further remove the TOC concentration in the anaerobic effluent, thereby the final effluent would contain less than 50 mg/L of COD before watercourse discharge.

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USE OF ALGAE IN THE MONITORING AND BIOREMEDIATION OF HEAVY METAL WASTES

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ABSTRACT

Algae including both the microalgae and macroscopic (seaweeds) species fulfil the criteria for good biological monitors of pollution in the aquatic environment. Algae bioaccumulate heavy metals to high levels without being damaged. Benthic species like diatoms and seaweeds are useful for indicating time-integrated changes in heavy metal concentrations in the water. Toxicity and bioaccumulation data of heavy metals in Malaysian species of microalgae and seaweeds are being generated through our research. Such data are useful for the development of water quality criteria and standards. Algal species sensitive to heavy metals make good bioassay organisms while more tolerant species are useful for long term biomonitoring.

Non-living *Chlorella vulgaris* biomass was tested as a biosorbent agent for heavy metals. Cadmium and nickel are adsorbed rapidly, reaching equilibrium in two minutes. The maximum binding capacity for cadmium and nickel was 293 and 380 μmol g^{-1} dry wt. at pH 5, respectively. Up to 85% desorption was achieved using HCl and EDTA at pH 2.

Similarly non-living biomass of *Sargassum baccularia,* a brown seaweed proved to be a good biosorbent for cadmium and copper. The adsorption capacity for cadmium and copper (II) at pH 5 was 0.81 mmol g^{-1} and 1.13 mmol g^{-1} respectively.

The following publications give details of some of our results.

Waste Management in Malaysia: Current Status and Prospects for Bioremediation
B. G. Yeoh *et al.* (Eds.). 1993. pp. 27-40

3

ALGAE AND HEAVY METAL POLLUTION

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INTRODUCTION

A 'biological indicator' may be defined as an 'organism which accumulates substances in its tissues in a way so as to reflect environmental levels of those substances or the extent to which the organism has been exposed to them' [1]. These organisms may also be termed 'bioaccumulators' and are useful for the detection and analysis of very low concentrations of substances in the environment.

The use of biological indicators or monitors has an advantage over direct analysis of *metals in the water [2]* for,

(i) the biologically-available metal can be measured directly using the organisms;
(ii) time-integrated estimates of available metal concentrations in water can be conducted;
(iii) process of concentration of water samples containing low quantities of metals is expensive and laborious;
(iv) presence of humic acid or other complexing or chelating agents may render some of the metal unavailable to the organisms, causing over-estimation of the toxicity of the substance; this can be avoided using the biological indicator;
(v) the form of the substance in the water affects its availability and toxicity to organisms;
(vi) large seasonal, and even diurnal variations in metal content exist making comparison of sites difficult.

Sediment analysis has also been used as an indication of metal content in the water [3,4,5]. Problems associated with it include [2]:

(i) it is difficult to calculate the metal content because the metal content is not only a function of the quantity of metal deposited but also a function of the ratio of metal deposited to the sediment deposited over a given time period;
(ii) particle size, form and size also affect the final concentrations of metals in the sediment , because charge, presence of certain ionic groups and the surface area :volume ratio affect the adsorption of the metal prior to particle settling;
(iii) differences in mobilisation rates also affect the final concentration;
(iv) the concentration of a metal increases with the organic content [total carbon] in a linear manner;
(v) it does not relate the metal concentration to the actual availability to the organisms.

Therefore the use of biological indicators are important when the objective in metal analysis is to determine the availability of the metal to the living biomass of a specific habitat and to predict the impacts of metal pollution, in particular the toxic heavy metals, on an ecosystem, and on man.

The characteristics of good biological indicators include [1,2,6] :

(i) the organisms should accumulate the pollutant without being killed or rendered incapable of sustained reproduction by the levels encountered;
(ii) the organisms should be sedentary to be indicative of the area of study;
(iii) the organisms should be abundant and widespread to facilitate comparisons between areas;
(iv) the organisms should have a long life to enable long-term monitoring;
(v) the organisms should be reasonably large to provide enough tissue for analysis;
(vi) the organisms should be easy to sample and hardy to survive in the laboratory;
(vii) the organisms should tolerate brackish water;
(viii) the organisms should exhibit a high concentration factor for metals;
(ix) a simple correlation should exist between the metal content of the organism and the average metal content in the environment;
(x) all organisms in the survey should exhibit the same correlation between their metal contents and those in the environment, at all locations studied, under all conditions;
(xi) the organisms should be readily identified.

Metals can originate from three possible routes : from solution, from ingestion of food and from ingestion of particulate material containing metals [2]. Not all indicator species can be used for all three types of metal load, and the indicator has to be chosen based on the targetted substance and its form.

Many algal species meet many of the requirements for biological indicators. Several toxicological studies of algae and heavy metals have been reported and reviewed [2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. Algal indicators are important as they form the basis of many food-chains.

ALGAE AS INDICATORS OF HEAVY METALS

Marine Ecosystem

Much of the studies relating marine algae, both microalgae and macroalgae, to toxic metals in the environment , have been carried out in temperate waters. As such, the commonly used macroalgal species include *Fucus vesicularis, Fucus serratus, Fucus spiralis, Laminaria digitata, Ascophyllum nodusom* and *Ulva lactuca* [17, 18, 19] Tropical species studied include *Enteromorpha intestinalis, Caulerpa racemosa, Bryopsis plumosa, Gelidiella acerosa, Cystoseira indica, Sargassum bracteolosum, Sargassum linearis and Dictyota dichotoma* [20, 21].

Algae reflect only the soluble metal content in the environment and not the total metal loads. Metals are lost slowly from many macroalgae, and because bound metals have long biological half-lives, a high degree of time-integration of the metals in the water is obtained [2].

The concentration of metals in macroalgae is affected by many factors:

(i) Species variations
Different species concentrate metals to different extents [20, 21, 22]. Under the same conditions, *Amphiroa anceps, Enteromorpha intestinalis* and *Caulerpa racemosa* exhibited minimum concentration of chromium, lead and cadmium while *Bryopsis plumosa, Gelidiella acerosa* and *Cystoseira indica* showed maximum concentration of the same metals [21]. The accumulation rate of chromium was higher than lead followed by cadmium in these species. Of four species of brown, three species of green and three species of red algae tested in Jordan, the red algae showed the highest concentration

of zinc, manganese, copper, cadmium, magnesium and iron [22].

(ii) Variation with age of thalli
Concentrations of zinc in *Laminaria digitata* and zinc, lead, copper, iron and aluminium in *Fucus vesiculosus* were higher in older parts of thalli [2]. The basal, mid and peripheral parts of the thalli of *Ulva lenestrata, Codium yezoense* and *Codium fragile* accumulated metals differently [23].

(iii) Seasonal variations
Fucus vesiculosos exhibited seasonal variation in accumulation of zinc, iron, aluminium and manganese [19,24]. Concentrations of the metals were generally high in summer and low in winter [17, 19]. This is attributed to varying metabolic activities, growth rates, climatic conditions and hydrobiologic conditions [17, 19].

(iv) Sampling position
The position on the shoreline at which the plants grow may affect the content of heavy metals [25]. Concentrations of zinc and cadmium were highest in *Fucus versiculosus* collected from the mid-range of the vertical shoreline distribution. Reasons suggested for this included differences in metal availability due to tidal exposure, salinity and metal stratification [24]. This was more evident in estuaries than the open coasts.

Freshwater Ecosystems

Both freshwater microalgae and macroalgae have been studied as to their uptake and tolerance of heavy metals. Among the microalgae studied are *Selenastrum capricornutum, Chlorella pyrenoidosa, Navicula cryptocephala, Achnanthes microcephala, Synedra acus, Synedra filiformis, Synechococcus* and *Closterium moniliferum* [26, 27, 28, 29, 30]. Filamentous forms which have been studied include *Cladophora glomerata, Nitella flexilis, Oedogonium rivulare, Chara vulgaris* and *Stigeoclonium tenue* [31,13,32,33].

Research on the freshwater algae have been more extensive than on the marine algae. However as with the marine algae, temperate species are better studied. Several species of freshwater algae have been used extensively in bioassays of toxic compounds in the aquatic ecosystem. *Cladophora glomerata* and *Stigeoclonium tenue* were recommended for monitoring heavy metals in U.K. waters [31, 34]. These two species exhibited great responses to small increases in zinc, cadmium and lead as shown by their steep slopes in the dose-response curves [32]. *Selenastrum capricornutum* was used to bioassay for toxic activity in two drainage basins in Quebec [26]. Freshwater algae can also be used to monitor and remove gold and uranium [35] and to remove heavy metals from electroplating wastes [36] and paper mill effluent [37].

Like the marine algae, species variation influenced concentration of heavy metals in the freshwater algae [32]. Seasonal variations also occurred. In summer, concentrations of 10-100 ppb cadmium, copper and mercury had little effect on the plankton biomass in the river. But in winter, heavy metals of the same concentrations resulted in biomass reductions [38].

The concentration of metals in algae grown in water contaminated with paper mill effluent, was as follows : lead > nickel > cobalt > manganese [37]. The metal concentrations increased with increasing concentrations of chloride, sulphate, total hardness and alkalinity. Blue-green algae and diatoms were more tolerant of high metal concentrations than green algae. *Scenedesmus quadricauda* when exposed to heavy metals was

found to have an adaptation to copper at one order of magnitude lower than that to mercury [39].

Chara vulgaris when exposed to cadmium, mercury and lead had a 50% growth inhibition in the apical tips of the internodes at 95 ppb cadmium, 150 ppb mercury, 330 ppb organic lead and 8000 ppb inorganic lead [33].

Table 1 lists some algae, mainly microalgae, with levels of heavy metals which cause inhibition of growth.

Table 1 Concentrations of Some Metals Which Cause Inhibition in Microalgae [11, 33]

Algae	Metal	Concentration causing inhibition
Marine:		
Tetraselmis suecica	Cd	> 1 ppm
Isochrysis galbana	Cu	> 47 ppm
Isochrysis galbana	Cd	> 15 ppm
Cyclotella nana	Cu	5 ppb
Synura sp.	Cu	0.75 ppm
Skeletonema costatum	Pb	100 ppb
Freshwater:		
Chara vulgaris	Cd	9.5 ppm
Chara vulgaris	org. Pb	330 ppb
Chara vulgaris	inorg. Pb	8000 ppb
Chlorella fusca	Cu	300 ppb
Selenastrum capricornutum	Cu	90 ppb
Selenastrum capricornutum	Cd	8 ppb
Selenastrum capricornutum [50% reduction]	Pb	140 ppb
Lyngbya nigra	Cu	0.45 uM
Chlorella vulgaris	Zn	25 ppm
Scenedesmus quadricauda	Cd	61 ppb
Euglena gracilis	Cd	1000 - 10000 ppb
Scenedesmus sp.	Ni	0.5 ppm

MECHANISMS INVOLVED IN THE UPTAKE AND ACCUMULATION OF HEAVY METALS BY ALGAE

Various mechanisms have been postulated and studied to provide a better understanding of the influence of heavy metals to algae.

Uptake and Accumulation of Heavy Metals

Passive and active mechanisms have been proposed to explain the uptake of heavy metals in algae. In passive uptake, metals are adsorbed onto the algal surfaces, even on dead cells. Zinc adsorption in *Synedra ulna* and *Phaeodactylum tricornutum* have been suggested [11].

Factors which influence uptake of metals include pH, age of algal cells, aeration of culture and presence of other metals [11]. The presence of high concentrations of

copper resulted in an increased uptake of nickel in *Scenedesmus* [11]. Cadmium and copper have inhibitory effects on phosphorus uptake in *Scenedesmus quadricauda* and the process is pH dependent [12]. Toxicity of cadmium to phosphorus uptake increased strongly [almost 200-fold] with increasing pH over the range 5.5 - 8.5. Hydrated Cd^{2+} was the dominant form. A 76-fold increase in copper toxicity was observed from pH 5.0-6.5 and hydrated Cu^{2+} was the dominant form.

Except for studies on *Chlorella*, *Hormidium rivulare* and *Scenedesmus quadricauda* [12] it has been observed that in acidic conditions metals were more toxic to algae while the reverse was true of alkaline conditions [11]. *Ankistrodesmus bibrainus* exhibited reduced toxic response to heavy metals with increased pH [40]. pH and redox potential are known to be responsible for the mobilization and immobilization of heavy metals like zinc, iron, manganese, copper, etc. [11]. Iron and manganese form hydroxides at alkaline conditions and in an oxidising environment [11]. These hydroxides precipitate various metal ions and thus decrease their availabilities.

Increasing hardness of water reduces toxity of heavy metals to algae. This occurs by precipitation and co-precipitation or formation of complex carbonate and hydroxy compounds of heavy metals with calcium and magnesium [41]. The heavy metals become unavailable through these processes. Also these complexes may not be able to permeate the cell membranes and therefore their toxicity is reduced.

Salinity has not been shown to have a significant effect on heavy metal uptake and toxicity except for copper [41]. An increase in salinity results in competition between adsorbed metals and dissolved ions. The latter in replacing the metals decrease their availabilities. The accumulation of zinc, manganese and cobalt was enhanced by decreasing salinity in *Scytosiphon lomentaria* and *Enteromorpha intestinalis* [42].

An increase in temperature has been found to cause an increase in toxicity as well as the reverse. While the latter is not easily explained, enhanced respiratory activities may explain the former [43].

Nitrate, phosphates and organic compounds in the water may also reduce metal toxicity to algae [11, 13]. Insoluble metal phosphates are formed which make the metal unavailable. Organic substances such as glycollic acid, fulvic acid, humic acid [44], amino-acids, etc. have metal-binding properties and form complex heavy metal compounds, thereby making them unavailable also.

Binding of Metals on Cell Surfaces

Functional groups on algal cell surfaces are able to bind metal ions [11, 14, 15, 45, 46, 47]. Lipids and polysaccharides which are cell wall constituents appear to be preferred sites for heavy metal interaction [11]. The lipids and polysaccharides contain amino, phosphate, carbonyl and cysteinyl ligands which act as diffussion barriers. The ligands also form sites for metal ion binding.

Two marine species, *Chlorella stigmatophora* and *Chlorella salina* were monitored using anodic stripping voltametry (ASV) for the capacity of dissolved polysaccharides to bind copper, lead, cadmium and zinc [48]. The native polyssacharides produced by *C. stigmatophora* was highly charged and was efficient in metal complexing. The polyssacharides of the other species were weakly charged and did not prove useful for metal binding. The binding ability of the first species was due to the high uronic content of its polyssacharides. Heavy metals can also accumulate in sodium alginate in *Laminaria japonica, Fucus evanescens, Costaria costata , Pelvetia wrightii and Cystoseira crassipes* [49]. The range of zinc concentrations in the algae was 10.0

66.3 μgg^{-1} and of copper was 2.4 - 59.0 μgg^{-1} dry weight. The zinc in the dry residue of the algae ranged from 138 to 250 μgg^{-1} dry residue. Carrageenan was found to be a suitable binding agent for lead both *in vitro* and *in vivo* [50].

Extracellular poplypeptides, organic acids are also some extracellular products of algae which are used to complex and thus ameliorate the toxic effect of heavy metals [11].

Phytochelation Complexes

In studies on the detoxification of heavy metals by microalgae, the presence of a heavy metal binding protein was identified. Higher plants have been found to synthesize a series of cysteine-rich peptides, the phytochelatins, when exposed to heavy metals [51]. When several species of diatoms , *Chlorella, Euglena, Scenedesmus, Porphyridium* and *Sargassum* were exposed to heavy metals, they were observed to synthesize the phytochelatins [14]. The results showed that the ability to produce the phytochelatins was ubiquitous in algae and that all previously referred to 'metal-binding proteins' are phytochelatin complexes. The formation of the phytochelatins are induced, and via enzymic polymerization of peptide precursors. This is thus the mechanism employed by algae in the amelioration of heavy metal stress.

Polyphosphate Bodies

Lead, cadmium and zinc can be incorporated in polyphosphate bodies in algal cells [52, 53]. Metals incorporated this way may be transferred up the food-chain. Mobilization of polyphosphates in phosphorus-poor waters would internally mobilize the metal eventually leading to death of the organism or release of the metal into the environment. Heavy metals may be immobilized in the vacoules and be excreted back into the environment . They may also cause changes in the cells such as reduced protein synthesis, chlorophyll content and photosynthesis as shown with *Anacystis nidulans* and *Spirulina platensis* [54].

Chelation to Phenols Located in Physodes

Adsorbed cadmium was found to be localised in the physodes and cell walls in the outer layer of *Fucus vesiculosus* [55]. Physodes are small light-refracting bodies in algal cells. They contain fats, proteins, tannins, terpenes, nitrogenous compounds, glycosides and most importantly phloroglucinol-like polyphenols [56, 57, 58, 59]. This was shown in studies using X-ray microanalysis in the electron microscope. Physodes in *Fucus vesiculosus* were found to contain high amounts of phenol [2 - 3% dry weight] [58].

Phenol content in *Ascophyllum nodusum* increases with age of tissue and increasing salinity [59]. However when tissues reached three-to four years, the accumulation of phenols decreased. *Fucus vesiculosus* contain about 400% more phenols than *Ascophyllum*. Phenols from these two species had decreasing affinities for different divalent metals as follows : copper>lead>nickel>zinc>cobalt>cadmium. Phenols have highest affinity for copper. Phenols were also accumulated in soft vegetative apical parts of plant susceptible to herbivory [60]. This phenotypically plastic trait varies with differences in photosynthetic activity of thalli and availability of nutrients. Phenol content in *Fucus vesiculosus* was found to increase under nitrogen deficiency which suppresses plant primary production [61]. Phenolic content however was not correlated to carbon content but was negatively correlated to the nitrogen:carbon ratio.

USE OF ALGAE AS BIOMONITORS OF HEAVY METALS IN MALAYSIA

Information on heavy metal contamination in the Malaysian environment has been on the increase in the last decade, with the increasing awareness of toxic and hazardous pollution. Heavy metal pollution in our rivers and coastal waters [62, 63, 64, 65] and marine resources [66, 67, 68, 69, 70] is being assessed.

Coastal monitoring of Peninsular Malaysian waters gave results with regard to heavy metal contamination as shown in Table 2 [71].

Table 2 Heavy Metal Concentrations in Malaysian Waters

Metal	Range (ppm)
lead	0.02 - 0.76
cadmium	0.14 - 0.67
mercury	0.0002 - 0.21
chromium	0.01 - 0.38
copper	0.013 - 0.64
nickel	0.016 - 0.84
arsenic	0.08 - 0.60

Seaweeds or the marine macroalgae have many advantages over other biological monitors for heavy metals. They do not regulate or limit the uptake of heavy metals [18] . They can accumulate heavy metals to very high levels without damage. Being attached and benthic, they can be used for long-term monitoring of environmental conditions. In the temperate waters, the commonly used species for heavy metals detection and monitoring include *Fucus vesiculosos, Ascophyllum nodosum, Enteromorpha* and *Ulva fasciata* [18, 72, 73]. A large store of information exists on the influence of heavy metals on these species, their tolerance, mechanisms and rates of uptake of the metals and the toxicity effects. A mathematical model for uptake of heavy metals by *Ascophyllum nodosum* [72] as well as a mass transport model for metal uptake by *Ulva fasciata* grown at different rates [74] have been tested.

Comparitively, very little work has been carried out on heavy metal accumulation by tropical seaweeds. Much of the work has been conducted in India [21] while a few isolated reports on the use of *Ulva reticulata* are from Malaysia [69, 74, 75]. *Ulva reticulata* was exposed to cadmium, chromium, cobalt, lead, zinc, manganese and nickel at concentrations of 50, 100, 200, 300 and 500 ppm over 48 hours [74]. The bioconcentration factor for the 50 and 500 ppm concentrations were 36 and 21 times for cadmium, 18 and 11 times for chromium, 136 and 23 times fro cobalt, 124 and 24 times for lead, 48 and 4 times for zinc, 144 and 35 times for manganese, and 146 and 10 times for nickel respectively. Table 3 gives the content of the heavy metals in some Malaysian algae. Table 4 gives the heavy metal content in temperate waters and temperate seaweed species, for comparison.

Table 3 Heavy Metal Contents of Some Malaysian Seaweeds [69]

Algae	Cd	Cr	Cu	Fe	Pb	Zn
			($\mu g g^{-1}$ dry weight)			
CYANOPHYTA						
Pelagothrix clevei	4.73	17.03	3.78	946.00	17.03	38.03
RHODOPHYTA						
Acanthophora orientalis	7.15	trace	trace	486.29	5.72	34.32
Gracilaria sp.	13.17	42.13	10.53	631.92	31.60	63.19
Hypnea sp.	3.74	trace	trace	1008.59	44.83	59.77
Jania sp.	9.01	18.02	4.50	878.67	29.29	27.04
Laurencia sp.	5.93	21.33	4.74	1007.23	8.30	14.22
PHAEOPHYTA						
Colpomenia sinousa	5.63	9.01	4.50	8445.00	9.01	27.02
Dictyota bartayresii	13.82	trace	49.74	15473.92	49.74	210.00
Padina tenuis	7.11	25.60	5.69	3327.72	17.06	45.51
Sargassum grevillei	6.44	10.61	5.15	347.84	5.15	15.46
CHLOROPHYTA						
Caulerpa racemosa	10.28	54.83	6.85	5106.23	41.12	71.97
Cladophora fascicularis	9.25	33.30	7.40	4735.49	12.95	38.85
Enteromorpha flexousa	16.32	58.77	26.12	7117.26	58.77	104.47
Valonia fastigiata	10.43	trace	16.69	5821.06	14.61	47.99
Valoniopsis pachynema	8.29	trace	11.05	13816.00	19.34	58.03
Concentrations in: sea water (ppm)	below detect. level	0.18	below detec. level	below detec. level	0.18	0.04
: sediment at 1cm depth	25.00 (ppm)	17.50	9.0	17800	33.50	89.25

Table 4 Heavy Metal Contents of Some Temperate Seaweeds [2]

Algae	Cd	Cr	Cu	Fe	Pb	Zn
			($\mu g g^{-1}$ dry weight)			
RHODOPHYTA	0.86	-	12.9	2809	-	32
Porphyra sp.	0.97	-	23.3	3800	10.5	177
PHAEOPHYTA						
Fucus vesiculosus	21.00	4.50	14.3	204	3.2	330
Ascophyllum nodosum	1.80	-	96.00	40	2.2	278
Laminaria digitata	4.30	-	36.00	-	37.00	290
CHLOROPHYTA						
Enteromorpha sp.	13.00	-	65.00	-	1200	1820
Ulva lactuca	2.00	-	26.00	-	18.00	160

The use of biological indicators in pollution monitoring is gaining importance. It can be developed further to present a treatment system for heavy metal discharges, where the marine macroalgae are utilized to remove heavy metals prior to their discharge. However much research has to be conducted to provide the information needed . The areas where research is required include:

(i) an inventory of the seaweeds in Malaysian waters;
(ii) the biology of local species;
(iii) response of local species to varying levels of heavy metals; tolerance levels ; accumulation rates; excretion rates; effects of physico-chemical factors;
(iv) heavy metal content and contamination of our estuarine, coastal and open waters;
(v) heavy metal content of seaweeds, plankton and other marine life in Malaysian waters.

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Heavy Metal Accumulation Patterns in Selected Seaweed Species of Malaysia

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Abstract. Seaweeds collected from Malaysian waters were found to bioconcentrate heavy metals to high levels. Seven species; *Chaetomorpha linum, Padina tetrastomatica, Sargassum baccularia, S. siliquosum, Gracilaria changii, G.edulis* and *G.salicornia*, were used in static studies to characterise the heavy metal accumulation patterns in the seaweeds. This was done by incubating the seaweeds in a range of metal concentrations in sea water and by determining the metal content, at fixed time intervals, in the seaweeds over a 24 h exposure period. Five metal accumulation patterns were observed in this study : Pattern (1) An initial rapid uptake, followed by a gradual accumulation till 24 h ; (2) A continuous gradual accumulation pattern for the entire 24 h ; (3) An initial rapid uptake, followed by a release-uptake pattern before a steady state concentration or gradual accumulation continued till 24 h ; (4) An alternating uptake-release pattern throughout the 24 h ; (5) An initial net accumulative pattern, followed by a continuous regulatory discharge till 24 h.

INTRODUCTION

Seaweeds fulfill several selection criteria of good biomonitors listed by Rainbow & Phillips (1993), making them cost-effective monitors of bioavailable metals in marine waters.

Seaweeds are used to quantify heavy metal bioavailability in temperate waters (Seeliger & Edwards, 1977; Melhuus *et al.*, 1978; Bryan, 1983). Metal content in seaweeds from the tropical region have been reported (Denton & Burdon-Jones, 1986; Ho, 1987; Ganesan *et al.*, 1991; Kureishy, 1991; Kesava Rao, 1992; Rajendran *et al.*, 1993; Karez *et al.*, 1994; Sheila *et al.*, 1994).

In Malaysia, a survey of the heavy metal content in seaweeds showed that of sixty-four seaweed samples (belonging to 21 species) analysed, some were found to be potential biological indicators of selected heavy metals (Sheila *et al.*,1994). These species had high accumulation of the heavy metals. The contents of Fe, Zn, Mn, Cd, Cu, Cr and Pb in the seaweed tissues range from 100 - 5225, 4.5 - 135, 8.5 - 1200, 1.0 - 5.0, 1.0 - 12.5, 0.5 - 21.5 and 2.0 - 21.5 µg/g dry weight respectively.

To be useful as biomonitors, seaweeds should be strong net accumulators of the metals concerned and should not regulate the total concentration of an element in the body tissues when exposed to different metal bioavailabilities (Rainbow & Phillips, 1993). A net metal accumulation pattern results when seaweeds are unable to match rates of excretion of metals to rates of uptake (Rainbow *et al.*, 1990). In field surveys, the metal accumulated by seaweeds is a time-integrated measure of the supply of bioavailable metal and may not necessarily correlate with average ambient metal concentration over a recent time period. Seaweeds respond essentially to dissolved metal sources only. Despite the relatively low concentrations of metals in the surrounding environment, seaweeds take up and accumulate them in tissues to concentrations which are many orders of magnitude above ambient levels. This makes the organism fall along a gradient of metal accumulation strategies from regulators, through partial regulators, weak net accumulators and strong net accumulators (Rainbow, 1993).

The characterisation of Malaysian seaweed species based on metal accumulation abilities and

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strategies would assist in the selection of biomonitors for use under local conditions.

In this paper the accumulation of four heavy metals (Cu, Zn, Mn and Cd) in seven seaweed species in the course of 24 h exposure was studied to characterise the metal accumulation patterns. The patterns would suggest the accumulation strategies hence explaining the uptake mechanisms.

MATERIALS AND METHODS

Seven species from three classes of seaweeds were used in this study (as shown in Table 1).

Preparation of seaweeds and seawater. Healthy plants were collected from the sites at low tide. Seaweeds were carefully removed and specimens were placed in plastic bags and stored in the shade until they were brought back to the laboratory on the same day. In the laboratory, the seaweeds were cleaned thoroughly with seawater to wash off associated epiphytes and debris. Seawater for the experiments was collected from a non-polluted source. The seawater was filtered through 0.45µM Millipore Membrane filters. Cleaned seaweeds were conditioned in filtered seawater in aerated plastic aquaria. The aquaria were placed on lighted shelves with an average light intensity of 33.65 µmol photon m^{-2} s^{-1} with 12:12 h Light-Dark cycle, with average water temperature of 28°C. Each aquarium had been washed in 20% nitric acid.

Metal range determination studies. The seven seaweed species were exposed to a range (1, 5, 10, 20, 50 and 100 mgl^{-1}) of the test metals in a 24 h static toxicity test. Results indicated that at concentrations ranging from 1-10 mgl^{-1}, there were no significant differences in growth ($p < 0.05$) compared to the control in all the seaweeds and metal tested. Growth in each seaweed was determined using the following parameter; *Chaetomorpha linum* - chlorophyll a content; *Padina tetrastomatica*, *Sargassum* spp., *Gracilaria* spp. - dry weight.

Test solutions. Increasing concentrations of Cu, Zn, Mn and Cd from 0 (Control), 1, 5 and 10 mgl^{-1} were prepared in filtered seawater (salinity of 30 parts per thousand and pH of 8.2) with $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$ and $CdCl_2.H_2O$ respectively. Concentrated nitric acid (Analar Grade) was used to dissolve any precipitate formed (final pH of test solutions were as follows: Cu concentrations, pH=6.5; Zn concentrations, pH=7.8 ; Mn concentrations, pH=8.0 ; Cd concentrations, pH=7.8).

Uptake studies. 28 sets of static tests were conducted. 1.8 litres of the various Cu, Zn, Mn and Cd concentrations were measured into acid-washed 2 L glass beakers. Every concentration was carried out in duplicate. In each beaker 50 g of seaweed was incubated over an exposure time of 24 hours. Beakers were illuminated with 33.65 µmol photon m^{-2} s^{-1} (12:12 h Light-Dark cycle) light at 28°C. At fixed time intervals of exposure to the metals, 3 g of the incubated seaweeds were removed from each beaker. Water samples (10 ml) were also taken to monitor metal levels in the incubation medium and stored at 10°C. Seaweed samples were throughly washed in distilled-deionised water and dried under dust free conditions.

Analytical procedure. Sufficient material to give at least 500 mg dry wt. of seaweeds were dried at 60°C for 4 hours and powdered in a blender. An aliquot of 500 mg powder of each seaweed sample

Table 1 : Seaweed species and collection sites

Seaweed	Collection site
Chlorophyta	
Chaetomorpha linum (O.F.Mueller)Kuetzing	Port Dickson
Phaeophyta	
Padina tetrastomatica (Hauck)	Port Dickson
Sargassum siliquosum (J.Agardh)	Port Dickson
Sargassum baccularia (Mertens)C.Agardh	Port Dickson
Rhodophyta	
Gracilaria changii Xia & Abbott(Abbott,Zhang and Xia)	Carey Island, Morib
Gracilaria edulis (Gmelin) Silva	Morib
Gracilaria salicornia C.Agardh	Morib

was digested with 15 ml concentrated (Analar Grade) Nitric Acid, a slight modification of the technique by Say *et al.*(1990). The digested sample was then filtered through a filter paper (Whatman filter paper No.4) and made up to 50 ml with distilled-deionised water. The resulting solutions were stored in acid washed polyethylene bottles at 10°C. The concentrations of Cu, Zn, Mn and Cd in seaweeds were determined by aspirating the samples to an Inductively-Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) model 2000 (BAIRD), with background correction (purified argon gas as the plasma) and expressed as µg metal g^{-1} dry weight of seaweed. No detectable amounts of Cu, Zn, Mn and Cd were found in the reagent blanks. Quality assurance procedures included replicates, standard addition tests and analysis of the certified reference material NIES No.9 *Sargasso*. Seawater samples were diluted 50 times before aspirating to the ICP-AES. Quality assurance procedures included replicates and standard addition test. The values were expressed as mg metal l^{-1} of seawater.

RESULTS

Metal contents in seawood (µg metal g^{-1} dry wt. of seaweed) and metal concentrations in seawater (mg metal l^{-1} of seawater) reported at each time interval were averaged values from duplicate samples. Standard deviations ranged from 5-20 % of the means.

Metal uptake patterns

1. Chaetomorpha linum

a) Cu uptake (see Fig. 1a) : An initial rapid uptake was observed at all concentrations of Cu in *C. linum*. The initial rapid uptake, followed by a gradual increase till 24 h was observed, only at 1 mg^{-1}. At other concentrations a release-uptake pattern was evident after the initial uptake. Then gradual accumulation continued till 24 h. At 24 h, Cu content in tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}

b) Zn uptake (see Fig. 1b) : An initial rapid uptake of Zn, followed by a gradual accumulation till 24 h was only evident at 10 mgl^{-1} in *C. linum*. At other concentrations, a continuous gradual accumulation pattern for the entire 24 h was observed. At 24 h, Zn content in the tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

c) Mn uptake (see Fig. 1c) : An initial rapid uptake was evident at all concentrations of Mn in *C. linum*. This was followed by a gradual increase at all concentrations of Mn throughout the 24 h. Concentrations at 1 mg/l were higher than 5 mg/l for large portion of the experiment. At 24 h, Mn content in tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

d) Cd uptake (see Fig. 1d) : An initial net uptake was followed by a continuous regulatory discharge pattern at all concentrations of Cd in *C. linum*. At 24 h, Cd content in tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

2. Padina tetrastomatica

a) Cu uptake (see Fig. 2a) : An initial rapid uptake, followed by a release-uptake pattern persisted before accumulation continued till 24 h at all concentrations of Cu in *P. tetrastomatica*. At 24 h, Cu content in tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

b) Zn uptake (see Fig. 2b) : An initial rapid uptake, followed by a release-uptake pattern persisted at all concentrations of Zn in *P. tetrastomatica*. Then either a steady state concentration or gradual increase continued till 24 h. At 24 h, Zn content in tissues increased in the following order of concentrations: 1 < 5 < 10 mgl^{-1}.

c) Mn uptake (see Fig. 2c) : An initial rapid uptake was evident at all concentrations of Mn in *P. tetrastomatica*. Then, a release-uptake pattern persisted before accumulation continued till 24 h, only at 10mgl^{-1}. At other concentrations, increasing Mn levels continued till 24 h after the initial rapid uptake. At 24 h, Mn content in tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

d) Cd uptake (see Fig. 2d) : An initial rapid uptake was followed by gradual accumulation till 24 h at all concentrations of Cd in *P. tetrastomatica*. At 24 h, Cd content in tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

3. Sargassum siliquosum and S. baccularia

a) Cu uptake (see Fig. 3a & 4a) : Both *Sargassum* species showed an initial rapid uptake which was followed by a release-uptake pattern at all concentrations of Cu. Then, either a steady state concentration or gradual accumulation continued till 24 h. At 24 h, Cu content in both seaweed tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

b) Zn uptake (see Fig. 3b & 4b) : An initial rapid uptake was followed by a gradual accumulation throughout the 24 h at all concentrations of Zn except at 1 mgl^{-1} in *S. siliquosum*. At 1 mgl^{-1}, this seaweed showed a gradual accumulation pattern for the entire 24 h. *S. baccularia* showed a similar accumulation pattern as observed for Cu uptake. At 24 h, Zn content in both the seaweed tissues increased in the following order of concentrations : 1 < 5 < 10 mgl^{-1}.

c) Mn uptake (see Fig. 3c & 4c) : *S. baccularia* showed an initial rapid uptake which was followed by a gradual accumulation pattern at all concentrations of Mn whereas in *S. silliquosum* this pattern was only observed at 10 mgl^{-1}. At other concentrations in *S. silliquosum*, a continuous gradual accumulation persisted during the entire 24 h. At 24 h, Mn content in both the seaweed tissues increased in the following order of concentrations : $1 < 5 < 10\ mgl^{-1}$.

d) Cd uptake (see Fig. 3d & 4d) : An initial rapid uptake was observed at all concentrations of Cd in both the species. This was followed by a gradual accumulation pattern till 24 h, only in *S. silliquosum*, while *S. baccularia* showed either a steady state concentration or gradual increase. At 24 h, Cd content in both the seaweed tissues increased in the following order of concentrations : $1 < 5 < 10\ mgl^{-1}$.

4. Gracilaria changii , G. edulis and G. salicornia

a) Cu uptake (see Fig. 5a , 6a & 7a) : An initial net accumulative pattern was followed by a continuous regulatory discharge throughout the 24 h at all concentrations of Cu in the three species of *Gracilaria*. At 24 h, Cu content in all the species tissues increased in the following order of concentrations : $1 < 5 < 10\ mgl^{-1}$.

b) Zn uptake (see Fig. 5b , 6b & 7b) : An initial net accumulative pattern, followed by a continuous regulatory discharge at all concentrations of Zn was only observed in *G. changii* and *G. edulis*. *G. salicornia* showed an initial rapid uptake which was followed by a gradual increase throughout the 24 h. At 24 h, Zn content in all the species tissues increased in the following order of concentrations: $1 < 5 < 10\ mgl^{-1}$.

c) Mn uptake (see Fig. 5c , 6c & 7c) : At all concentrations of Mn, the three species showed an alternating uptake-release throughout the 24 h. At 24 h, Mn content in all the species tissues decreased in the following order of concentrations : $1 < 5 < 10\ mgl^{-1}$.

d) Cd uptake (see Fig. 5d , 6d & 7d) : *G. changii* showed an alternating uptake-release pattern throughout the 24 h at all concentrations of Cd. But both *G. edulis* and *G. salicornia* showed an initial rapid uptake followed by a gradual accumulation till 24 h. At 24 h, Cd content in all the species tissues increased in the following order of concentrations: $1 < 5 < 10\ mgl^{-1}$.

Seawater metal content

Metal concentrations in the seawater medium of the 28 sets of experiments are shown in Figures 8 - 14.

DISCUSSION

The present study showed that the patterns of heavy metal accumulation in seaweeds are mainly dependent on the metal and seaweed species. Five patterns of heavy metal accummulation were observed;

Pattern 1 : An initial rapid uptake, followed by a gradual accumulation till 24 h.

Pattern 2 : A gradual accumulation for the entire 24 h.

Pattern 3 : An initial rapid uptake, followed by a release-uptake pattern before a steady state concentration or gradual accumulation continued till 24 h.

Pattern 4 : An initial net accumulative pattern, followed by a continuous regulatory discharge till 24 h.

Pattern 5 : An alternating uptake-release pattern throughout the 24 h.

Pattern 1, 2 and 3 are categorised as net accumulative patterns. Pattern 4 is a clear continuous regulatory process. Pattern 5 seems to be a combination of the net accumulation pattern and pattern 4. The findings of the 28 sets of experiments carried out are summarised in Table 2.

The effect of metal concentration on accumulation patterns was only found in seaweeds with net accumulative patterns (as shown in Table 2).

Initial rapid accumulation/uptake was evident in most of the patterns observed; the rapid uptake occurred within the first hour of exposure. The uptake of Zn in *Padina gymnospora* (Karez *et al.*, 1994) and that of Cu, Mn, Ni, Pb and Zn in *Sargassum pallidum* (Tropin and Zolutukhina, 1994) has been shown to follow similar trend. The rapid uptake corresponds to passive uptake involving cell surface adsorption and simple diffusion into cells or intracellular spaces (Karez *et al.*, 1994); identified as first phase process. An initial rapid accumulation is followed by a slower uptake, identified as second phase process, or in some cases a continuous or non-continuous excretion (regulatory measures) in the seaweeds. Regulatory mechanisms of heavy metal uptake have been reported in microalgae (Maeda & Sakaguchi, 1990).

Heavy metal accumulation patterns in individual species

Chaetomorpha linum Four metal accumulation patterns were observed : i) An initial rapid uptake followed by increase throughout the 24 h ; ii) A rapid uptake followed by a release-uptake pattern before accumulation continued till 24 h ; iii) A continuous gradual uptake pattern during the entire

Table 2. Summary of the results of the 28 sets of experiments conducted in the study

Accumulation pattern	Seaweed species						
	C.l	P.t	S.s	S.b	G.c	G.e	G.s
1	Cu (1 mgl^{-1})* Zn (10 mgl^{-1})* Mn	Mn Cd	Zn Mn (10 mgl^{-1})* Cd	Mn	-	Cd	Zn Cd
2	Zn	-	Zn (1 mgl^{-1})* Mn	-	-	-	-
3	Cu	Cu Zn Mn (10mgl^{-1})	Cu	Cu Zn Cd	-	-	-
4	-	-	-	-	Mn Cd	Mn	Mn
5	Cd	-	-	-	Cu Zn	Cu Zn	Cu

*: Concentration of metal specified; C.l.: *Chaetomorpha linum*; P.t.: *Padina tetrastomatica*; S.s.: *Sargassum siliquosum*; S.b.: *Sargassum bacculoria*; G.c.: *Gracilaria changii*; G.e.: *Gracilaria edulis*; G.s.: *Gracilaria salicornia*

24 h ; iv) A net uptake followed by a continuous regulatory discharge pattern. The first 3 patterns are indicative of net accumulation. The net accumulative patterns were only observed for exposures to Cu, Zn and Mn. These patterns in *C. linum* suggest the potential of high bioaccumulation and unregulation of metal uptake. Ganesan *et al.* (1991) reported that *Chaetomorpha antennina* and other seaweeds readily accumulate Cu from seawater and could indicate the contamination levels. High metal accumulation attributes to metal ions being irreversibly held within cell wall matrix of thallus (Kesava Rao, 1992).

After 24 h, at all concentrations tested, metal content in tissues of *C. linum* increased with increasing external metal concentrations. Wong *et al.* (1979), Ho (1987) and Rajendran *et al.* (1993) indicated that higher seawater metal levels lead to a greater bioaccumulation of metals in seaweeds while working with *Chaetomorpha* sp..

Continuous regulatory discharge patterns observed in this species when exposed to Cd, suggest a metal excretion mechanism which resulted in reducing bioaccumulated concentrations in the tissues (Rainbow & Phillips, 1989).

Padina tetrastomatica. Two metal accumulation patterns were observed : i) A rapid uptake followed by gradual increase throughout the 24 h; ii) An initial rapid uptake followed by a release-uptake pattern, before accumulation continued till 24 h. The two patterns are indicative of net accumulation. After 24 h, metal content in the seaweed tissues increased with increasing external metal concentrations.

A similar study was carried out by Karez *et al.*(1994) using *P. gymnospora* exposed to Zn for 48 h. The study showed that the metal could be strongly bound to cellular sites with subsequent release not possible. This resulted in a net accumulation pattern for Zn in the species. The study also showed that Zn accumulation increased with external Zn concentrations in seawater. The author concluded that in the field, *P. gymnospora* could indicate the maximum available metal concentration in the water. Such accumulation patterns could be due to the saturation of the regulation mechanism and this prevents excretion of excess metal (Rainbow *et al.*, 1990).

Sargassum species. Both the *Sargassum* species in the present study showed a net metal accumulation pattern for all the metals and metal concentrations tested. Three forms of net metal accumulation patterns were observed : i) An initial rapid uptake followed by gradual increase throughout the 24 h ; ii) A continuous gradual uptake during the entire 24 h ; iii) A rapid uptake followed by a release-uptake pattern before a steady state concentration or gradual accumulation continued.

Net accumulation patterns have also been shown in *Sargassum pallidum* exposed to Cd, Cu, Mn, Ni, Pb and Zn (Tropin and Zolutukhina,

1994). Such accumulation patterns may be due to the strong polyanionic groups of the sulphated polysaccharides and alginic acid in brown seaweeds. Metal ions bind strongly to the polyanionic groups and therefore, subsequent release is not possible (Bryan, 1969).

After 24 h, metal contents in the seaweeds increased with increasing external metal concentrations. Such high accumulation abilities and a non-regulative characteristic in *Sargassum* in response to metal ions suggest that *Sargassum* is a potential indicator of heavy metal pollution, especially in tropical waters. In comparison, *Fucus, Laminaria digitata* and *Ascophyllum nodosum* are brown seaweeds that are commonly used in temperate regions in heavy metal studies and have been shown to be good indicators of the bioavailable forms of metals in seawater (Bryan, 1969; Munda, 1982; Ho, 1984).

Gracilaria species

In *Gracilaria*, the following metal accumulation patterns were shown: i) An initial rapid accumulation was followed by a gradual increase throughout 24 h; ii) An alternating uptake-release pattern for the entire 24 h; iii) An initial net accumulation pattern was followed by a continuous regulatory discharge throughout 24 h. The first pattern is indicative of a net accumulation.

After 24 h, the Mn contents in *Gracilaria* increased with decreasing external Mn concentrations. Such trends were not observed in other metals tested.

The alternating uptake-release pattern observed in *Gracilaria* spp. (Mn) and *G. changii* (Cd) could be due to a non-continuous regulatory mechanism that serves to maintain the ambient tissue metal concentration (Rainbow *et al.*, 1990). On the other hand, continuous regulatory patterns were observed with Cu (all three species) and Zn (only in *G. changii*). The continuous regulatory patterns may be due to excretion of excess metals to reduce the bioaccumulated metal concentrations in seaweeds.

In *G. edulis* (Cd) and *G. salicornia* (Zn and Cd), metal uptake was probably not regulated and this contributed to the net accumulation patterns observed. Such accumulation patterns are advantageous if the seaweed is to be used as a metal indicator as more metals can be absorbed. The high affinities for heavy metals in *Gracilaria* is attributed to the high cellular content of the polysaccharide that exist in red seaweeds (Munda & Hudnik, 1991).

Comparison between species

The net accumulation patterns were observed for the four metals tested (as shown in Table 2), only in *P. tetrastomatica, S. siliquosum* and *S. baccularia*. This observation suggests an accumulation strategy that is similar within the three brown seaweeds. *C. linum* showed net accumulation patterns for the metals exposed except for Cd. Similarity of accumulation strategy is indicated for these Chlorophyta and Phaeophyta exposed to Cu, Zn and Mn. The *Gracilaria* species failed to show any net accumulation patterns for Mn and Cu. This suggests a similar accumulation strategy within the *Gracilaria* genus.

CONCLUSION

1. This study revealed five patterns of heavy metal accumulation in the Malaysian seaweeds tested;

Pattern 1 : An initial rapid uptake, followed by a gradual accumulation till 24 h.

Pattern 2 : A continuous gradual accumulation pattern for the entire 24 h.

Pattern 3 : An initial rapid uptake, followed by a release-uptake pattern before a steady state concentration or gradual accumulation continued till 24 h.

Pattern 4 : An initial net accumulative pattern, followed by a continuous regulatory discharge till 24 h.

Pattern 5 : An alternating uptake-release pattern throughout the 24 h.

2. Patterns 1,2 and 3 are grouped as net accumulative patterns on the whole, while pattern 4 is a clear indication of a regulatory process.

3. Both the net accumulation patterns and the increasing trends obtained between metal content in seaweed tissues and external metal concentrations, suggest the following seaweeds as potential indicators of the selected heavy metals :.

Indicator	Metal
Chaetomorpha linum	Cu, Zn and Mn
Padina tetrastomatica	Cu, Zn, Mn and Cd
Sargassum siliquosum	Cu, Zn, Mn and Cd
Sargassum baccularia	Cu, Zn, Mn and Cd
Gracilaria edulis	Cd
Gracilaria salicornia	Zn and Cd

4. The occurrence of continuous metal regulatory in seaweeds in this study is summarised below.

Seaweed	Regulated metal
C. linum	Cd
G. changii	Cu and Zn
G. edulis	Cu and Zn
G. salicornia	Cu

S6-5 Toxicity of four heavy metals on four economically important tropical phytoplankton species

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An increase in the incidence of heavy metal pollution in the Malaysian marine environment largely due to industrial activities has resulted in levels exceeding the Proposed Interim Standards for Marine Water Quality. Toxicity tests were conducted to measure the chronic toxicity of cadmium, copper, manganese and arsenic to four economically important, local and tropical marine phytoplankton: *Chaetoceros calcitrans*, *Isochrysis galbana*, *Tetraselmis tetrahele* and *Nannochloropsis oculata* . Phytoplankton, being primary producers are highly relevant for the assessment of potential effects in the environment. The phytoplankton growth tests were carried out at 28±1.0°C, using synthetic seawater (pH 8.0±0.5 and salinity 30±2.0ppt), under continuous illumination of 50.4 $\mu molm^{-2}s^{-1}$. Growth was estimated by OD measurements and cell counts in 96 hour static toxicity tests. Growth (increase in OD and cell number) relative to the control enabled the determination of IC_{50}, the inhibition concentration of metal estimated to cause a 50% reduction in growth of phytoplankton relative to the control. Generally, for all species tested, the sequence in metal toxicity was $Cu^{2+} > Cd^{2+} > Mn^{2+} > As^{5+}$. The algae were all sensitive to copper but relatively tolerant to manganese and arsenic. *C. calcitrans* and *I. galbana* were more sensitive to cadmium than *T. tetrahele* and *N. oculata*. Cadmium chloride was used as the reference toxicant in all toxicity tests. The cadmium IC_{50} values for *C. calcitrans*, *I. galbana*, *T. retrahele* and *N. oculata* are 0.1 ppm, 0.1 ppm, 11.2 ppm and 5.8 ppm respectively. Sensitive strains will be useful bioindicators of heavy metal pollution while the tolerant ones will be useful in long term monitoring.

Biosorption Of Cadmium And Copper By *Sargasum Baccularia*

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ABSTRACT

The biomass of nonliving, dried brown marine alga Sargassum baccularia demonstrated high equilibrium uptake of cadmium and copper from aqueous solutions. Adsorption of these metals was quantitatively evaluated using the Langmuir adsorption isotherm. Maximum cadmium and copper adsorption capacity of this biomass was 84.4 mg Cd/g biomass and 63.5 mg Cu/g biomass respectively.

The accelerated pace of industrialization in Malaysia has increased the number of polluting sources and pollution levels. Currently, there is a widespread problem in the treatment, storage and disposal of waste containing heavy metals. The metal finishing industry topped the list by contributing 43.7% of the nation's total toxic and hazardous waste in 1985 (DOETH, 1987). Although treatment facilities have been installed in medium to large-scale industries, in compliance to the Environmental Quality (Scheduled Wastes) Regulations (Govt. of Malaysia, 1989), the small scale industries are unable to do so due to financial constraints. As such there is an urgent need for a cost-effective treatment system for toxic metal removal.

Algae have been used as biological indicators for heavy metal pollution (Phang, 1993). Recently there has been an interest in employing dead biomass of algae for bisorption of heavy metals as a form of wastewater treatment method. The charged functional groups on the algal surface can act as a biological ion-exchange resin and subsequently this technology can replace the commercial ion-exchange material which is not economical especially for dilute wastwaters.

Brown seaweed *Sargassum baccularia* was harvested from Cape Rachado in Port Dickson, washed, dried and ground to produce biomass particles measuring between 500-710 microns. Batch, adsorption experiments were performed in 100 ml Erlenmeyer flasks employing 0.1 g biomass in a series of metal solution (50 ml) within the concentration range of 50-400 mg/ml. The pH of the solutions were 5.0. Metal-free and biomass-free controls were also set up. Separation of the biomass from the test solution was by filtration, using cellulose acetate membrane filters (0.45 micron pore size)

and a 20 ml disposable syringe. The filtrates were diluted accordingly and subject to metal analysis by the ICP-AES method.

In order to understand the biosorption phenomenon, critical adsorption parameters such as the solution pH and the kinetics were investigated. The solution pH is important as it determines the metal speciation and the chemical state of the functional groups responsible for metal binding on the algal surface. The equilibrium data for cadmium and copper adsorption under various initial pH values has been presented in the form of the Langmuir isotherms in Figures 1 and 2. The Langmuir equation is as follows:

$$q_{eq} = (q_{max} \, k \, C_{eq}) / (1 + k \, C_{eq})$$

where q_{eq} is the solid phase equilibrium metal concentration (mmol/g biomass), C_{eq} is the liquid phase equilibrium metal concentration (mM); q_{max} and k (mM) are the model parameters which represent the maximum adsorption capacity and the Langmuir equilibrium constant, respectively.

The correlation coefficients calculated for these isotherms satisfactorily (> 0.9) indicate that the adsorption data follow the Langmuir model. The general trend for both the metals is that with increasing pH, the q_{eq} values also increase. Optimal pH for cadmium and copper adsorption was found to be pH 5.0.

The isoelectric point for algal surfaces is pH~3 and at values higher than this the surface acequires a net negative charge through the loss of protons (Crist et al., 1981). This condition promotes adsorption whereby the positive ions Cd^{2+} and Cu^{2+} electrostatically bind to the negatively charged surface. Functional groups responsible for metal binding on the surface are the unprotonated carboxyl oxygen and sulphate groups of algal polysaccharides. The low binding that occurred at pH 2.0 was due to specific adsorption, which is independent of the surface charge of the biomass surface. Such bonds are formed by the metal ions with amino and carboxyl groups, the imidazole of histidine and the nitrogen and oxygen of the peptide bond, all of which are part of the cell wall protein components.

Kinetics of metal adsorption was investigated as a short equilibrium time between the waste effluent and the biomass is highly desirable for treatment plants. The contact time also determines the size of the equipment (reactor), which in turn affects the capital and operating costs of the process. The time taken to reach equilibrium for cadmium and copper adsorption are 60 minutes and 2 hours respectively.

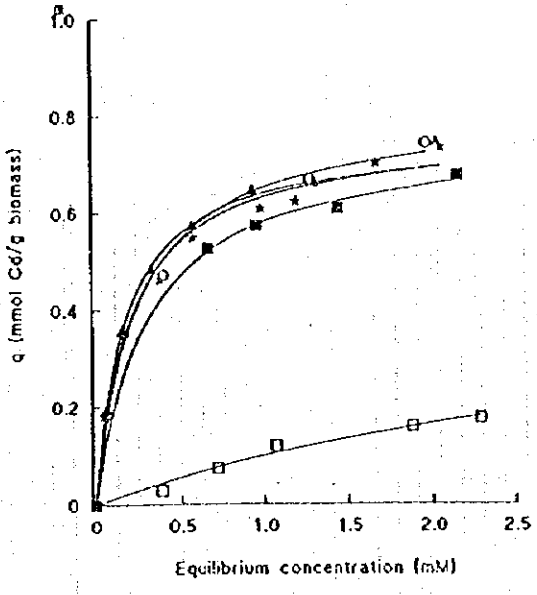
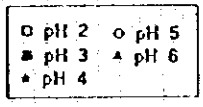


Figure 1 : Effect of initial solution pH on cadmium adsorption in S.baccularia.



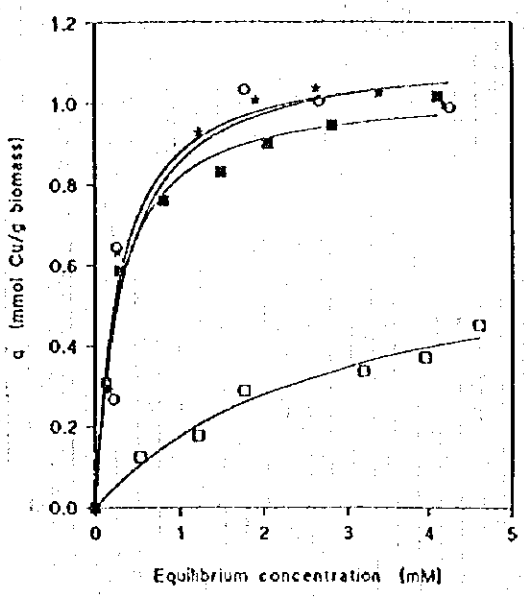
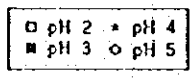


Figure 2 : Effect of initial solution pH on copper adsorption in S.baccularia.



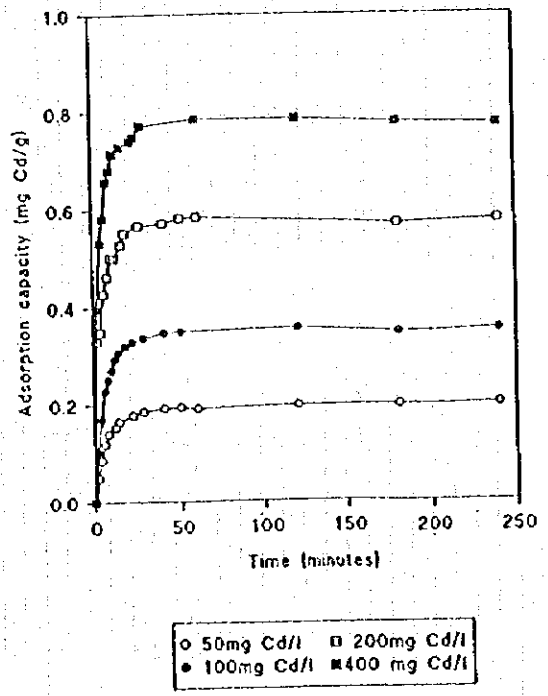


Figure 3: Cadmium adsorption profile with time, at different initial metal concentration.

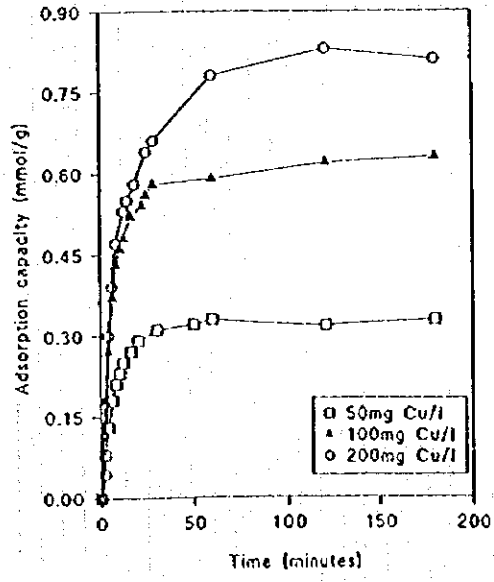


Figure 4 : Copper adsorption profile with time at different initial metal concentrations.