5.0 DISCUSSION

The characteristics of TL used in this study were similar to the results reported by Agamuthu (1999) as shown in Table 4. For example, studies on treated leachate (TL) obtained from the Air Hitam Sanitary Landfill shows pH value in TL ranged between 6.0 to 8.8. The pH of the raw leachate was typically around 5.8 and the pH of the aerated lagoon effluent was around 8.0. Landfill leachates are generally of greater organic strength than municipal wastewater. Leachate can contain very high levels of ammoniacal-nitrogen, up to 100 times greater than the concentrations normally found in sewage. The NH_3-N and PO_4 content in TL ranged from 3.2 to 151.7 mgL^{-1} and 2.4 to 8.2 mgL^{-1} respectively. The COD content in TL ranged between 860.0 to 8066.7 mgL^{-1} and TSS value ranging from 555.3 to 3651.1 mgL^{-1}. Based on COD, NH_3-N and PO_4 content in TL, C:N:P ratio value in the TL was ranging from 81: 26 : 1 to 4607 : 58 : 1. Leachate from municipal solid wastes commonly contains high concentration of soluble matter and inorganic ions such as chlorides, sulphates and metal (mainly iron, sodium, potassium, calcium and zinc) (Agamuthu & Nather, 1997). Results from the analysis of heavy metals contained in TL showed, the heavy metal values in TL in Batch I and Batch VIII were higher than the National Guidelines for Drinking Water Quality (Appendix 4).

The leachate could be highly toxic and contain high concentrations of organic and metal compounds, as well as pathogens, which can pollute water courses and groundwater (Adam *et al.*, 2001), and if the leachate is discharged directly into water bodies, it could impact aquatic life and degrade water quality. Therefore TL have to be treated to reduce the polluting compounds in the leachate before discharge to the iver system. The present study has shown that landfill leachate could be used as a nedium to grow microalgae using the High Rate Algal Pond (HRAP) System.

.1 TREATED LEACHATE (TL) AS GROWTH MEDIUM FOR MICROALGAE

In this study, TL was used as a substrate to support growth of microalgae. The C:N:P values in TL ranged from 81 : 26 : 1 to 4607 : 58 : 1. The C:N:P ratio of 58: :1 is suitable for microalgal growth (Phang, 2001). However based on the C:N:P values in every batch of TL collected, the carbon and NH_4-N were not limiting utrients but PO_4 content in TL was the limiting nutrient for algal growth. The eachate should be nutritionally balanced to support microbial life. Studies in Europe s well as North America, have shown that the leachate is usually deficient in the hosphate but not nitrogen. For efficient aerobic treatment it is generally accepted that BOD_5 : N : P ratio of about 100 : 5 : 1 must be maintained in the influent stream. Therefore it is essential to supplement the phosphate for successful aerobic biological reatment.

.1.1 Preliminary toxicity test

Studies on selection of microalgae species for growth shows, in the reliminary toxicity test, algal species which are sensitive to heavy metals make seful indicators of the metals and may be used as the test organisms in toxicity tests o generate data for use in the formulation of water quality criteria and standards Samina, 1999). Of eleven species of microalgae tested, *Chlorella vulgaris* (UMACC 01), *Scenedesmus* sp. (UMACC 039) and *Synechococcus* sp (UMACC 075) were nost sensitive to Cu toxicity and may be used as indicator for Cu. *Chlorella* sp.

(UMACC 078), *Chlorococcum* sp. (UMACC 110) and *Oocystis polymorpha* (UMACC 153) were most sensitive to iron (Fe) toxicity. *Ulothrix* sp. (UMACC 071) and *Euglena* sp. (UMACC 058) can be used as indicator for Mn. *Ankistrodesmus convolutes* (UMACC 101) was most sensitive to cobalt (Co) toxicity while *Ankistrodesmus arcuatus* (UMACC 170) was most sensitive to chromium (Cr) toxicity.

Studies on 96h IC_{50} values for toxicity testing with $CdCl_2$ on six microalgae which have been selected before shows *Oocystis polymorpha,* (UMACC 153) was very resistant to the Cd with IC_{50} value at 59.05 ppm and *Chlorococcum* sp. (UMCC 110) was less resistant to the Cd with IC_{50} values at 7.86 ppm. However, of the Malaysian isolates, *Ankistrodesmus convolutus* (UMACC 101) was found to be tolerant to heavy metals and was used for detailed toxicity test. The least tolerant Malaysian species was *Chlorococcum* sp. (UMCC 110).

The order of toxicity based on IC_{50} values for toxicity test with heavy metal in *Ankistrodesmus convolutus* (UMACC 101) is Zn = Mn > Cu > Cd > Cr > Co > Fe. This indicated that *Ankistrodesmus convolutes* (UMACC 101) is tolerant of Mn and Zn and Fe was the most toxic metal for *Ankistrodesmus convolutus*.

5.1.2 Laboratory studies on screening of microalgae for growth in TL.

Generally, from the results microalgae can grow very well in control medium followed by 25%, 50%, 75% and 100% TL medium. In control medium the chl *a* values were highest. It is because in this medium there were no contaminants that will inhibit the growth. In 25% TL medium, chl *a* values ranging from 1475.67 to

12.67mgm^{-3} and in 50% TL medium, chl *a* values ranged between 546.33 to 0 mgm^{-3}. Chl *a* values ranged from 48.33 to 0 mgm^{-3} in 75% TL medium. In 100% TL medium, chl *a* values were the lowest. This is because the TL contained toxic compounds in high levels that inhibited the algal growth.

During the study, in the first 4 days, some of the algae grew in the various TL concentrations and the chl *a* values increased. During this period the algae will optimally use the nutrients in the TL. Some algae however did not grow in the TL during the first 4 days because they were not tolerant of the toxic compounds in the TL. From day 4 until day 8, some of the algae continued to grow. These algae are tolerant of the toxic compounds in the TL.

Results showed that the chl *a* values were highest in UMACC 058 where this algae can grow very well in the 25% and 50% TL medium with chl *a* values of 713.33 mgm^{-3} and 546.33 mgm^{-3} respectively on day 8. The growth rate of UMACC 058 at 50% TL medium was highest compared to other species tested, (μ = 0.1294 day^{-1}). The IC_{50} values also show that the UMACC 058 had the highest value of 51.27%. This shows that this algae is very resistant to toxic compounds in the TL and it can be use to treat TL. Chl *a* content was the lowest in the UMACC 110 and this algae cannot grow well in TL. The chl *a* values in UMACC 110 which were grown in 25% TL medium ranged between 12.67 mgm^{-3} to 256.67 mgm^{-3}. The IC_{50} values also showed that the UMACC 110 has the lowest value of 13.15%.

In the Batch II experiment, BBM medium was used as the control to compare algal growth. From the results the algae tested grew well in BBM medium followed by 0%, 25%, 50%,75% and 100% TL medium. UMACC 039 can grew well in TL obtained from the Air Hitam landfill. The IC_{50} values showed that UMACC 039 had the highest value of 37.62%. The chl *a* values in UMACC 039 which grew in 0%, 25% and 50% TL medium ranged between 642.67 mgm^{-3} to 95.33 mgm^{-3}. Same as in Ampang Jajar TL, UMACC 110 cannot grow well in the TL medium. The chl *a* values in UMACC 110 which grown in 0% and 25% TL medium ranged between 43.00 mgm^{-3} to 296.33 mgm^{-3}. The IC_{50} values also showed that UMACC 110 has the lowest value with 14.62%.

In the Batch III experiment, BBM medium was used as the control to compare algal growth. From the results the algae tested grew well in BBM medium followed by 25%, 50%,75% and 100% TL medium. Overall, the chl *a* values in this batch were lower than in Batch II. In Batch III, the IC_{50} values showed that UMACC 001 had the highest value of 46.62% followed by UMACC 074, UMACC 058 and UMACC 110. Based on chl *a* content, UMACC 058 had the highest value. The chl *a* values in UMACC 058 which grew in 25%, 50%, 75% and 100% TL medium ranged between 0.45 mgm^{-3} to 0.98 mgm^{-3}. UMACC 074 cannot grow in the TL medium. The chl *a* values in UMACC 074 which grew in 0%, 25%, 50%, 75% and 100% TL medium ranged between 0.10 mgm^{-3} to 0.26 mgm^{-3}. The IC_{50} values also showed that UMACC 110 has the lowest value with 14.62%. Therefore the order of suitability for growth in treated leachate is as follows : UMACC 058 (*Euglena* sp.) > UMACC 039 (*Scenedesmus quadricauda*) > UMACC 001 (*Chlorella vulgaris*) > UMACC 101 (*Ankistrodesmus convolutus*) > UMACC 074 (*Oocytis* sp.) > UMACC 110 (*Chlorococcum oviforme*).

5.2 EFFECT OF TREATED LEACHATE (TL) SUPPLEMENTATION IN HRAP CULTURES

5.2.1 Growth of micralagae

Growth is defined as an irreversible change in the size of a cell, organ or whole organism. It may also be the increase in cell number without changes in volume or weight. Commonly growth is the increase in the amount of living material (protoplasm) which leads to an increase in cell size and ultimately cell division. The increase in protoplasm is brought about as water, carbon dioxide and inorganic salts are transformed into living material. Growth occurs only in living cells by metabolic processes involved in the synthesis of proteins, nucleic acids, lipids and carbohydrates at the expense of metabolic energy provided by photosynthesis and respiration (Janick, 1979).

Carbon, nitrogen and phosphorus (C,N,P) are the macro-nutrients for the growth of microalgae. The microalgae in HRAPs utilize both inorganic carbon (CO_2 and HCO_3) and organic carbon. HRAPs has high organic loads and this organic carbon is used by algae either photoheterotrophically or heterotrophically in the deeper parts of the ponds (Abeliovich 1980, 1986). Inorganic carbon in HRAPs is derived from both atmospheric CO_2 and CO_2 produced by bacterial respiration. The bacterial generated CO_2 is quite small in amount while about 25 to 50% of the algal carbon is derived from heterotrophic utilization of the organic carbon. Cultures of microalgae in HRAPs are very efficient at organic carbon utilization (Martinez *et al.*, 1987).

The bacteria in HRAPs are not only CO_2 generator, but there is also evidence that heterotrophic bacteria can reduce algal growth (Dor and Svi, 1980), while at the same time, algae can also produce some inhibitory substances to bacterial growth (Dor, 1980). The main role of bacteria in HRAPs is to break down the complex organic molecules to make them available to the algae.

Ammonia concentration in the pond is an important factor for the successful management of the HRAPs. About 2.0 mM total ammonia (NH_4 + NH_3) at pH 8.1 has been shown to inhibit photosynthesis O_2 evolution by about 50% in many algal species (Abeliovich and Azov, 1976; Azov and Goldman 1982) and high concentrations of ammonia can lead to death of the algae (Borowitzka and Borowitzka, 1988). The total ammonia concentration generally should not exceed 1.5mM. Photosynthetic CO_2 uptake will raise the pH in the pond and if there is a high concentration of total ammonia, i.e.> 2.0mM photosynthesis is inhibited when the pH reaches about pH 8.1. When the ammonia concentration is high, careful balance should be maintained between its concentration and BOD, to enable the continued lowering of pH via respiration; i.e. if ammonia concentration is high then the BOD should also be high (Abeliovich, 1983).

The growth of microalgae in the HRAP culture in this present study was measured based on chl *a* content, optical density at 620 nm, dry weight and cell count. Studies on the growth of microalgae in the HRAP shows, overall the chl a content, OD_{620nm} and cell count values in the pond increased for the first 15 days after the TL was introduced into the pond. This is because the initial increase in nutrient contents enabled the algae to grew. Levels of toxic compounds were low so as not to inhibit algal growth. Then after two weeks, growth decreased. Between day 30 until day 194, the algal growth fluctuated where the chl *a* content, OD_{620nm} and cell count values increased and decreased throughout. Low fluctuations show adaptation of algal growth to tolerance of the TL. Also the C : N : P values in TL were variable. For example, in the TL of Batch I and Batch III, the C : N : P ratios were 197 : 19 : 1 and 81 : 26 : 1 respectively and showing that NH_3-N was the limiting nutrient for growth. In many wastewaters, N_2 is the limiting factor for algal growth especially at high degrees of pollution (Forsberg, 1976). The level of nitrogenous compounds in the medium is one of the most important factors affecting microalgal growth. Jeanfils *et al.* (1993) showed that *Chlorella vulgaris* grows optimally when NO_3^- is supplied at 6 to 12 mM and growth is not inhibited even at 97 mM NO_3^-. Cells may continue to divide after the depletion of nitrogen, probably by recycling and redistributing the nitrogen stored. The limited nutrients in the TL will reduce the growth rate of microalgae and this condition will decrease the chl *a* content, OD_{620nm} and cell count values in the pond. In Batch II and IV, NH_4-N was not the limiting nutrient for algae growth. Therefore growth of algae in the pond increased. Generally, the growth of algae in T_1 pond (using selected algae) was higher than T_0 pond (pond water) at the loading rate of 1% TL.

At the loading rate of 2% TL, between day 194 and day 306, the chl *a* content, OD_{620nm} and cell count values of T_0 pond was higher than T_1 pond. The chl *a* content in T_0 pond, OD_{620nm} and cell count values increased for the first 40 days after the new loading rate was introduced, then it decreased slowly. The chl *a* content in T_1 pond decreased slowly and become constant. This indicated that the algae in T_1 pond did not respond well to the new loading rate. This is because, when the loading rate was increased, the pollutant content and nutrient levels increased. Therefore the algae in T_1 pond could not tolerate the new loading rate for the first few days and after about

27 days later, the algae adapted and growth stabilized. Meanwhile in T_0 pond, when the nutrient level increased, the algal growth increased too but decreased slowly after 40 days because the algae began to be affected by the corresponding increase in toxic compounds in the TL.

When the loading rate was doubled to 4% TL, for the first 50 days the chl *a* content, OD_{620nm} and cell count values increased in both ponds, after which the algae decreased in growth. At this high loading rate, the algae could not tolerate the high content of toxic pollutants in the pond. High levels of nutrients such as ammonia will also inhibit algae growth. Ammonia concentration in the pond is an important factor for the successful management of the HRAPs. About 2.0 mM total ammonia (NH_4 + NH_3) at pH 8.1 has been shown to inhibit the photosynthesis (Abeliovich & Azov, 1976; Azov & Goldman 1982) and high concentrations of ammonia can lead to death of the algae (Thomas *et al.*, 1980; Borowitzka & Borowitzka, 1988). The total ammonia concentration generally should not exceed 1.5mM.

Plant growth and development are influenced by physical, chemical and biological components in the plant environment. Some factors influencing algal growth are:

a) Abiotic Factors: Light, temperature, nutrient concentrations (especially N, P, and organic C), O_2, CO_2, pH, salinity and toxic chemicals.

b) Biotic Factors : Pathogens (bacteria, fungi, viruses) predation by zooplankton and competitions.

c) Operational Factors : Mixing, dilution rate, depth, addition of bicarbonate or CO_2 and harvesting frequency.

Any factor in the plants environment that is less than optimum, whether it is deficient or in excess, will limit plant growth (Ames and Wayne, 2002).

The major external influencing factors for algal growth are irradiance and temperature (Goldman, 1979). During the studies the temperature in both ponds ranged between 27 to 33° C and the irradiance ranged from 7.26 to 60.52 $\mu mols^{-1}m^{-2}$. The growth rate of algae increases with increasing temperature until the optimum temperature is reached. The effect of temperature on algal growth rates normally follows Van`t Hoff's rule i.e. a doubling for each 10 °C rise in temperature within the range of temperature tolerance. The average minimum and maximum ambient temperature for algal growth is 24 to 34 °C. The interaction between light and temperature have been observed in outdoor cultures (De Pauw *et al.*, 1980). The productivity of biomass in outdoor cultures is affected by temperature. For example, the net productivity of *Spirulina platensis* is 23% higher at 35 °C than at 25 °C, possibly due to the higher loss of night biomass at the lower temperature (Torzillo & Vonshak, 1994).

The range of pH tolerance and the optimal pH levels for growth vary from one species to another. Different algae have different pH optima. Blue green algae like *Spirulina platensis* and desmids prefer alkaline conditions (10.5) to grow well (Richmond & Grobbelaar, 1986) while green algae like *Chlamydomonas* sp. can tolerate pH as low as 4.0.

During the study, the average pH values in T_1 pond and T_0 pond were 7.5 and 8.2 respectively. pH in HRAPs is usually between pH 7.5 to 10. pH values less than bout pH 6.0 may lead to algal death. For optimal photosynthesis in HRAPs, pH alues may reach >pH 10 without having any adverse effects on the stability of the lgal biomass production.

Both photosynthesis and the uptake of inorganic carbon are affected by xternal pH. Photosynthetic rate usually decreases at pH levels which are suboptimal or growth. The influence of pH on photosynthesis is related to its effect on the nzymes involved, which function optimally within pH 7.5 to 8.5 (Eric *et al.*, 1995). Caplan (1981) suggested that the inhibition of photosynthesis at pH >9.5 is due the ower rate of carboxylation.

pH values were found to correspond to the dissolved oxygen (DO) level and olar irradiance. pH was in alkaline range when the DO and solar irradiance were igh, that is at active photosynthesis. The photosynthetic utilization of CO_2 from the nedium releases hydroxyl ions, as shown in the following equation :

$$CO_3^{2-} + H_2O \leftrightarrow CO_2 + 2OH^-$$

High O_2 concentration (>30 mgL^{-1}) should be avoided which may lead to hoto-oxidative damage of the algal cells. This situation can be avoided with proper ixing which increased the transfer of the supersaturated oxygen from the water to e atmosphere and also transfer the O_2 for bacterial respiration (Abeliovich, 1986). he net productivity of an algal culture is directly correlated to the gross rate of CO_2 xation or O_2 evolution (photosynthesis) and the rate of respiration. Photosynthesis d respiration are dependent on temperature but only CO_2 fixation and O_2 evolution e both light-and temperature–dependent (Torzillo & Vonshak, 1994).

In this present short-term study, only the effect of loading rate was investigated. Results show that, without any nutrients supplementation, or optimization of any physical-chemical parameters, the highest loading rate which allowed algal growth is between 2 and 4% of TL. Further studies have to be conducted to improved the HRAP conditions to allow for higher loading rates to be used.

5.2.2 Pollution reduction

Leachates contain many organic and inorganic compounds which are toxic to aquatic life discharged to waterways. It is hoped that treatment with the HRAP system can reduce the contamination of toxic compounds in the leachate.

Wastewater treatment with algae and to remove nutrients such as N and P and to provide O_2 for aerobic bacteria was proposed over 30 years ago by Oswald and Gotaas (1957). This system was designed primarily to reduce the organic load (Biochemical Oxygen Demand, BOD) or inorganic load (Chemical Oxygen Demand, COD) in wastewater.

During the growth of microalgae in wastewater the oxygen derived from algal photosynthesis is available for bacterial respiration which subsequently reduces the BOD of the effluent. In addition the nitrogen and phosphorus are assimilated by the microalgae and this may have important implications for the trophic status of the receiving water bodies. Sheleft *et al.*, (1978) stated that microalgae incorporate nitrogen and phosphorus into the biomass. They showed that the increase in biomass yielded proportional increase in nutrient removal from effluents.

The pollutant reductions were found to be dependent upon the initial pollutant concentrations in the leachate. This was clearly shown in the changes of COD, NH_4-N and PO_4 levels in the present study. Table 15 shows the reduction in pollution parameters in the HRAPs during the study. Overall the COD content in the TL ranged between 860 to 8066 mgL^{-1} and after treatment in the HRAP, the COD levels in both ponds were reduced to 40 to 2160 mgL^{-1} with percentage reduction of 16.27 to 95.34%. The highest COD reduction was recorded at the loading rate of 1% TL with percentage reduction ranging from 21.34 to 98.95%. The NH_4-N content in TL (initial level) ranged from 3.20 to 151.66 mgL^{-1} and it was reduced to 0.02 to 42.1 mgL^{-1} with percentage reduction at 87.72 to 99.90%. The highest NH_4-N reduction was recorded at the loading rate of 4% TL with percentage reduction ranging from 87.72 to 99.83%.The highest PO_4 reduction (69.74 to 98.38%) was recorded at the loading rate of 2% TL, when the influent PO_4 value ranged between 0.21 to 8.18 mgL^{-1}. In terms of the overall performance, the HRAP system can successfully reduce the polluting parameters of the TL. The final effluent quality of the main pollutants namely COD, NH_4-H and PO_4, passed the standards for wastewater discharge set by the DOE (Appendix 1).

Removal of NH_4-N from the effluent occurs in two ways, algal utilization and ammonia stripping (volatilization). Ammonia stripping takes place under strong alkaline conditions and elevated temperature (Reeves, 1972). Since the pH of the present HRAP cultures ranged from 4.86 to 9.37, ammonia stripping was not expected to occur. Thus the NH_4-N removal was totally dependent on algal assimilation. The prolonged algal growth enhanced the NH_4-N reduction.

Table 15: Summary of the reduction in pollution parameters when the microalgae were grown in TL supplementation.

LOADING RATE	COD			NH_4-N			PO_4		
	Initial (mgL^{-1})	Final (mgL^{-1})	% reduction	Initial (mgL^{-1})	Final (mgL^{-1})	% reduction	Initial (mgL^{-1})	Final (mgL^{-1})	% reduction
1%	1093- 8066.	40 – 860	21.34-98.95	3.20-151.66	0.11 – 42.21	63.39-99.90	4.37 –8.18	0.62 – 15.26	5.25-89.60
2%	860 -2590	80 – 720	30.77-97.52	9.0 – 95.76	0.14 – 1.25	79.65-99.27	0.78-2.38	0.03 – 1.08	69.74-98.38
4%	2580 - 3040	40 - 2160	16.27-95.34	6.27 – 12.29	0.02 – 0.77	87.72-99.83	0.21-0.29	0.01 – 0.17	37.93-95.86

Leachate normally contains nitrogen in the organic and ammonium forms. Transformations of nitrogen forms that may occur in biological treatment systems are illustrated in Figure 70. These transformation include:

i. Hydrolysis and bacterial breakdown of organic nitrogen to ammonium

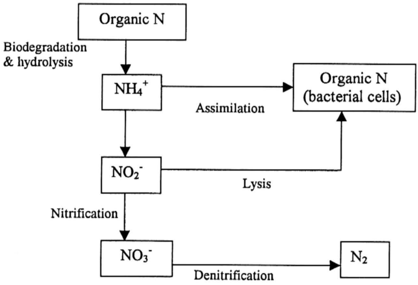
ii. Assimilation of ammonium nitrogen in bacterial cells

iii. Release of ammonium due to cell lysis

iv. Nitrification of ammonium to nitrites and nitrates

v. Denitrification of nitrates to nitrogen gas

Figure 70: Nitrogen transformations in biological treatment systems



Nitrogen entering the leachate system in the organic or ammonium forms may be removed through assimilation or oxidized to nitrate through nitrification. Nitrates can subsequently be denitrified to nitrogen gas. Nitrification is the biological oxidation of ammonia to nitrate with nitrite formation as an intermediate. The microorganisms involved are the autotropic genera *Nitrosomonas* and *Nitrobacter* which carry out the reaction in two steps:

$$2NH_4 + 3O_2 \xrightarrow[\textit{Nitrosomonas}]{} 2NO_2^- + 2H_2O + 4H^+ + \text{new cells}$$

$$2NO_2^- + O_2 \xrightarrow[\textit{Nitrobacter}]{} 2NO_3^- + \text{new cells}$$

Since a buildup of nitrite is rarely observed, it can be concluded that the rate of conversion to nitrite controls the rate of the overall reaction.

The cell yield for *Nitrosomonas* has been reported as 0.05 to 0.29 kg NVSS.kg^{-1} NH_4-N and *Nitrobacter* 0.02 to 0.08 kg NVSS.kg^{-1} NO_2-N. Here NVSS represents nitrifiers volatile suspended solids. The empirical overall (oxidation and synthesis) reaction is:

$$2NH_4 + 1.83O_2 + 1.98\, HCO_3^- \rightarrow 0.98\, NO_3^- + 0.021\, C_5H_7NO_2 + 1.88\, H_2CO_3 + 1.04\, H_2O$$

Thus the stoichiometric equation for nitrification indicates that for 1kg of ammonia nitrogen removed approximately:

- 4.3 kg of O_2 are consumed
- 7.1 kg of alkalinity are destroyed
- 0.15 kg of new cells are formed
- 0.08 kg of inorganic carbon are consumed.

Denitrification is the biological conversion of nitrate-nitrogen to more reduced forms such as N_2, N_2O and NO. The process is brought about by a variety of facultative heterotrophs which can utilize nitrate instead of oxygen as the final electron acceptor. The stoichiometric reaction describing denitrification depends on the carbonaceous matter involved. Thus, for methanol, which is the most extensively used and studied external carbon source, the empirical reaction including synthesis is:

$$NO_3^- + 1.08\ CH_3OH + 0.24\ H_2CO_3 \rightarrow 0.056C5H_7NO_2 + 0.47\ N_2 + 1.68\ H_2O + HCO_3^-$$

This reaction indicates that for 1 g of nitrate-nitrogen that is denitrified

- 2.47 g of methanol (or approximately 3.0 g of BOD) are consumed
- 0.45 g of new cells are produced
- 3.57 g of alkalinity are formed.

Phosphate removal in HRAP includes salt precipitation and algal utilisation. Similar to the ammonia removal, enhanced algal growth or biomass production by TL addition has yielded better phosphate reduction. Since the carbon, NH_4-N and PO_4 are the major components in algal biomass, the C:P or N:P ratio in the wastewater could affect the phosphate uptake by algal cells.

Addition of TL into the treatment ponds increased the C:P ratio and thus the phosphate removal. This was shown in the HRAP at the loading rate where the TL was added in the morning and made available for algal assimilation.

TL contains phosphorus in the ortho, condensed and organic forms. Transformation of phosphorus forms that may occur in biological treatment plants are illustrated in Figure 71. These transformations include:

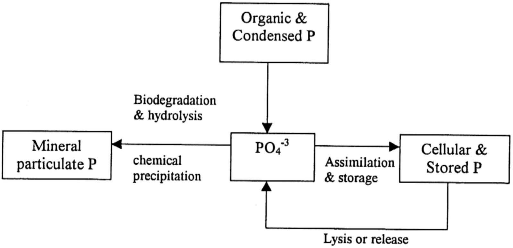
Hydrolysis and bacterial breakdown of organic and condensed phosphorus to orthophosphate

. Assimilation or storage of orthophosphate in bacterial cells

i. Released of orthophosphate (due to lysis and other reasons)

i. Chemical precipitation with natural occurring or added cations

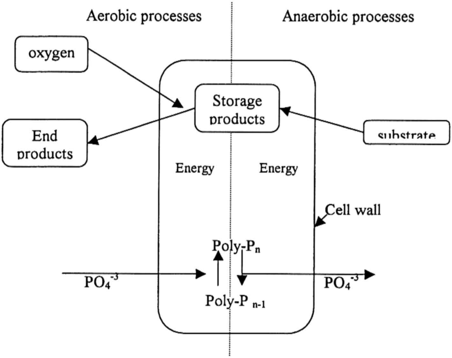
Figure 71: Phosphorus transformations in biological treatment systems



In contrast to nitrogen, which can be removed from wastewater as a gaseous roduct, phosphorus removal can only be achieved through its incorporation in articulate matter such as bacterial cells or mineral precipitates (Martin, 1991).

All biological phosphorus removal processes involved the alternate exposure the activated sludge to anaerobic and aerobic conditions. The anaerobic contact ne in this system functions as a biological selector for phosphorus-storing microorganisms. It provides a competitive advantages to those microorganisms since they can take up carbonaceous substrate in this zone before other microorganisms can. The energy required for this uptake is derived from the depolymerization of polyphosphates which were generated previously under aerobic conditions. A simplified illustration of the commonly accepted mechanism is depicted in Figure 72.

Fig 72: Simplified illustration of biological phosphorus removal mechanisms



It is generally accepted that the carbonaceous substrate necessary for preferential uptake by phosphorus removing organisms should consist of simple, readily degradable compounds. In domestic wastewater such compounds are the volatile fatty acids (VFAs) which are the fermentation products of soluble organics. Thus, in the absence of VFAs in the raw wastewater, the anaerobic basin must also function as an aerobic fermenter where soluble organics are converted to VFAs. The molar ratio of VFA utilization to phosphorus release varies from 0.6 to 1.3.

Phosphorus uptake in the aerobic basin is related to the biodegradation of carbonaceous substrates. These substrates consist of storage products as well as residual organics which were not taken up in the anaerobic basin. The phosphorus uptake rate was found to be zero-order with respects to phosphorus when the incoming BOD consists of simple organic compounds, and first-order when the influent BOD is made of more complex organics (Tracy & Flammino, 1987).

The presence of nitrates in the anaerobic basin may interfere with phosphorus removal, since denitrifying organisms can compete with phosphorus removing organisms for the available organics. It was shown, however that phosphorus removing organisms such as *Acinetobacter* and others are capable of denitrification (Lotter, 1984) and uptake of phosphorus can take place in a denitrifying environment (Gerber *et al.*, 1986). Based on energetic considerations, Tracy and Flammino (1987) concluded that under a high denitrification rate sufficient ATP (adenosine triphosphate) is generated to drive the polyphosphate polimerization reaction so that phosphate uptake and denitrification take place simultaneously.

Mixing speed and paddle immersion–depth also play an important roles on removal of pollutants. Chui (1993) showed that the HRAP operated at 20 rpm and blade immersion depth of 0.1 m produced the best reduction in terms of TS, TVS, COD, PO_4,NH_4^+ and NO_3^- from rubber effluent. This indicates that a higher mixing rate closer to the pond's surface resulted in higher reductions of the pollution parameter.

The removal of heavy metals from polluted wastewater is certainly a promising field for the use of microalgae and cyanobacterial biomass produced in wastewater. Microalgae such as *Chlorella, Scenedesmus, Chlamydomonas,* and *Oscillatoria,* have been used for the removal of heavy metals. For example, alginate and polyacrylamide immobilized *Chlorella vulgaris, Chlamydomonas* and have been used for the remove many metals including Cu, Pb, Zn and Au (Wong and Tam, 1997). Analysis of heavy metal contents in the algae cells and effluent in the present study shows that the Zn, As, Cd, Cu, Cr, Pb and K contents were higher than the heavy metal content in Drinking water standards (Appendix 4). This is because of bioaccumulation processes which occurred in the HRAP where, during the studies we did not discharge the effluent in the pond. Therefore the heavy metals in the effluent and in the algal cells remained in the ponds. Harvesting (removal) of the biomass (algae and bacteria) would remove these contaminants from the final discharge.

5.3 TREATMENT EFFICIENCY

To a certain extent, supplementation of TL exerted positive effects on the algal biomass generation and recovery of nutrients from the TL using a HRAP system. Introduction of TL increased the C:N or C:P ratio of TL resulting in a better growth medium for *Chlorella vulgaris* and *Spirulina* sp. Overall treatment efficiencies of pollution parameters achieved were, COD (16.27 to 98.95%), NH_4-N (63.39 to 99.90%) and PO_4 (5.25 to 98.38%). This showed that culture of selected microalgae in HRAP is a good system to treat TL. The HRAP system has been reported to be a very good and an efficient and low cost treatment system to treat agro-industrial wastewater.

5.4 SEMIDIURNAL STUDIES

The semidiurnal studies recorded the changes of the various physical and growth parameters during the 12 light hours (0700h – 1900h). The parameters centered around the algal photosynthetic activity. The semidiurnal changes in solar radiation and temperature were quite consistent during the study. The maximum solar irradiance and temperature were recorded in the afternoon (1100h – 1400h) whereas the minimum occurred in the early morning (0700h) and in the evening (1900h).

The semidiurnal changes in pH value during the study showed that pH increased gradually from morning when algal photosynthesis was just about to start at 0700h. Whereas the highest pH value was attained in the afternoon when the photosynthesis was at its peak. However the pH did not decline till the original level as in the morning. Variation in pH were probably caused by photosynthetic uptake of CO_2 and bicarbonate, the latter being substituted for hydroxyl ion, which explains the high pH value during periods of intense photosynthetic activity during the day (Ruttner, 1963).

Dissolved oxygen (DO) content is an indication of oxygen availability in the pond culture. DO is depend on solar irradiance and the oxygenation processes which, in present studies, consisted of algal photosynthetic oxygenation and partly mechanical aeration. Despite the continuous mixing, the cone shape semidiurnal DO curves were recorded in all of the studies. The rise in DO concentration was probably caused by the net production of oxygen through photosynthesis and the fall in DO during the evening by the respiratory uptake of oxygen by the pond biota, especially the microalgae (Edwards & Sinchumpasak, 1981).

The cell density did not show any marked semidiurnal change pattern. Despite an almost constant cell number recorded through the day in the semidiurnal studies, the chl *a* concentration and OD_{620nm} values increased with time. The maximum values were attained in the afternoon and the minimum was attained in the morning and evening, showing suitable algal growth and photosynthesis regimes in the HRAPs.