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WATER AND ENVIRONMENTAL ENGINEERING
Tampere University of Technology

UNIDO
CONTRACT No. 96/024/ML
PROJECT DP/ETH/93/005

Unpublished Report
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RESEARCH AND EXPERIMENTAL INVESTIGATION ON TANNERY EFFLUENT TREATMENT BY ADVANCED INTEGRATED WASTEWATER POND SYSTEM (AIWPS) IN ETHIOPIA

FINDINGS
OF
THE EXPERIMENTAL INVESTIGATION

(FINAL REPORT)

May 1999

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This report is based on a three-year research work carried out at Modjo Tannery by the Institute of Water and Environmental Engineering, Tampere University of Technology (TUT), Tampere, Finland. The research was made possible by financial assistance from the United Nations Industrial Development Organization (UNIDO) and the Academy of Finland. The financial supports are greatly acknowledged.

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SUMMARY

BACKGROUND

Tanning is an old craft and is still one of the major industrial establishments in many developing countries. Modern tanning in Ethiopia dates back to the mid-twenties with the establishment of the first tannery, circa 1925. Ethiopia having the largest livestock population in Africa, possesses huge potential for hides and skins. As a result, the processed hides and skins and leather products are the leading export items next to coffee. In view of the huge potential and increased activities from the public and private sectors in the leather market, water pollution from discharge of untreated tannery effluents is becoming a matter of increasing concern. At present no tannery treats its effluent properly. A 1993 survey put the yearly pollution load generated by public tanneries at 2,400 tons of BOD and 4,900 tons of COD (Tadesse 1993). The present pollution load may have exceeded that of 1993 as many tanneries have been established ever since that time.

Although there are a number of high-tech treatment alternatives for tanning wastes none are, as yet, appropriate for Ethiopia mainly due to their prohibitive capital costs, high operation and maintenance costs and the need for skilled manpower. It was against this background that this research was initiated three years ago to establish a low cost and appropriate treatment alternative for the tanning industry. The research was conducted on a pilot Advanced Integrated Wastewater Pond System (AIWPS) constructed in the premises of Modjo tannery, Ethiopia.

This report compiles the findings of a three-year experimental study carried out in Modjo tannery by the Institute of Water and Environmental Engineering of Tampere University of Technology, Tampere, Finland. The research was funded by the United Nations Industrial Development Organization, UNIDO and the Academy of Finland.

Discussion in the report falls into five main sections: description of the pilot experimental plant, the start-up procedures, the experimental methods used, results obtained and problems encountered.

FINDINGS

Pollutants removal

The pilot AIWPS attained a high level of treatment efficiency comparable to that of advanced treatment methods. Subjected to volumetric BOD loading of 278 g/m^3-d in its integrated fermentation pit and aerial BOD loadings of 1236, 228, and 185 kg/ha-d in its advanced facultative, secondary facultative and maturation ponds, respectively, the system achieved an overall BOD removal efficiency of 98%. The removal for COD was 97%. At intermediate loading rate (phase 2), the system has attained an overall BOD and COD removal efficiencies of 87% and 90%, respectively. At overloading condition (phase 3), the overall BOD and COD removal efficiencies were 92% and 86%, respectively.

The removal efficiencies for solids under normal operating condition were: 77% for total, 92% for suspended, 64% for dissolved and 93% for volatile. The relatively low removal efficiency recorded for dissolved solids was due to high sodium chloride concentration in the raw effluent which is generally not amenable to a any biological treatment. Overloading has resulted in a lower overall removal efficiency of solids.

Nitrogen which is one of the key pollutant nutrients of a tanning waste was analysed in terms of ammonia, nitrite and nitrate. An 85 % of the influent ammonia was removed by the system under the normal loading rate. A significant increase of ammonia took place from influent to effluent in AFP during both the intermediate and overloading conditions. It is possible that the sustained loading condition to which AFP was subjected might have increased the anaerobicity there by increasing anaerobic degradation of organic nitrogen to ammonia. In all the three loading rates, the highest ammonia removal occurred in the secondary facultative pond (50-59%) followed by maturation pond (20-29%). Such high removal efficiency observed in the algal ponds could largely be attributed to algal uptake. Influent nitrate concentrations were erratic during the course of the study, and the mean values of nitrate varied from 7 to 19 mg/l. Influent nitrate concentrations are probably due to those contained in the tannery process water. As the main mechanism of ammonia removal in the algae ponds was more photosynthetic assimilation than nitrification the nitrate levels in aerobic ponds has not increased. The nitrate concentration of the finally treated effluent over the three loading rates had shown little variation (2-2.5 mg NO_3^-/l). The apparently high overall nitrate removals of 71 and 87% obtained during phase 2 and phase 3, respectively should be examined in relation with high influent nitrate concentrations. Nitrite, due to its unstable and transitory nature, can not be detected.

Chromium that allegedly defames tanneries was removed by 99%. Overall chromium removal efficiency was 86 % during the intermediate loading and overloading phases.

Sulphur compounds that put tanneries into disrepute (as sources of malodorous and poisonous H_2S gas) were effectively removed. Under normal operating conditions, the overall sulphate and sulphide removals of the system were 80% and 99%, respectively. Sulphate removal in AFP, varied from 79%-93%. In the bio-chemical transformation of sulphur compounds, no offensive odour was produced. This was achieved by a controlled culture of a consortium of photosynthetic sulphur bacteria in the facultative pond overlying anaerobic fermentation pit. The overall sulphide removal efficiency remained high even at increased organic loading rates (96% for F2 and F3). There was a shift in highest sulphide removal efficiency from the secondary facultative pond (98%) during phase 1 to maturation pond (68% and 82%) during phase 2 and phase 3. This indicates that insoluble sulphides moved from the anaerobic pond into the maturation pond in substantial quantities. The percentage of hydrogen sulphide to non-odorous sulphides was less than 9% during the three experimental phases. Such low level emission did not, however, pose any odour problem. The finding was contrary to the general belief that asserts anaerobic ponds as useless for industrial wastes.

Phosphorus removal was only 33%. This was by no means unsatisfactory, when compared with the performance of other related treatment methods. Apart from that achieved by chemical precipitation, phosphorus removal by conventional biological treatment methods is generally lower than carbon and nitrogen. In one of AIWPS units we found microbial removal efficiency of 65%, 59%, and 37% for BOD, NH_3-N and total phosphorus, respectively. This was a very high uptake, indeed, for phosphorus and the result was comparable to microbial luxury uptake experienced in some efficient bio-reactor, namely the activated sludge process.

The fact that sodium chloride is not amenable to any biological treatment method was clearly demonstrated by low overall removal percentage (21-39%). It is, therefore, a good strategy to segregate salt laden effluent from the general effluents and treat it separately, perhaps, in solar ponds for eventual recovery of crude salt. A gradual build up of electrical conductivity was observed as both volumetric and organic loadings increased.

Environmental conditions

This focuses on the media environmental conditions of the system, namely temperature, pH and dissolved oxygen. The system, especially integrated fermentation pit, has a high buffering capacity against any conceivable fluctuation of pH. The pH in the fermentation pit was maintained to a very narrow range (7.2-8.2) at all times, in spite of the wide fluctuation in the feed (9.4-11.4). The system temperatures remained fairly constant the averages being 21 °C for fermentation pit, 22 °C for the secondary facultative pond and 23 °C for the maturation pond. The depth variation of dissolved oxygen, pH and temperature in the advanced facultative pond was minimal. Lack of any pronounced thermal stratification in the advanced facultative pond was suggestive of some form of internal mixing, perhaps by the biogas evolving from the sludge layer. The oxygen concentrations in the upper water layers (30 cm) of the secondary facultative and maturation ponds were on average above 8 mg/l. During the peak photosynthesis (14:00-16:00h), oxygen concentration in algal ponds exceeded the saturation level by nearly 300% under the normal operating condition. Diurnal variation of temperature, pH and dissolved oxygen in algal ponds followed more or less similar trends, with peaks occurring between 1300 and 1500 hours. The dissolved oxygen concentration was found above the saturation level in all but the overloading phase. Under the overloading condition the dissolved oxygen concentration was 55-75% of the saturation level.

Biogas

The AIWPS biological waste treatment system offered a high potential for energy in addition to its high pollutant removal efficiency. This supplemental energy is derived from the biogas generated from the fermentation pit of the advanced facultative pond. This spin-off benefit makes the AIWPS method more attractive to tanneries than its counterpart conventional biological treatment methods. The biogas will find many applications in tanning industries which include but not limited to: (i) supplementary energy source for boilers, (ii) drying recovered wool and (iii) incineration of excess solids, etc. This could offer enormous saving in annual operational costs. Part of this energy may also be used to heat up the fermentation pit thereby insuring process stability and increase biogas out put during cold seasons.

At normal operating condition, where volumetric BOD and COD loading rates in the fermentation pit reached 75 g/m^3-d and 565g/m^3-d respectively, the daily average production was 50 l/d. The biogas conversion ratios during the same period were 0.350 m^3/kg BOD removed and 0.165 m^3/kg COD removed. The conversion ratios were quite high considering the sub-mesophilic operation temperature (19-21 °C) of the fermentation pit. The results are comparable to conversion ratio of 0.160 m^3/kg COD obtained for an anaerobic filter and 0.21 m^3/kg COD obtained for an UASB reactor. As the organic loading increased, biogas production gradually decreased. At three-fold increase of organic loading the biogas production has ceased altogether. The main culprit for upset in biogas production during overloading condition was the high ammonia concentration within the AFP. A 76% increase in ammonia concentration was observed during overloading condition. In spite of the decrease in biogas production, BOD and COD removal efficiencies in the AFP remained fairly high. The fact that anaerobic system continued to perform well in terms of organic removal while methanogenesis was upset indicates that some kind of biological degradation, other than sedimentation, was still active.

Ecosystem

The biological community of the AIWPS pond system was rich, with remarkable synergy among the key players. The first reactor in the series being anaerobic, was occupied by anaerobic and facultative group of bacteria. Effective anaerobic digestion in the fermentation pit relied on correct balance between acid formers and methane producers. Other syntrophic bacteria that grow in association with fermentative bacteria, particularly in sulphate rich media, were the sulphate reducing bacteria. Their balance with methane producers was equally important. The water overlying the fermentation pit was dominated by anoxygenic photosynthetic sulphur bacteria, who did a wonderful deodorizing job against hydrogen sulphide. The middle and last units of the system, being facultative and aerobic algal ponds, respectively, rely on mutualistic relationship between algae and bacteria. Zooplanktons and zoobenthos had a role of controlling the levels of lower organisms thereby influencing the succession of species in the last units of the system. Although it is difficult to study every microbe in the ecosystem, some attempt was made to screen out the dominant genera of zooplankton, zoobenthos, phytoplankton and sulphur bacteria.

The most dominant algal species found in the secondary facultative and maturation ponds at the normal loading rate were *Phacus longicauda* and *Euglena cf. Viridis* . Algae of the genera *Chlorella*, *Chlamydomonas* and *Chlorogonium* were also present. The algal standing crops of 1,500-3,000 µg chlorophyll a /l obtained for maturation and secondary facultative ponds were within concentrations of healthy ponds which lie between 500 and 3,000 µg chlorophyll a per litre.

The study on zooplankton and zoobenthos indicated that no potential grazer that endangered algae existed in the two algal ponds. It was also confirmed that no malaria vector (*Anopheles gambiae*) inhabited the algal ponds.

Purple bacteria of the genus, *Thiocystis* were the dominant among anoxygenic photosynthetic sulphur bacteria found in the advanced facultative pond. Some colourless sulphur bacteria of the genus *Thiobacillus* and purple nonsulphur bacteria of genera *Rhodosprillium* and *Rhodopseudomonas* were also identified. These sulphur bacteria played an important role in combating any odour nuisance due to hydrogen sulphide.

The algal ponds continued to perform well even under overloading condition. Algological examination on samples from the secondary facultative and maturation ponds showed that the most tolerant *Chlamydomonas fenestrata* continued to dominate during heavily loaded operation. *Chlamydomonas f.* dominance was attributed to its high mobility and ability to maintain itself in the euphotic layer of the loaded ponds. The other speciality of *Chlamydomonas f.* is its high tolerance for toxic inhibition, especially, that of sulphide.

1 INTRODUCTION

The United Nations Industrial Development Organization (UNIDO) contracted the Institute of Water and Environmental Engineering of Tampere University of Technology, Tampere, Finland under Contract No. 96/024/ML, to carry out Research and Experimental Investigation on Tannery Effluent Treatment in Advanced Integrated Wastewater Pond System (AIWPS). The Contract was part of the Project DP/ETH/93/005 to Ethiopia.

This final report is submitted to the UNIDO's Contracts Unit in Vienna, Austria. It covered the findings of a three-year experimental investigation carried out on pilot-scale AIWPS constructed at Modjo tannery, Modjo, Ethiopia.

2 DESCRIPTION OF THE PILOT EXPERIMENTAL PLANT

The pilot treatment plant constructed at the premises of Modjo Tannery, Modjo, Ethiopia for the study of tannery effluent treatment was of a two-stage process comprising a pre-treatment and a biological treatment facility. At the pre-treatment stage, the raw effluent from the tannery's production hall flowed through two coarse bar screens and one fine bar screen before it passed on to primary and secondary settling basins. The screens retain fleshings and floating hair that have escaped from the lime fleshing and paint and machine unhairing processes. After the screening, further removal of suspended solids was achieved in two horizontal settling basins. No coagulants were employed in the settling basins. With lime mediated combined tannery effluent having high pH (normally above 9), solids removal as high as 35% was easily achieved without coagulation. Jar test carried out using Alum (aluminium sulphate coagulant, $Al_2(SO_4)_3.14H_2O$) proved non-worthy. Conveyance of the raw tannery effluent from the production hall via the bar screens to the settling basins was by gravity.

The biological treatment facility, known as Advanced Integrated Wastewater Pond System (AIWPS), consisted of three biological reactors arranged in series. The first reactor in the line was a primary facultative pond known as an Advanced Facultative Pond (AFP). Treatment in this reactor was through anaerobic bio-degradation in the bottom fermentation pit followed by further aerobic stabilization in the overlying facultative pond. The pre-treated effluent from the settling basins was pumped directly into the fermentation pit using a peristaltic pump. The digested waste then overflowed into the overlying facultative pond. The AFP was squarish in plan with surface dimensions 3 m x 3 m and liquid volume of 20.7 m^3 at 2.3 m depth. A cylindrical fermentation pit with base diameter of 1.6 m and wall height of 2 m was incorporated in the floor of the AFP. The walls of the fermentation pit have extended 1 m below and 1 m above the AFP bottom. Under such arrangement, the top of the fermentation pit wall was 1.3 m below the water surface of the AFP. This vertical clearance was sufficient for the installation of submerged gas canopy that converges emerging biogas into surface gas collector. The gas canopy also prevented the intrusion of dissolved oxygen into the fermentation pit as a result of wind-induced vertical mixing.

A 4 m heigh,150 mm diameter galvanized steel casing supported the surface gas collector and four 12 mm diameter mixing bars on a thrust bearing welded 15 cm below the top. The lower end of the casing was slotted in order to allow sludge and digested waste to enter the casing. The casing, in addition to serving as a central support column, facilitated abstraction of sludge samples and insertions of oxygen, pH and temperature probes for the daily process monitoring. The sludge deposit in the fermentation pit was de-gased regularly by turning the surface gas collector. The rotational motion created on the surface was transferred to 11 mixing spikes at the bottom by four 12 mm diameter rods running along the casing. The biogas produced from the fermentation pit was collected through two vents provided at the top of the gas collector and the casing. The vent on the casing collected biogas as it emerged from the sludge-water interface, while the vent on the surface collector captured biogas after it has ascended through the overlying water column. Two level gas collections permitted assessment of the relative purity of the biogas from the two vents. There were five major gaseous components in the biogas: CH_4, CO_2, H_2, N_2 and H_2S. Of all these gases, CH_4 has a high energy value as it readily combusts. The rest were regarded as impurities. CO_2 and H_2S were usually consumed by photosynthetic sulphur bacteria that inhabited the upper water layer of the advanced facultative pond. Thus the biogas collected from the surface collector was expected to be more pure (high methane composition) than the biogas coming from the sludge water interface.

The partially treated effluent from AFP was drawn from a depth of 40 cm below the water surface and was introduced into the bottom of the Secondary Facultative Pond (SFP). The SFP was rectangular in plan with surface dimensions 3.2 m x 1.6 m and liquid volume of 7.68 m^3 at 1.5 m depth. The mode of treatment in this reactor was through metabolic activity of aerobic and facultative bacteria in the upper and middle layers and strict anaerobes at the bottom. The intermediate facultative zone fluctuated diurnally, depending on the presence of dissolved oxygen fed from the upper layer. Oxygen for biodegradation of the waste by pond bacteria was mostly generated by algal photosynthesis. This symbiotic relationship between algae supplying oxygen for the bacterial oxidation of organic waste and bacteria producing carbon dioxide for algal photosynthesis was crucial for the entire purification process in this reactor. The last reactor in the series was a Maturation Pond (MP). The effluent from SFP was drawn from a depth of 20 cm below the water surface and introduced into the bottom of MP. The maturation pond was provided largely as a tertiary treatment process removing nutrients and algae from the final effluent.

Purification in this reactor was by and large through aerobic bacterial oxidation. The MP was rectangular in plan with surface dimensions of 2m x 1m and liquid volume of 2 m^3 at 1 m depth. The treated effluent was finally drawn from 15 cm below the water surface. Such inlet-outlet arrangement avoided all conceivable manner of short circuiting as the waste was fed by gravity into each reactor through 3/4 inch galvanized steel pipe.

Odour suppression provision by way of recirculating warm, oxygen rich water from maturation pod back to the surface water layer of AFP was originally envisaged. But, lack of any offensive odour from AFP proved the provision unnecessary and hence stopped altogether.

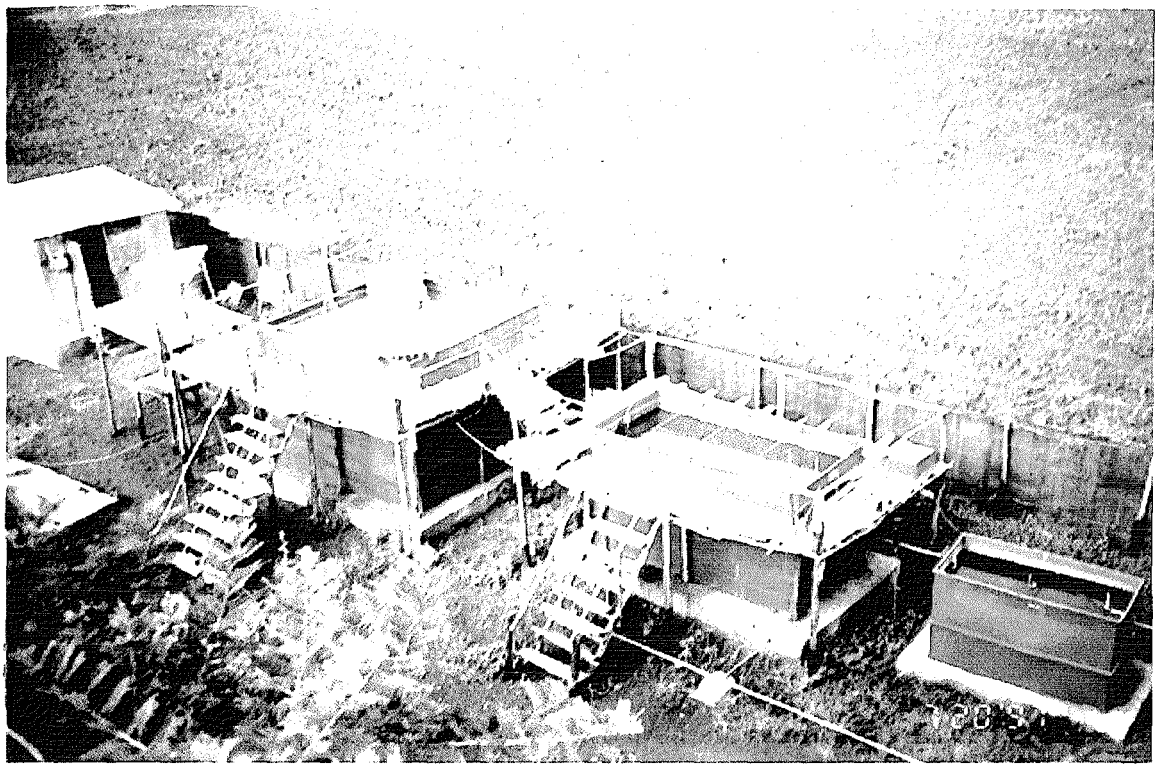


Plate 1. Bird's eye view of the pilot AIWPS reactors.

Research site layout and detailed engineering drawings of the pilot experimental plant were included in the second interim report (October 1996). Since some modifications were made on the AIWPS reactors, after submission of the second interim report, sectional details of Advanced Facultative Pond (AFP), Secondary Facultative Pond (SFP) and Maturation Pond (MP) are only included in this report. The sectional details are attached as Annexe 1.

Summary of the physical dimensions of AIWPS biological reactors is given in Table 1 below.

Table 1. Physical dimensions of AIWPS reactors.

Reactors	Reactor Geometry	Reactor Overall Dimensions	Reactor Depth	Water Column Depth	Mid-depth/ Surface Area	Liquid Volume
		(m) x (m) x (m)	(m)	(m)	(m^2)	(m^3)
Fermentat-ion Pit(FP)	Cylindrical	ϕ1.6 x 2	2	2	2	4
Overlying Facultative Pond(OFP)	Squarish	3 x 3 x 2.5	2.5	2.3	9	21
Advanced Facultative Pond(AFP)	Squarish	3 x 3 x 3.5	3.5	3.3	9	23
Secondary Facultative Pond(SFP)	Rectangular	3.2 x 1.6 x 1.8	1.8	1.5	5	8
Maturation Pond(MP)	Rectangular	2 x 1x 1.3	1.3	1	2	2

3 START-UP OF AIWPS BIOLOGICAL REACTORS

3.1 Considerations in reactor start-up

Start-up is the initial operational procedure carried out in commissioning new biological reactors to establish the necessary microbial population to effect waste stabilization. As the raw tannery effluent lacks the microbes for the biological degradation of the waste, an acclimatized inoculum consisting of bacterial flora adapted to tannery waste should be used as a seed material. In starting-up a new biological reactor, the following factors need to be seriously considered:

- Source and nature of inoculum material
- Volume and number of inoculations
- Feed organic loading
- Hydraulic retention time (HRT)
- Nutrients and conducive environmental factors

Source and nature of inoculum

Selection of a proper inoculum source is important to obtain rapid rector start-up and minimize the time required for the initial microbial establishment. Usually a bacterial flora adapted to the target wastewater (i.e. tannery waste) should be used. If this is not possible, the reactor can be seeded with a mixture of various inocula in order to get a rich and active microbial community. As much as possible, granular sludge should be used to inoculate anaerobic reactors. But, when granular sludge is not available a well digested, flocculant, non-granular sludge (e.g., septic tank sludge, waste activated sludge, or even cow manure) can be used.

Volume and number of inoculation

In general a right amount of inoculum material should be added to start new biological reactors. The amount of inoculum sludge needed to start-up anaerobic digestors has not been well defined. Some investigators put inoculum volume required to 10-30% of the reactor volume. Care should, however, be taken not to overseed units, that is, the amount of seed to start must not be greater than can be grown naturally from the waste being treated.

Feed organic loading

During the start-up period, the initial organic and hydraulic loading should be kept at minimum. Care should be taken not to overfeed reactors. At the beginning, anaerobic reactors should be fed diluted waste with sludge loading less than 0.1 kg COD/1kg VSS-d. It is considered a good feed strategy to gradually increase the flow rate to the reactors in 5-15% increments.

Hydraulic retention time (HRT)

Longer hydraulic retention time (HRT) is normally required during start-up periods, so as to prevent early wash-out of inoculated biomass. Start-up of anaerobic reactors is generally time consuming because of the slow growth rate of acetogenic and methanogenic bacteria.

Nutrients and conducive environmental factors

The addition of both macro-nutrients (C, N & P) and micro-nutrients (Fe, Ni, Co & Mo) during the start-up is necessary if the target waste is deficient in them. A balanced C:N:P ratio for the growth of anaerobes is in the range of 100: 1-10: 1-5. Supplementation of key micro-nutrients (Fe, Ni, Co & Mo) is also necessary, particularly during start-up.

For achieving a fast growth of the starter culture, optimum environmental conditions should be maintained. Reactor temperature (>20 °C) and pH 6.8-7.5 are favourable for growth of most microbial population. In addition, inhibitory substances should be avoided. High concentrations of salts (Na, K, etc.);NH_4^+ (>1000 mg/l) and sulphide could cause start-up failure if care is not taken to minimize their effects. The above factors were seriously considered in the start-up the AIWPS reactors.

3.2 Start-up of Advanced Facultative Pond

Advanced Facultative Pond was initially filled with 50% acclimated tannery waste sludge from near by tannery and equal amount of mixture of Modjo river water near the tannery discharge point, sewage waste and raw tannery effluent. The content was then left to standstill for nearly two months. For some reason the reactor did not "wake up" as evidenced by lack of emerging bubbles from the reactor that herald methane fermentation. Some adverse conditions might have contributed to the very slow start-up. One possibility would be the unusual low temperature experienced during the start up period (October to December 1997).

After waiting for two weeks and when we were sure that further manipulation did not result in quick start-up we emptied the reactor and reseeded it again. During the reseeding we have added granular septic tank sludge into the fermentation pit instead of tannery sludge. The overlying facultative pond was filled with more than 60% sewage waste and 40% acclimated tannery waste. Within one and half month gas bubbles began to emerge as a sign of early onset of methane fermentation. The reactor was then fed intermittently with a mixture of sewage and tannery waste. At the beginning, biogas evolution was very low and as result recording with the gas meter was impossible. The biogas production has then increased steadily over four months period following the reseeding.

3.3 Start-up of Secondary Facultative and Maturation Ponds

Start-up of the two algae ponds was relatively easy. The secondary facultative pond was seeded with 52% algae rich pond water from Addis Ababa sewage treatment facility at Kality, 42% acclimated tannery waste, 2% septic tank sludge and 4% borehole water. The maturation pond was, however, inoculated with 90% algae rich pond water from Addis Ababa sewage treatment facility, 9% tannery waste and 1% septage. At the beginning, the reactors turned brown and remained turbid for about two weeks before they gradually cleared off and developed green colour of blooming algae. The brown colour was imparted from the septage and tannery waste that was vigorously mixed during filling. The algae population was fully established within two months. The pond water inoculum that was already rich in active micro-algae took relatively shorter time to establish than the bacteria in the primary facultative pond. The ponds were then feed with stabilized effluent from primary facultative pond.

4 EXPERIMENTAL INVESTIGATION

The experimental work involved such routines as sample collection, transportation and delivery to laboratories, insitu measurements carried out for process monitoring, laboratory analyses conducted on selected parameters for performance evaluation and further scrutiny of the results obtained. All these activities are discussed below.

4.1 Sapling procedure

The sampling procedure adopted comprised collection, preservation and transportation of samples to laboratories. At the beginning, weekly composite samples were thought to produce most representative samples. But, sample deterioration and especially loss of hydrogen sulphide during compositing and storage reverted us to grab sapling. Samples were collected from five sampling points. The first sampling point (SP1) was in the flow distribution box, from which homogenized raw tannery waste was collected. Pre-treated effluent sample was collected from the second sampling point (SP2) located at the outlet of the secondary settling basin. Effluent samples from advanced facultative pond, secondary facultative pond and maturation pond were collected from sampling points SP3, SP4 and SP5, respectively. Besides, column samples were collected from each AIWPS reactor for sulphide analysis and biological examination of phytoplankton, zooplankton zoobenthos and photosynthetic sulphur bacteria.

Grab samples of raw tannery waste and effluent from each reactor were collected between 0900 to1100 h. each week in 3 litre plastic containers. The samples were then transported in ice box and delivered to the laboratories within 2 hours of collection. Sulphide analysis necessitated collection in separate plastic bottles where samples pH were adjusted to above 9 using NaOH to keep sulphide in ionized forms ($S^{=}$, HS^{-}). The sampling day in each week was varied (i.e., Monday in week 1, Tuesday in week 2 and so on) in order to take into account most of the weekly variation in influent and effluent quality. Column samples for biological examinations were taken once during each experimental phase.

Plate 2. Labeled sample containers used during the experiment.

The chain of custody of samples was controlled by filling a submission form during each delivery. Sample submission and laboratory report forms are attached as Annexe 2.

4.2 Reactor performance monitoring

Two main tasks were carried out routinely for assessing the performance of AIWPS biological reactors: (i) on-site measurements, and (ii) laboratory analyses of selected parameters.

4.2.1 On-site measurements

On-site measurements were carried out to monitor the operational conditions of the reactors. As the results are obtained fairly quickly, remedial action can be taken on the spot on any malfunctioning reactor. The measurements include:

- Mean daily temperatures of air and reactors content
- Diurnal variation of temperature, dissolved oxygen and pH
- Profiles of temperature, dissolved oxygen and pH
- Biogas production
- Reactor feed rates, and
- Effluent flow measurement

Mean daily temperatures of air and reactors contents

The mean daily temperature was measured by maximum-minimum thermometer suspended at mid-depth of each reactor by means of a polystyrene float. The thermometers were suspended at 1.15 m, 0.75 m and 0.5 m below surface water levels of the primary facultative pond, secondary facultative and maturation pond, respectively. The mean air temperature was taken from a maximum-minimum thermometer hung at sampling platform 2.75 m above ground level. Thermometers were set at 0800-0900 h every morning and readings taken 24 h later. The data was recorded on a form which is attached as Annexe 3.

Diurnal variation of temperature, dissolved oxygen and pH

The diurnal variations of temperature, dissolved oxygen and pH were measured at one hour interval on samples taken at outlet level of each reactor. Dissolved oxygen and temperature were measured using a Hach model 16046 portable dissolved oxygen meter and pH using an INVENTRON pH/mV meter fitted with a combined pH electrode. A form prepared for recording the the diurnal variation of temperature, dissolved oxygen and pH is attached as Annexe 3.

Profiles of temperature, dissolved oxygen and pH

As dissolved oxygen, temperature and pH measurements at outlet levels vary throughout the day and as such do not provide useful information on each rector's performance, vertical variations (profiles) of the parameters were taken, at least once in week. Profiles were obtained at 0800, 1200 and 1600 h using the same DO and pH meters. Dissolved oxygen and pH electrodes were tied on a long wooden pole and lowered to the reactor bottom and readings were taken by gently raising the pole 5 cm each time (Plates 3&4). Readings were later converted to measure with reference to surface water level. The profiles were useful to determine the degree of vertical mixing and the extent of thermal stratification in each reactor. A form used for recording profile measurement is attached as Annexe 3.

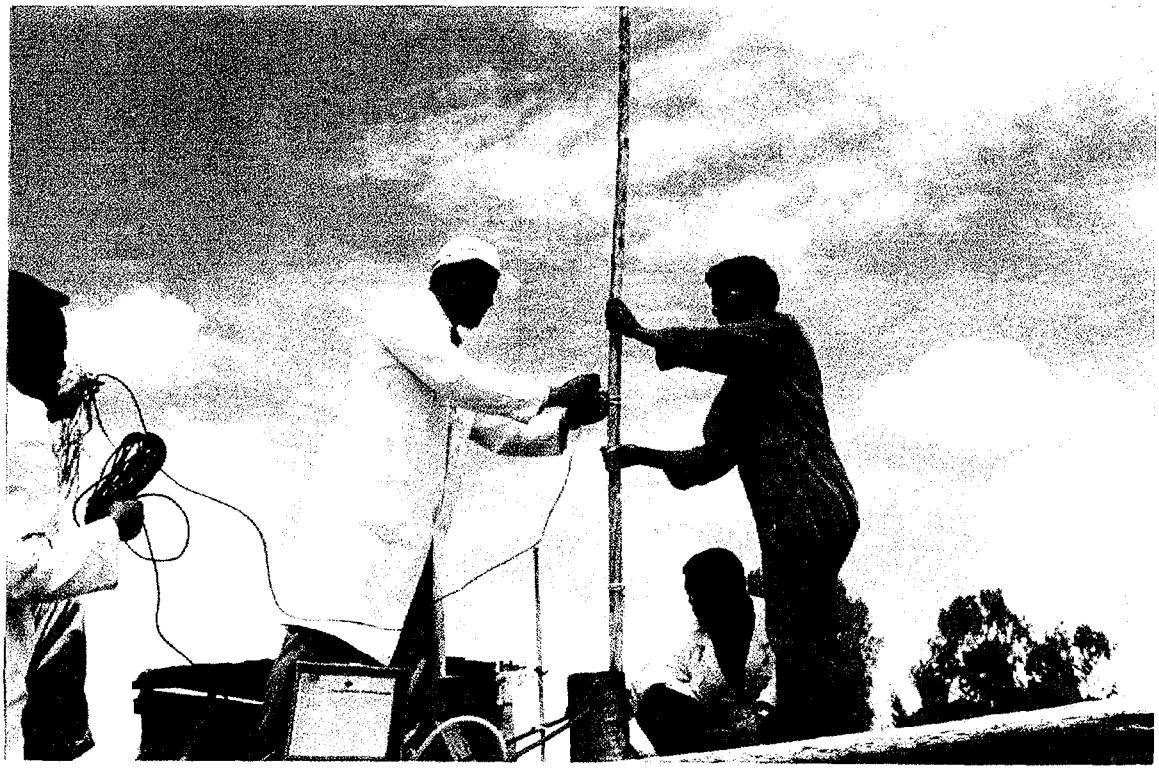


Plate 3. Tying electrode cable on a graduated wooden pole.

Plate 4. Dissolved oxygen profile measurement using a Hach model 16046 portable DO meter.

Biogas production

Biogas produced from the fermentation pit was recorded using water filled Ritter gas meter that could give reading to one hundred of a litre. Readings were taken every hour over 12 hours period. Gas samples were collected in one litre gas bags for determination of the percentage composition of the biogas in a Gas Chromatography. In addition to quantitative analysis, on-site flame tastes were done every day to check the quality of produced gas, as higher CH_4 proportions were ascertained by the blue colour of the flame. Daily biogas recording is maintained in a form attached as Annexe 3.

Reactor feed rates

Pre-treated tannery waste was pumped into the fermentation pit using one Masterflex peristaltic feed pump (Masterflex, Cole-Parmer Instrument Co. Chicago, Illinois, USA). The amount of wastewater pumped into the fermentation pit was noted from calibration curve which gives flow rate to each corresponding pump head rotation per minute (rpm). The switch setting was changed once every experimental phase and the data was recorded in a form attached as Annexe 3.

Effluent flow measurement

The tannery effluent flow was measured in a 90° Vee-notch weir fitted in the flow distribution box. Water head over the weir was measured every hour in 12 hours period and the data was entered into a form for latter computation of average daily flow. The form is attached as Annexe 3.

4.2.2 Laboratory analysis

The laboratory tests comprised routine analysis of the parameters tabulated below. The physico-chemical tests were analysed in the central laboratory of the Addis Ababa Water and Swerage Athority, while biological examinations of the reactors' eco-system were done in the Department of Biology of Addis Ababa University.

Table 2. Parameters on which routine laboratory tests were performed.

No.	Category	Parameters
1	Physico-chemical	BOD (filtered and unfiltered) COD (filtered and unfiltered) SOLIDS Total solids Suspended solids Dissolved solids Volatile solids Settleable solids Ammonia-N Nitrate-N Chromium III Chromium VI Sulphate Hydrogen sulphide Total sulphide Total phosphorus Chloride Electrical conductivity Biogas[1]‡
2	Biological	Phytoplankton Zooplankton & zoobenthos Photosynthetic sulphur bacteria and other Sulphur bacteria

‡/ Quantitative biogas analysis could not be done due to lack of Gas Chromatography with Thermal Conductivity Detector (TCD), instead on-site flame tests were routinely performed.

Biochemical Oxygen Demand (BOD)

The 5 day biochemical oxygen demand at 20° C (BOD_5) was one of the parameters routinely analysed throughout the experiment. Its importance lies in that it closely mimics the microbes in their metabolic degradation of the waste in AIWPS reactors. The tests were performed according to Standard Methods, section 5210 B (APHA et al., 1992) where the analytical determinations of dissolved oxygen of diluted samples were done by the azide modified Winkler method. To insure that tests provide valid results, six different dilutions were set up in duplicate, five for samples and one for seed control. The first six bottles were titrated immediately for initial dissolved oxygen determination, DO_0. The other six bottles were incubated at 20° C and then titrated for remaining five day dissolved oxygen, DO_5. Tests on samples from the biological reactors (SP3, SP4 and SP5) were done without seeding, as samples contain acclimatized microbes in sufficient number. However, samples from raw and pre-settled wastes (SP1 and SP2) were seeded with acclimated waste from the pilot secondary facultative pond. In both cases nitrification was inhibited by Allyl Thio Urea added into dilution water in the amount 10 mg/l. In addition, nutrients and buffer chemicals specified in Standard Methods were added into dilution water to maintain conducive pH range 6.5-7.5 and furnish the required macro-and micro-nutrients to the organisms. Sample dilutions were established in such away that a residual DO of at least 1 mg/l and DO uptake of at least 2 mg/l are achieved after 5 day incubation. Dilutions that resulted in reliable BOD results were: 0.05-0.08% for SP1, 0.08-0.25% for SP2, 0.5-1% for SP3, 2-4% for SP4, 4-20% for SP5, 5-13% for SP4(filtered) and 5-20% for SP5 (filtered). BOD results were then calculated by subtracting 5 day dissolved oxygen from initial dissolved oxygen taking into account the dilution and DO uptake by seed control, where sample are seeded.

Chemical Oxygen Demand (COD)

The chemical oxygen demand was determined from the amount of potassium dichromate (K_2CrO_7) needed to oxidize the sample. The parameter is a measure of the amount of oxygen required for chemical oxidation of organic matter. The test was performed according to Standard Methods, section 5220 B (APHA et al., 1992). Percentage dilutions that resulted in reliable COD results were: 4 for SP1, 10 for SP2, 40 for SP3, 50 for SP4 and 100 for SP5. Under those dilutions the interference of chloride was highly minimized, even without adding H_gSO_4. But, about 1g H_gSO_4 was added for each 50 ml sample to ensure that mercuric ions are present in sufficient excess to inactivate chloride ions. The difference between the volume of dichromate that remains in a blank and the volume in the sample gives the dichromate consumed, which is converted to an oxygen demand using the relationship given in the Standard Methods, section 5220 B (APHA et al., 1992).

Solids

Total solids

For the determination of total solids, 200 ml homogenized samples were taken in porcelain dish and evaporated at near 103° C. After much of the water has been evaporated the residue was dried further for one hour at 103° C and the dish was cooled in a desiccator and weighed. The total solids was then calculated according to the Standard Methods section 2540 B (APHA et al., 1992).

Suspended solids

The suspended solids is generally determined, in vacuum filtration, by filtering the sample through a glass fibre filter. However, frequent clogging of the filter forced us to resort to centrifugation. A 100 ml sample was centrifuged at 3000 rpm for 15 minutes and the supernatant was removed and the residue dried at 103° C. The dried residue was then cooled in a desiccator and weighed. Suspended solids was then determined according to the relationship given in the Standard Methods, section 2540 D (APHA et al., 1992).

Dissolved solids

Although dissolved solids could be analysed by evaporating the filtrate obtained from suspended solids determinations, it was instead obtained as a difference between total solids and suspended solids.

Volatile solids

Volatile solids was determined by igniting the residue obtained from suspended solids determination in muffle furnace at 600° C. Ignition was resumed for 20 minutes and the loss of weight was registered as volatile solids. Volatile solids was calculated according to Standard Methods, section 2540 E (APHA et al., 1992).

Settleable solids

Settleable solids was determined by filling a 1-litre Imhoff cone and allowing 1 h for sedimentation. The amount settled at the bottom was then expressed in millilitres per litre. A ready comparison of settleable solids values with other solids values, in terms of milligrams per litre, is possible by calculating the settleable solids as expressed in Standard Methods, section 2540 F (APHA et al., 1992).

Nitrogen

Ammonia-nitrogen

Ammoniacal nitrogen was determined colorimetrically using direct nesslerization method. A Hach DR/4000 spectrophotometer was used for the analysis. The analytical procedures followed in Hach manual were adopted from Standard Methods, section 4500-NH_3 C (APHA et al., 1992). The method nullifies interference due to colour and hardness by using a mineral stabilizer and dispersing agent. Dilution that produced reliable results varied between 2%-4%.

Nitrate-nitrogen

Nitrate-nitrogen was determined colorimetrically using the cadmium reduction method, Standard methods, section 4500-NO_3^- (APHA et al., 1992). A Hach DR/4000 spectrophotometer was used for the analysis. All possible interference from NO_2^- and chloride were eliminated by pre-treating the sample before analysis.

Chromium

Chromium III

Trivalent chromium (Cr^{3+}) was determined by oxidizing all Cr^{3+} present in the sample to Cr^{+6} and spectrophotometrically comparing it with an unoxidized sample. Sample oxidation was performed by digesting in the solution of concentrated nitric acid and perchloric acid using the Digesdahl Digestion Apparatus. The oxidized sample, having only hexavalent chromium (Cr^{6+}), served as a blank against which the amount of (Cr^{3+}) in the original sample can be measured directly. This method was an adaptation from the Standard methods, section 3500-Cr (APHA et al., 1992). Chromium concentration was read from a Hach DR/4000 spectrophotometer at wavelength of 595 nm.

Chromium VI

The hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in acid solution. The method was adapted from the Standard methods, section 3500-Cr (APHA et al., 1992) and measured using a Hach DR/4000 spectrophotometer at wavelength of 540 nm. A Hach formulation called Chroma Ver 3 chromium reagent containing an acid buffer combined with diphenylcarbazide gives purple colour when hexavalent chromium is present. Separate determination for hexavalent chromium was made to counter check the trivalent chromium concentration from the total chromium determinations obtained after sample digestion. The apparent low concentration of hexavalent chromium found in the tannery waste made this test unnecessary for indirect determination of trivalent chromium. Tanneries use salts of trivalent chromium in the tanning process.

Sulphate

The sulphate determination was based on barium sulphate turbidimetric method of the Standard methods, section 4500-SO_4^{2-} (APHA et al., 1992). A Hach formulation reagent called Sulfa Ver 4 contains barium with a stabilizing agent was used in the preparation of the sample. The Sulfa Ver 4 reagent forms a milky precipitate of barium sulphate with sulphate. The turbidity formed is proportional to the sulphate concentration. Sulphate concentration was read from a Hach DR/4000 spectrophotometer at wavelength of 450 nm.

Total sulphide

Total sulphide which may be present in the sample as free sulphide ion (S^{2-}) or as dissolved hydrogen sulphide (H_2S and HS^-) was detrmined by Methylene blue method. The method was adapted from Standard methods, section 4500-S^{2-} D (APHA et al., 1992) and based on the reaction of sulphide, ferric chloride and N,N-dimethyl-p-phenylenediamine oxalate to produce methylene blue. Two Hach formulation reagents containing ferric chloride and N,N-dimethyl-p-phenylenediamine oxalate were added in equal amounts to the blank and sample and total sulphide was read from a Hach DR/4000 spectrophotometer at wavelength of 665 nm.

Hydrogen sulphide

Samples for sulphide and hydrogen sulphide determinations were separately collected and stored after adjusting the pH to above 9 using NaOH. This kept all sulphides in an ionized forms ($S^=$, HS^-) and loss of hydrogen sulphide minimized. Hydrogen sulphide was estimated from the plotted percentage distribution of dissolved sulphide between H_2S and HS^- given in the Standard methods, section 4500-S^{2-} F (APHA et al., 1992). The only data required for such determination were the sample pH, temperature and electrical conductivity which were measured at the time of analysis.

Total phosphorus

For total phosphorus determination, all phosphorus (organic as well as inorganic) must be first converted to reactive orthophosphate by oxidative digestion using potassium persulphate and sulphuric acid. Once all phosphorus was converted into orthophosphate, the orthophosphate (reactive phosphorus) of the expected total phosphorus was determined by ascorbic acid method of the Standard methods, section 4500-P E (APHA et al., 1992). The blue coloration formed from ascorbic acid reduction was analysed colorimetrically by Hach DR/4000 spectrophotometer at wavelength of 890 nm.

Chloride

Chloride was determined titrimetrically using mercuric nitrate method as a titrant and diphenylcarbazone as an indicator. The procedure is outlined in the Standard methods, section 4500-Cl^- C.

Electrical conductivity

Electrical conductivity was measured at 25°C using a WTW-8120 WEILHEIM conductivity meter.

Biological examination

Phytoplankton

The identification and relative abundance of the different species of microalgae in the facultative and maturation ponds were obtained by microscopic examination of samples taken directly from the pond water column. Both fresh and fixed Lugol's iodine and formalin specimens were examined. For microscopic analysis of species composition and cell density estimates, a small volume (up to 20 ml) of phytoplankton samples preserved with Lugol's solution was allowed to settle in a counting chamber. The sedimented algal particles were counted along five horizontal transects with an inverted microscope. Five field of vision, each with a surface area of 1.5386 mm^2 were included in each transect considered in the counting procedure.

Counting included the total phytoplankton communities and the number of the dominant species. Chlorophyll a estimation was done by first collecting the algae on 47 mm diameter Whatmann GF/C glass fibre filter and extracting chlorophyll a in 90% acetone. Absorbance was measured by spectrophotometer at 663 and 750 nm before and after addition of 1N HCl. The concentration of both chlorophyll a and phaeophytin a were calculated from the following equations (Wetzel and Likens, 1979):

$$\text{Chl} - \text{a}\ (\mu g / l) = \frac{11.3\ (E_{663b} - E_{663a})\ (v)}{(V)\ (Z)}$$

$$\text{Phaeophytin}\ (\mu g / l) = \frac{17.86\ (E_{663b} - \left[2.43(E_{663b} - E_{663a})\right]\ (v)}{(V)\ (Z)}$$

Where:

E_{663b} = Turbidity corrected absorbance at 663 nm before acidification
= $A_{663b} - A_{750a}$, where A is the absorbance value

E_{663a} = Turbidity corrected absorbance at 663 nm after acidification
= $A_{663a} - A_{750a}$

v = Volume of extract in ml

V = Volume of water filtered in liters

Z = Length of light path through cuvette in cm

Zooplankton & zoobenthos

The zooplankton and zoobenthos examination involve microscopic identification of detritivorous, predators and possible grazers. Fixed Lugol's iodine and formalin specimens were examined on pond water column samples collected using plankton sampling nets (Plate 5).

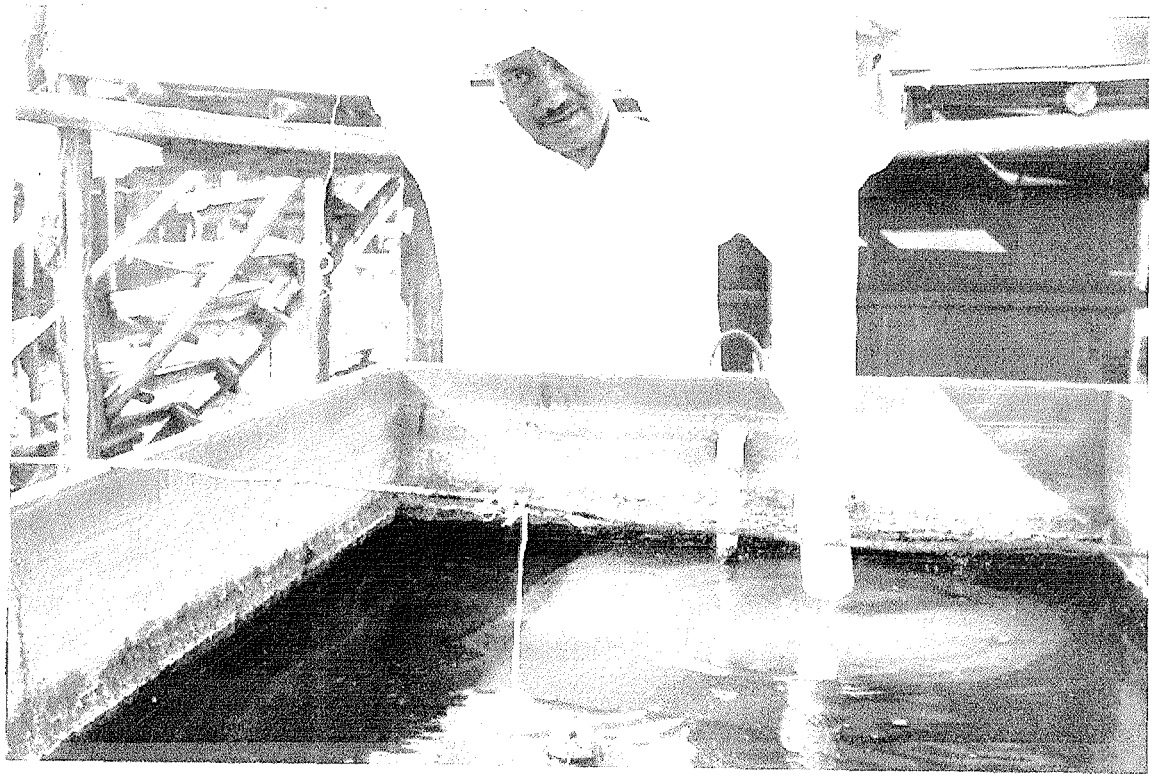


Plate 5. Sample collection for zooplankton and zoobenthos analyses.

Photosynthetic sulphur bacteria

Water column samples were taken from three depths (30 cm, 130 cm and 230 cm below the surface water level) of the advanced facultative pond where photosynthetic sulphur bacteria appeared to predominate. Representative column samples were abstracted by a Kemmerer sampler (Plate 6). Samples were serially diluted depending upon the expected population numbers and gram-stained. Gram-stained slides were then observed under oil immersion objective (100x) with low light intensity. The bacteria were then characterized on the basis of morphological characters (cell shape, cell size and arrangement, gram reaction and motility) to the genus level.

Pate 6. Column sample abstraction using a Kemmerer sampler.

5 EXPERIMENTAL RESULTS

5.1 Physico-chemical

Biochemical Oxygen Demand (BOD) removal

The variation of BOD along the sampling locations, removal efficiency, and the BOD loading rate in each reactor are given below.

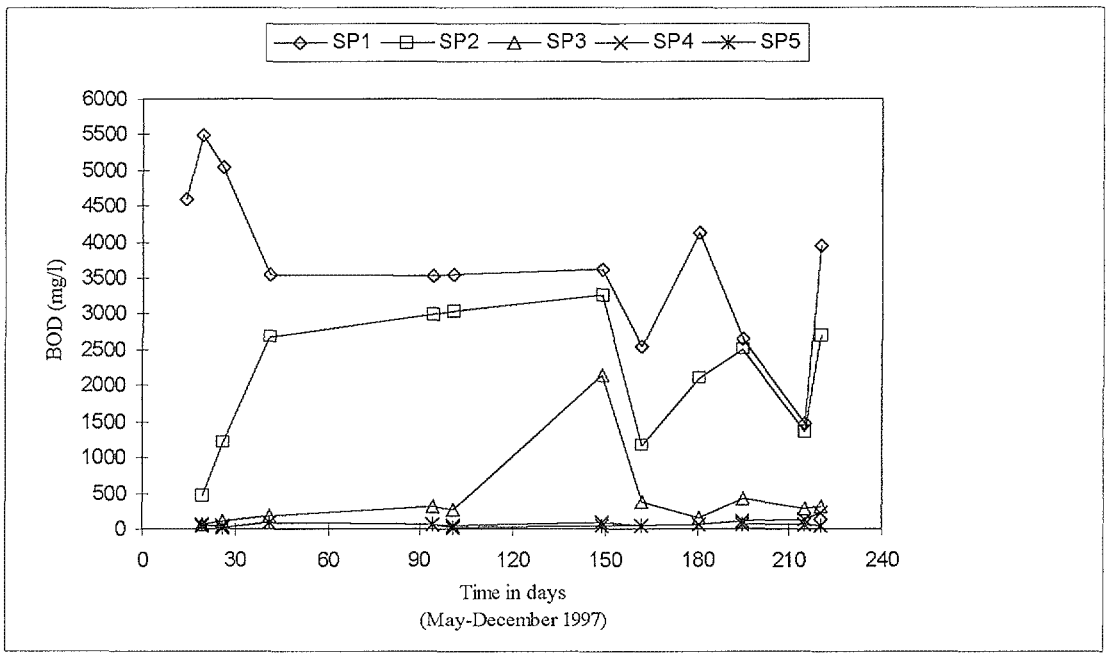


Figure 1. Variation of BOD along the sampling locations.

It is clear from Figure 1, that the variation of BOD of the raw tannery effluent (SP1) was high with values ranging from 4597 to 2552 mg/l. The variation remained high until the waste emerged from advanced facultative pond (SP3). The bio-chemical reactions in the reactors have helped to even out the variations greatly, as evidenced by nearly uniform BOD values from SP3 onwards.

Table 3. BOD removal efficiency.

Parameters	Units	Sampling Locations					Overall
		SP 1	SP 2	SP 3	SP 4	SP 5	
Mean flow rate	m^3/d	-	-	0.500	0.455	0.425	
Mean residence time	days	-	1	40	12	3	
Mean BOD	mg/l	3580	2225	250	87	52	
Percent BOD removal		0	37	88	65	40	98

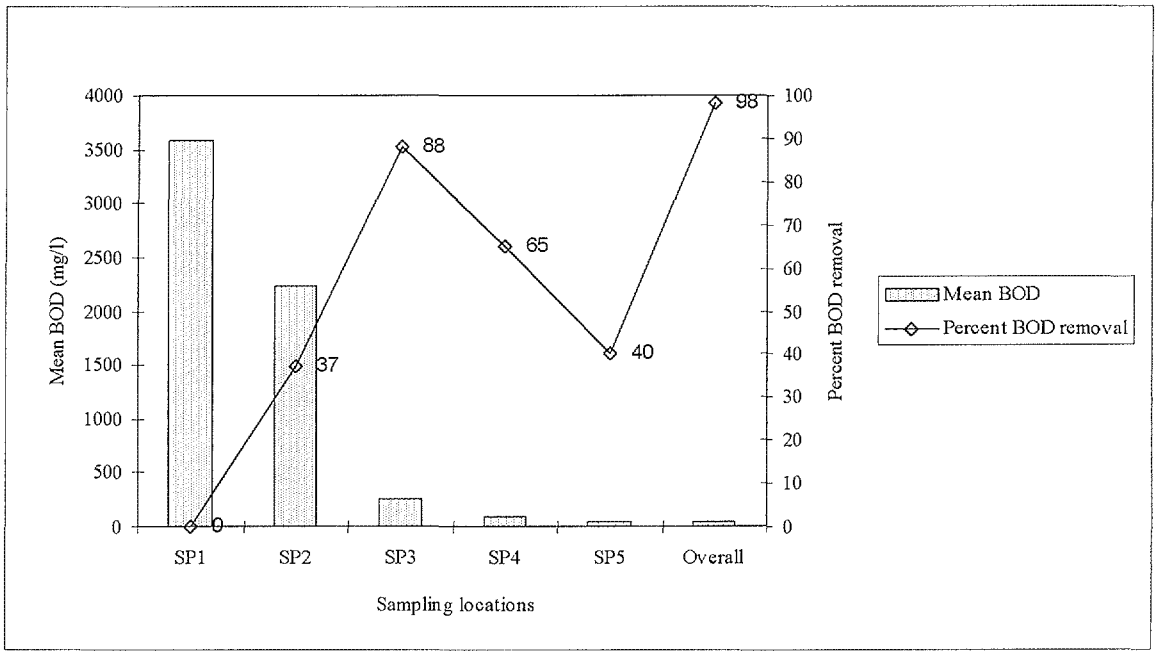


Figure 2. BOD removal efficiency.

The overall BOD removal efficiency of the system was remarkably high (98%), with influent BOD of 2225 mg/l at the inlet of advanced facultative pond reduced to less than 55 mg/l at the outlet of the maturation pond. It is also interesting to note that a one-day detention of the raw waste in sedimentation basin resulted in BOD reduction of over 30%. This generally corresponds to performances of conventional primary clarifiers. The result was largely attributed to exceptionally good settling properties of the combined tannery waste which was high in suspended solids and pH.

Much of the BOD reduction (88%) took place in an integrated anaerobic primary facultative pond. The secondary facultative pond and maturation pond attained removal efficiency of 65% and 40 %, respectively.

Table 4. BOD loading rate.

Parameters	Units	AIWPS Reactors			
		F. Pit	AFP	SFP	MP
Mean flow rate	m^3/d	0.500	0.500	0.455	0.425
Mean retention time	days	4	40	12	3
Surface BOD loading	kg/ha-d	5562	1236	228	185
Volumetric BOD loading	g/m^3-d	278	48	14	18

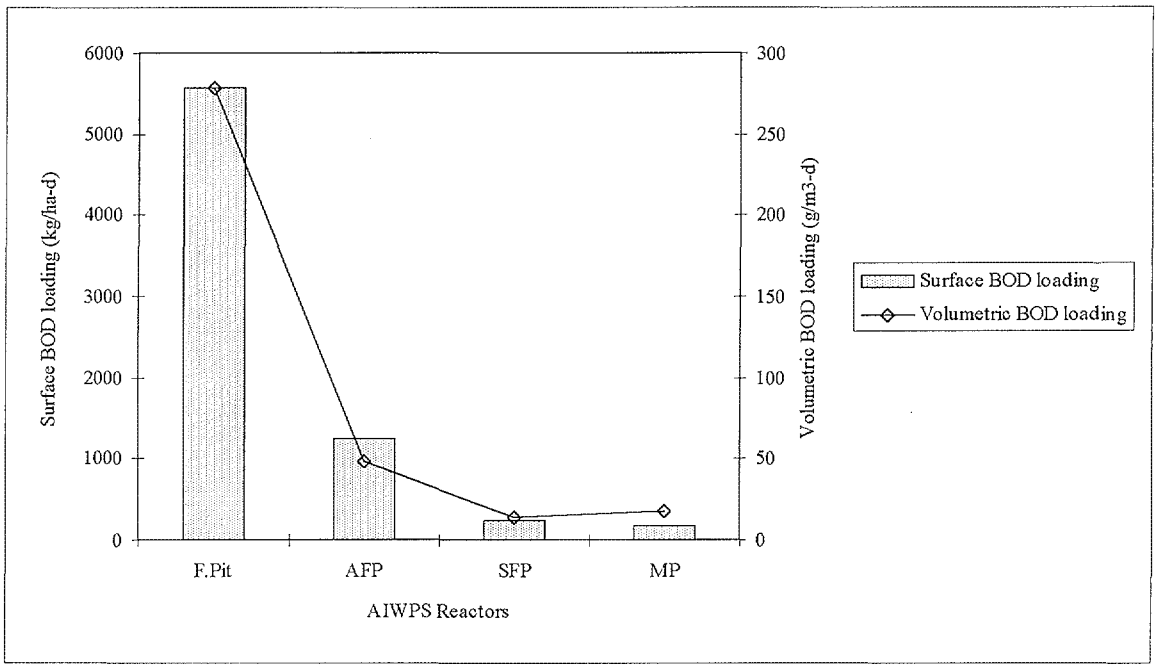


Figure 3. BOD loading rates.

For the temperatures experienced in the reactors, the surface BOD loadings of the secondary facultative and maturation ponds were well below the generally accepted permissible loading of 400 kg/ha-d. Similarly the volumetric BOD loading of integrated fermentation pit was below the permissible loading of 400 g/m^3-d set for conventional anaerobic ponds. The permissible loading rates quoted above are based on performances of lightly loaded pond systems receiving domestic sewage.

Although influent sulphate concentrations into the fermentation pit have exceeded 500 mg/l several times, no offensive odour of hydrogen sulphide was experienced during the experiment. This is a proof that the odour suppression provision incorporated in the primary facultative pond is working well.

Chemical Oxygen Demand (COD) removal

The variation of COD, removal efficiency and loading rates along the sampling locations of the reactors are given below.

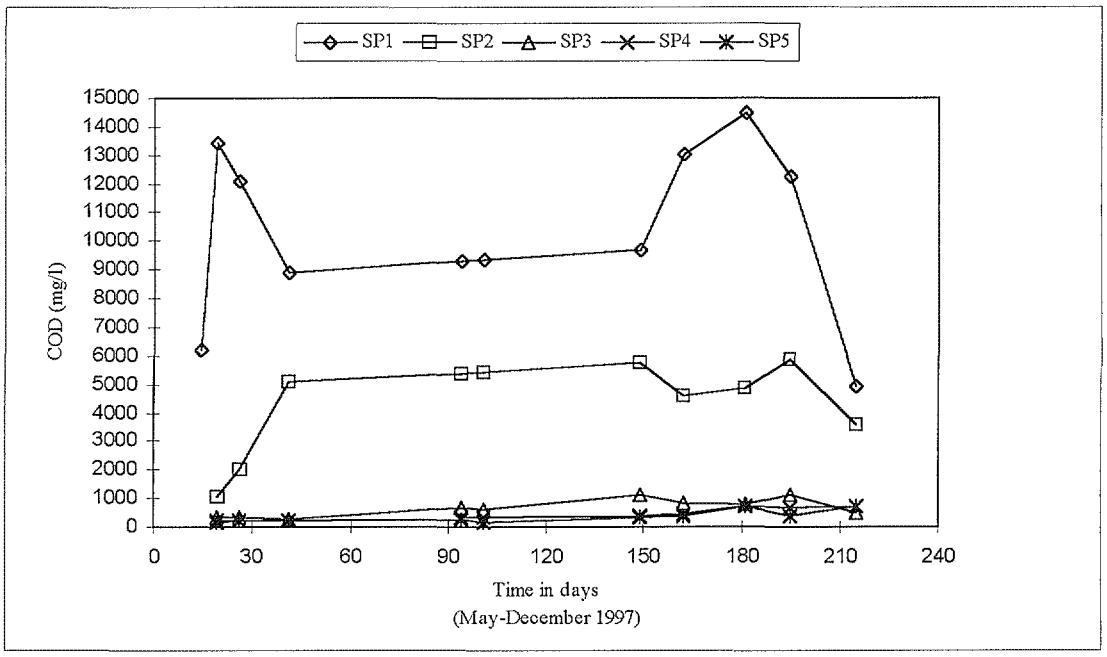


Figure 4. Variation of COD along the sampling locations.

The COD variation in the raw effluent was very high, with values ranging from 14,504 to 6,210 mg/l. Moderate variation was also noticed at the outlet of the sedimentation basin (SP2). The variation was damped out from SP3 onwards.

Table 5. COD removal efficiency.

Parameters	Units	Sampling Locations					Overall
		SP 1	SP 2	SP 3	SP 4	SP 5	
Mean flow rate	m^3/d	-	-	0.500	0.455	0.425	
Mean residence time	days	-	1	40	12	3	
Mean COD	mg/l	11,157	4,544	669	463	364	
Percent COD removal		0	60	85	31	21	97

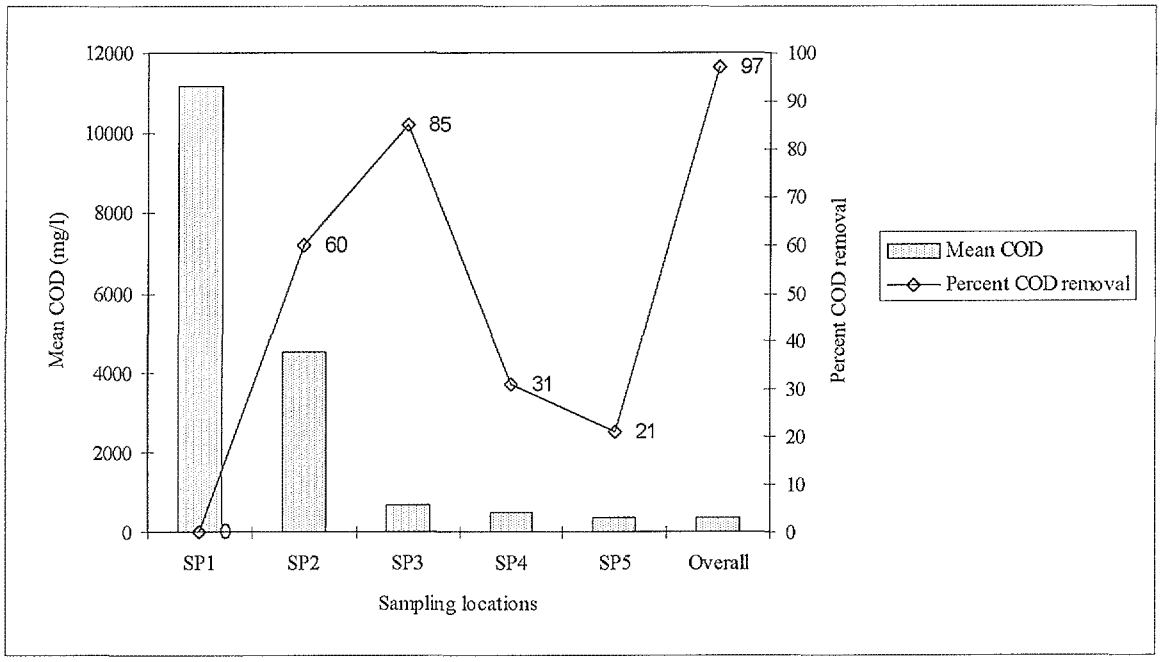


Figure 5. COD removal efficiency.

The overall COD removal efficiency of the system was high, indeed (97%), with influent COD of 4,544 mg/l at the inlet of advanced facultative pond reduced to less than 364 mg/l at the outlet of the maturation pond. The removal efficiency for COD, at one day retention time in the sedimentation basin, was higher than that achieved for BOD. This was because that much of the solids removed in the sedimentation basin were inorganic in nature. Moreover, the floating hair that contributed to much of the COD in the raw waste (SP1), was regularly skimmed off from the surface of the sedimentation basin immediatly after filling and as a result the COD in SP2 has greatly reduced. As with BOD, the highest COD removal (85%) was observed in the primary facultative pond.

Table 6. COD loading rate.

Parameters	Units	AIWPS Reactors			
		F. Pit	AFP	SFP	MP
Mean flow rate	m^3/d	0.500	0.500	0.455	0.425
Mean retention time	days	4	40	12	3
Surface COD loading	kg/ha-d	11,360	2,524	609	983
Volumetric COD loading	g/m^3-d	568	99	38	98

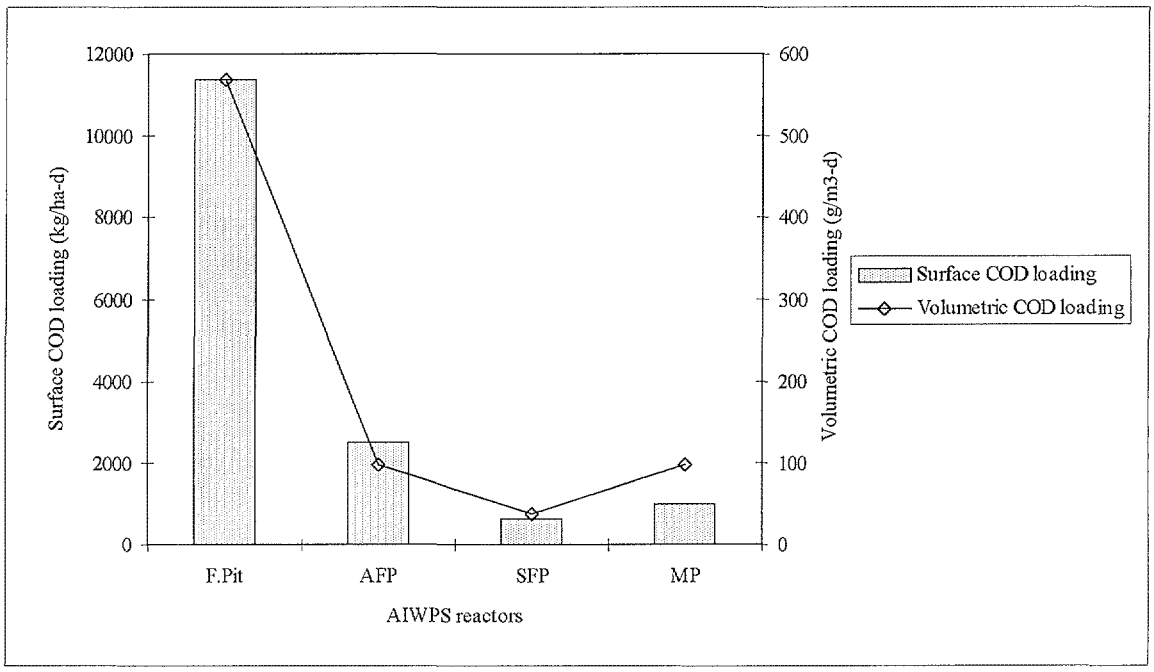


Figure 6. COD loading rate.

Solids removal

The variation of solids concentration along the reactors effluent sampling locations are shown in Figures 7-10.

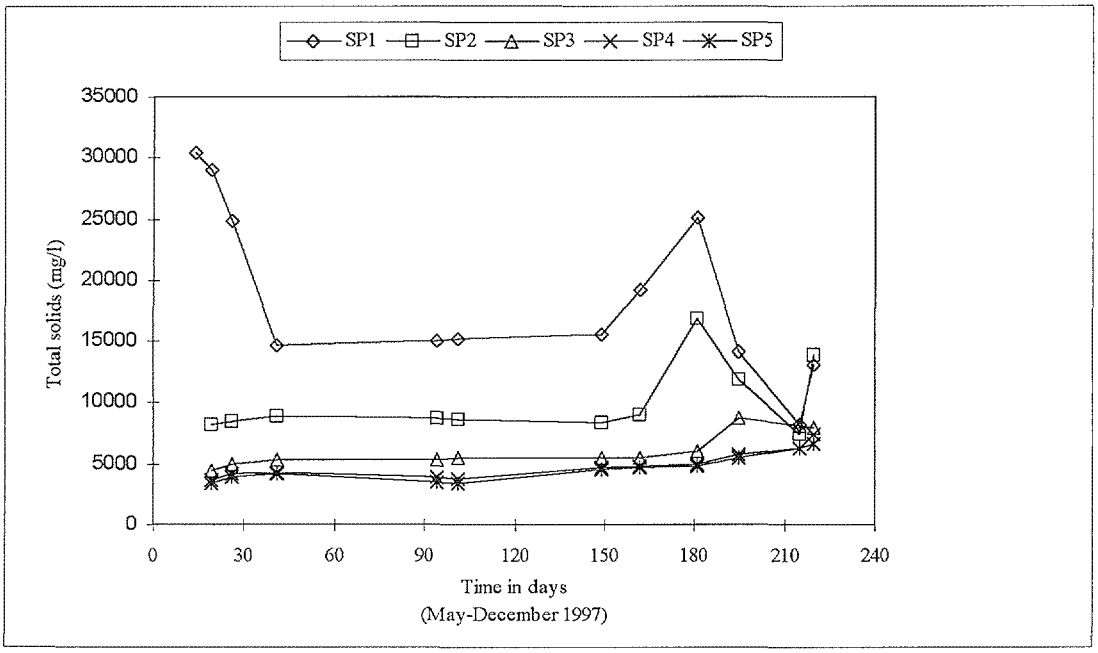


Figure 7. Variation of total solids along the sampling locations.

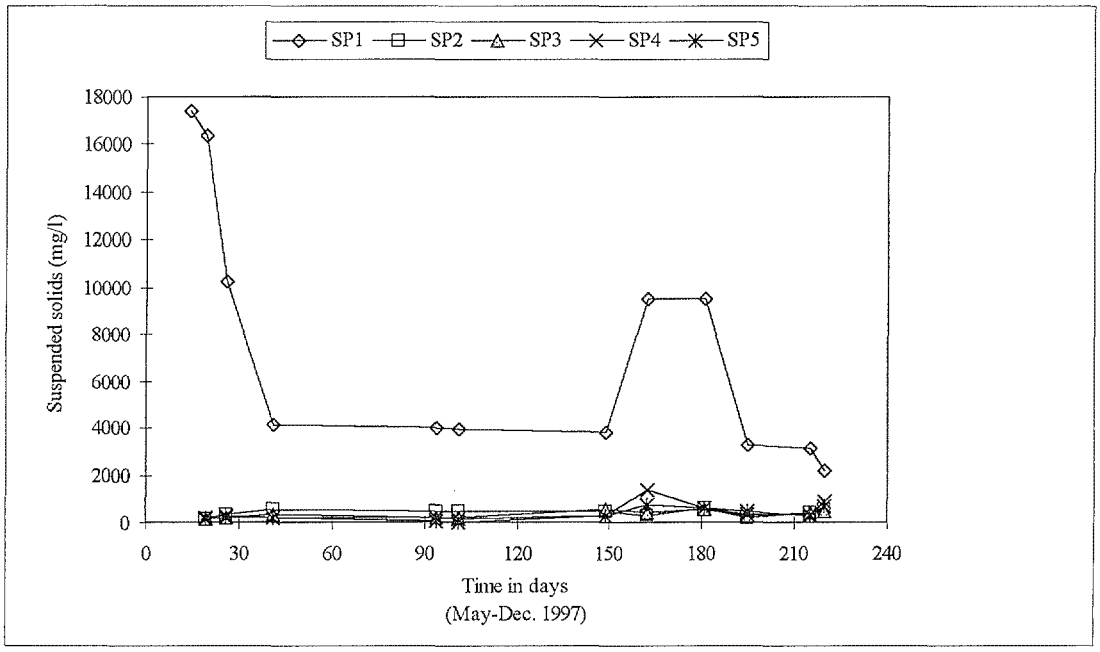


Figure 8. Variation of suspended solids along the sampling locations.

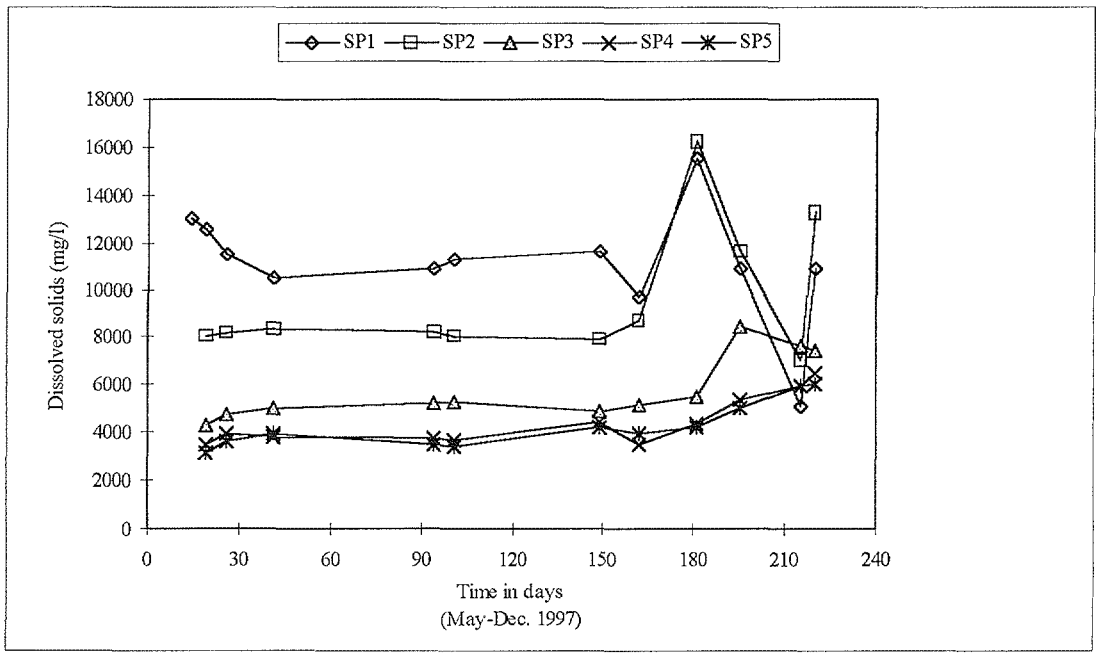


Figure 9. Variation of dissolved solids along the sampling locations.

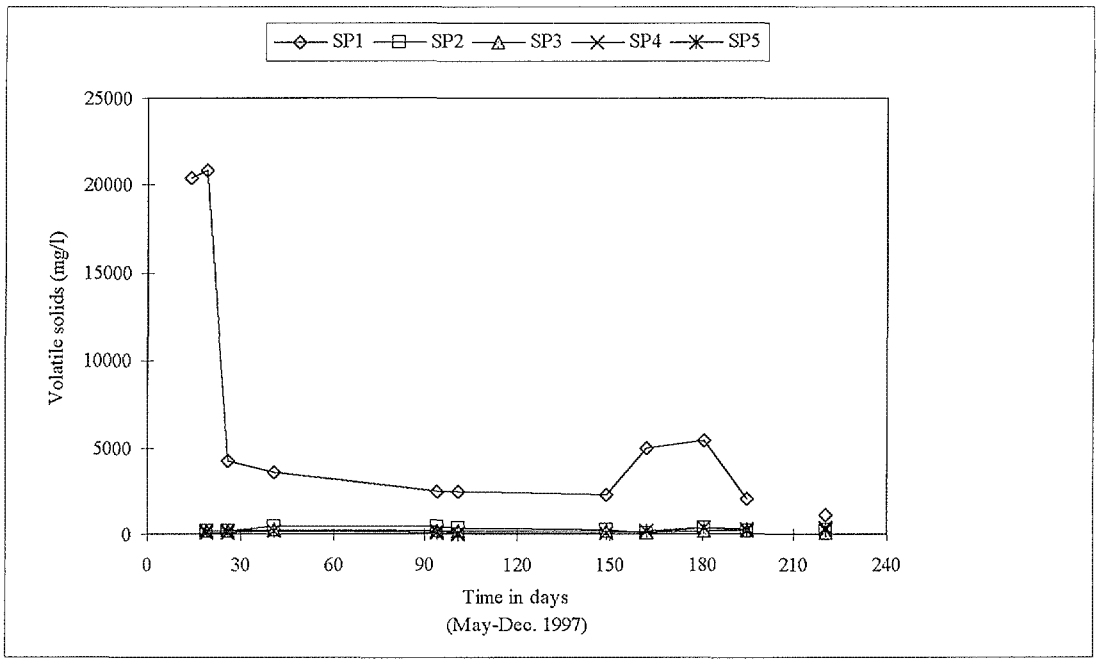


Figure 10. Variation of volatile solids along the sampling locations.

Total solids concentrations varied between 30,405-8,190, 13,850-7,435, 8,695-4,416, 7,405-3,605, 6,680-3,377 mg/l at SP1, SP2, SP3, SP4 and SP5, respectively. Suspended solids varied between 17,363-2,180, 620-166, 550-156, 1,340-125, 720-17 mg/l, dissolved solids between 15,550-5,070, 16,235-7,015, 8,415-4,260, 6,495-3,471, 6,000-3,160 mg/l and volatile solids between 20,864-1,140, 468-120, 292-110, 350-100, 360-13 at SP1, SP2, SP3, SP4 and SP5, respectively. The dissolved component of total solids was bigger than suspended as evidenced by high values of dissolved solids at all sampling locations.

Table 7. Solids removal efficiency.

Parameters	Units	Sampling Locations					Overall
		SP 1	SP 2	SP 3	SP 4	SP 5	
Mean flow rate	m^3/d	-	-	0.500	0.455	0.425	
Mean residence time	days	-	1	40	12	3	
Mean Total solids	mg/l	20,123	9,509	6,117	4,937	4,620	
% total solids removal		0	52	36	19	6	77
Mean SS	mg/l	5,071	418	339	279	386	
% SS removal		0	92	19	18	-	92
Mean dissolved solids	mg/l	11,856	9,148	5,778	4,488	4,266	
% dissolved solids removal		0	23	37	22	5	64
Mean volatile solids	mg/l	3,258	256	164	199	219	
% Volatile solids removal		0	92	36	-	-	93

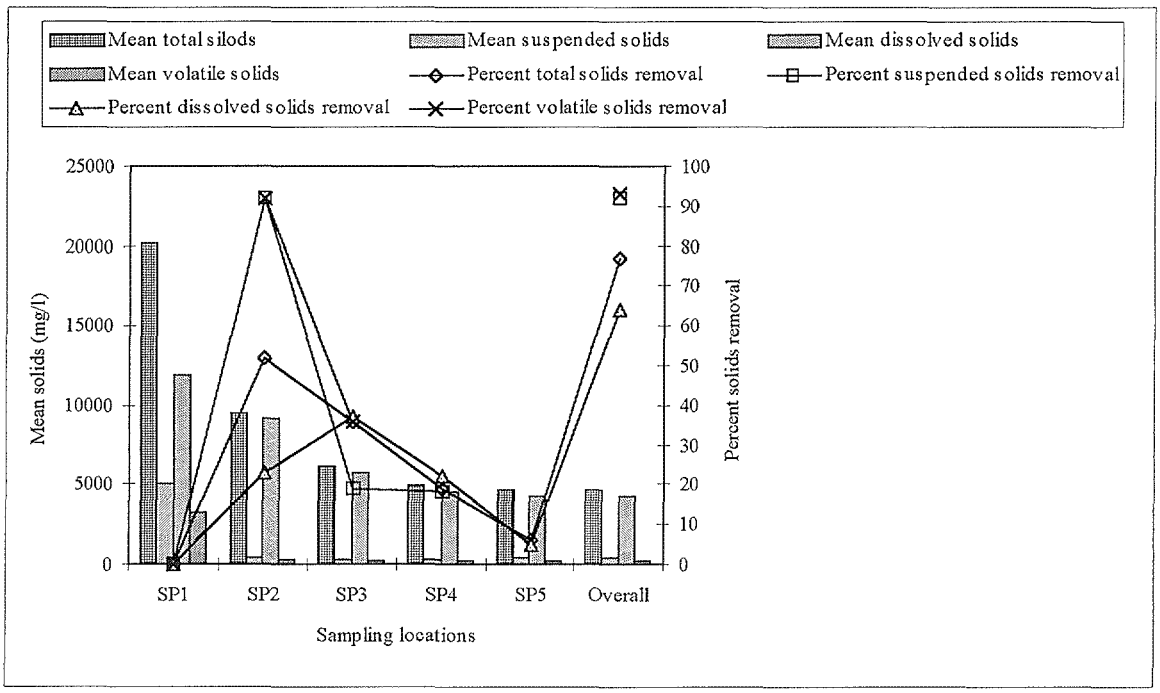


Figure 11. Solids removal efficiency.

Highest overall removal efficiencies were obtained on suspended and volatile solids, each with 92% and 93% removals, respectively. The overall dissolved solids removal is the lowest (64%), as most of the inorganic component, in particular, sodium chloride (common salt) passed directly through the reactors unchanged. It is interesting to note that almost all of the suspended solids and volatile solids were removed in the settling basins within one day detention time. This indicates the high settling characteristics of the raw combined tannery waste. The slight increases in suspended and volatile solids in the maturation pond were due to algal biomass.

Table 8. Solids loading rate.

Parameters	Units	AIWPS Reactors			
		F. Pit	AFP	SFP	MP
Volumetric total solids loading	g/m^3-d	1,189	207	348	1,049
Volumetric suspended solids loading	g/m^3-d	52	9	19	59
Volumetric dissolved solids loading	g/m^3-d	1,144	198	329	954
Volumetric volatile solids loading	g/m^3-d	32	6	9	42

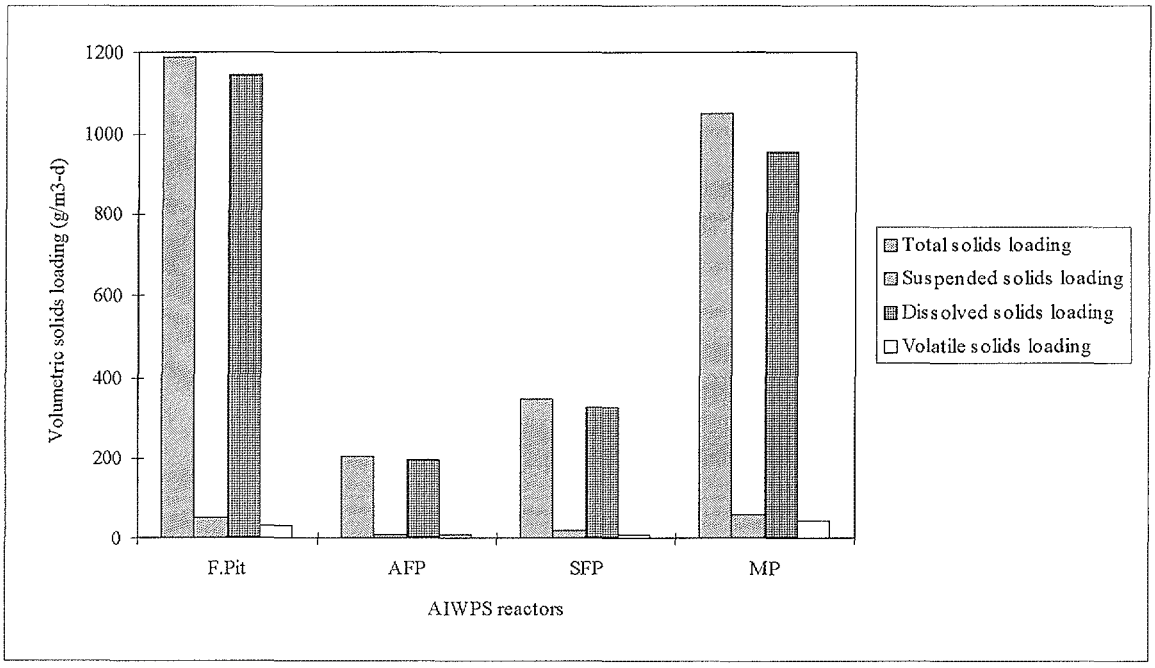


Figure 12. Solids loading rate.

The solids loading rates in the fermentation pit and maturation pond appeared nearly equal. In the fermentation pit, the high loading rate resulted from high solids concentrations. Although influent solids concentrations were the lowest in the maturation pond, its low reactor volume has contributed to higher loading rates than that in AFP and SFP.

Settleable solids in all the reactors sampling locations were negligible. Almost all settleable solids (>90%) were removed after one day detention in the settling basins.

Nitrogen removal

The two key species of dissolved nitrogen analysed in the experiment were ammonia-nitrogen and nitrate-nitrogen. The two were chosen for their importance as essential nutrients for microbial population of the rectors and their role as growth-limiting nutrients in the receiving water bodies. A complete picture of the various species of nitrogen can only be drawn through an investigation of the total dissolved nitrogen, which requires separate determination of dissolved organic nitrogen. Ammonia is a major nitrogen by-product of decomposing organic matter. Nitrate, which is derived from the nitrification of ammonia is an essential nutrient for the phytoplankton population of algal ponds. It was therefore of great importance to focus on these forms of nitrogen in the system.

The chief source of nitrogen in the tannery waste is the proteinaceous organic matter produced from the processed skin. Inorganic nitrogen, mainly ammoniacal nitrogen, can only be contributed from the deliming operations, if ammonia salts (ammonium chloride or ammonium sulphate) are used as deliming agent. The variation of ammonia and nitrate and their removal efficiencies are given in Figures 13-15.

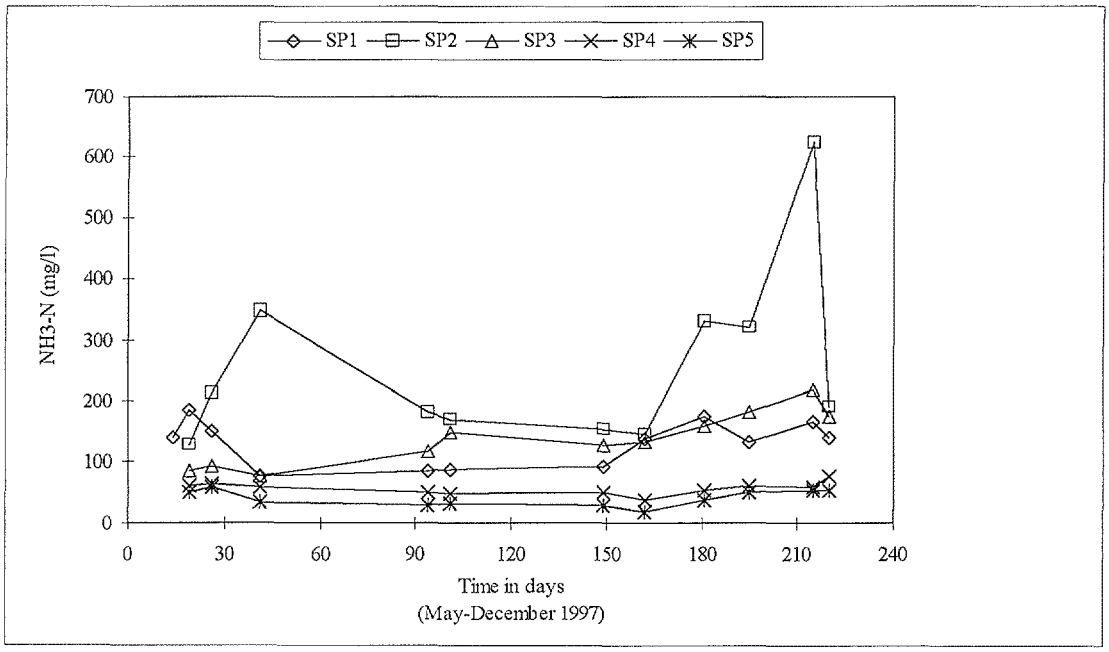


Figure 13. Variation of NH_3-N along the sampling locations.

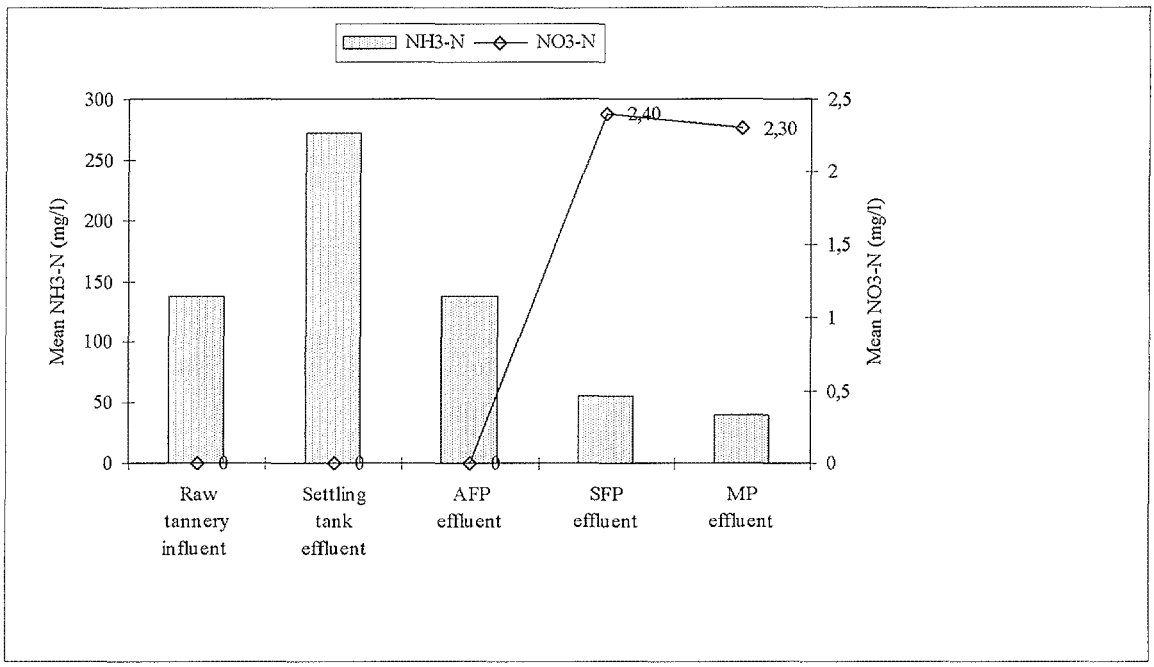


Figure 14. Mean NH_3-N and NO_3-N concentrations along the reactors effluents.

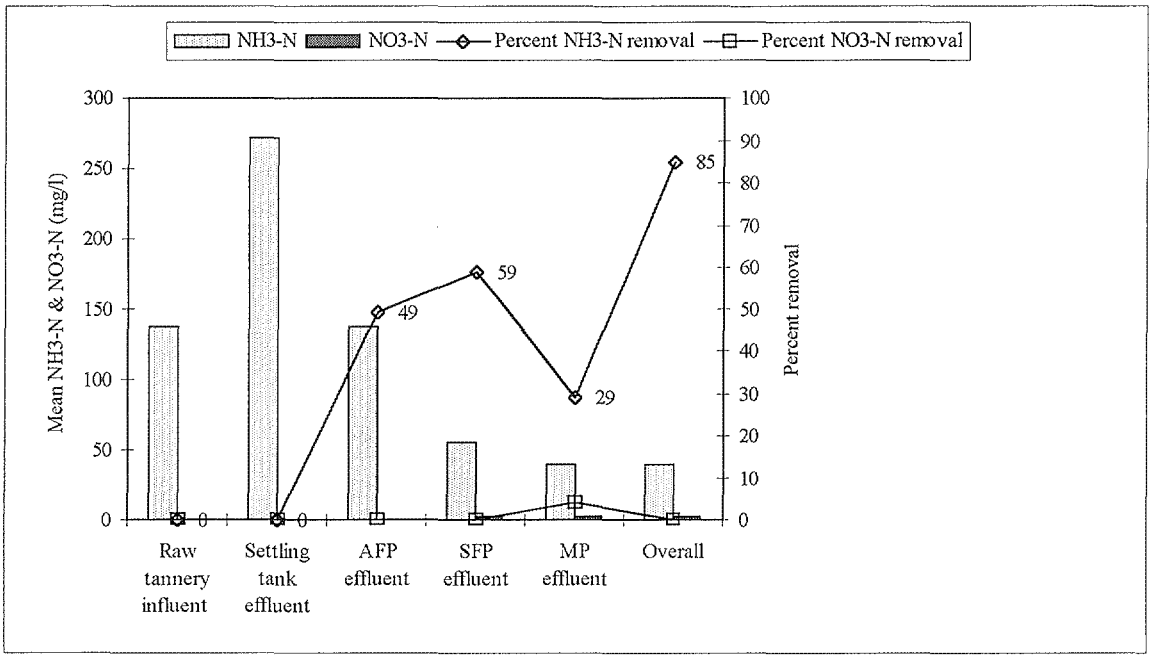


Figure 15. NH_3-N and NO_3-N removal efficiencies.

An overall ammonia-nitrogen removal efficiency of 85% was achieved in the system. This was quite satisfactory. The mechanisms by which nitrogen removal could be achieved are:

(i) Gaseous ammonia stripping to the atmosphere
(ii) Ammonia assimilation in algal biomass, and
(iii) Biological nitrification-denitrification

The experimental results depicted in the above charts are interesting and some explanation seems in order. Ammonia concentration has nearly doubled in the settling tank. This was something unexpected. It was unlikely that much of the organic matter decomposed in one day to generate as much twice ammonia. A Possible explanation for the increase would be ammonia released from the sludge at the bottom of the tank. The sludge was intentionally left to accumulate for some time to effect pre-acidification in the settling tank. When highly alkaline tannery waste (pH as high 11) was suddenly introduced into the tank ammonia trapped in the sediment began to emerge and increased its concentration in the overlying water column. This was what was possibly recorded in the tank's effluent sampling location, SP2.

Ammonia from the settling tank was reduced by nearly half in the advanced facultative pond (AFP). This was rather high reduction. Ammonia stripping could not be a possible reason for the reduction, as the reactor pH rarely exceeded above 8. At this pH, only 5% of the total dissolved ammonia is in a gaseous form and much remained in the solution as an ionized ammonia (NH_4^+). The environmental conditions (near to zero DO and low redox potential) prevailing in the fermentation pit do not either warrant autotrophic nitrification. It is clear from Figure 14 that autotrophic nitrification occurred in aerobic secondary facultative pond (SFP). So, ammonia reduction in AFP might be due to some form of heterotrophic nitrification-denitrification. But, the real puzzle here is how could nitrite and nitrate be formed in the first place under complete anoxic condition of the fermentation pit. Some investigators (Bergerova, 1975) have, however, demonstrated that heterotrophic nitrification could continue, even though small, at concentration of 0.3 mg O_2/l. This could have been ascertained from evolved N_2 of the biogas, had there been a gas chromatography to analyse the biogas.

The highest ammonia reduction in the system (59%) took place in the secondary facultative pond, where autotrophic nitrification was also occurred. The amount of nitrogen destroyed in the secondary facultative pond was about 81 mg NH_3-N/l. When this is compared with 2.4 mg NO_3^--N/l produced in the same reactor, the rate of nitrification was very low, indeed. So, the 59% ammonia reduction achieved in the secondary facultative pond may largely be due to photosynthetic assimilation and utilization of ammonia by algae. Most fast growing algae have a higher preference to ammonia than to nitrate as a nitrogen source. This was evidenced by the very low reduction of nitrate (only < 5%) in the maturation pond.

Chromium removal

Chromium waste liquor was comparatively small as the tannery's finished leather out put was less than 5% of the total. The flow of chromium waste into the treatment system was erratic and at times chromium was absent in the feed. Chromium sulphate ($Cr_2(SO_4)_3$) is the sole mineral tannage used invariably in all the tanneries including Modjo Tannery. Thus the resultant chromium in the effluent is in trivalent form. It's variations and removal efficiencies along the sampling locations is given below.

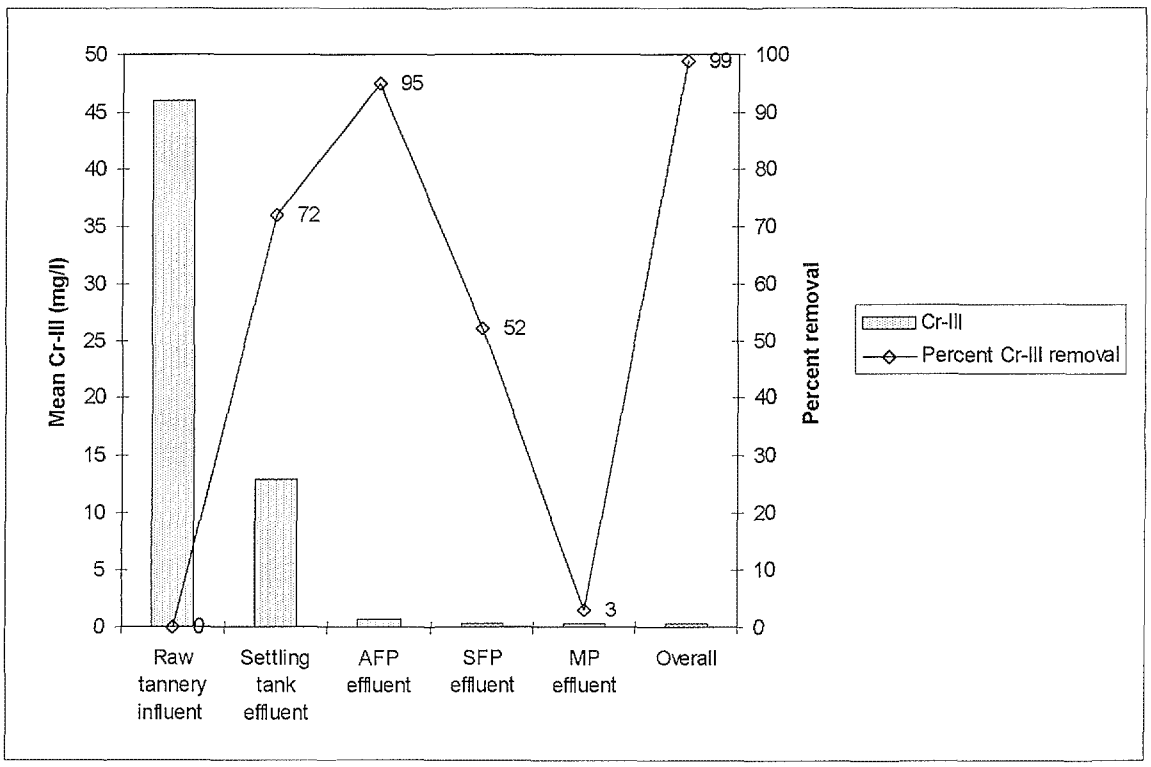


Figure 16. Cr-III removal efficiencies.

As shown in Figure 16, an overall Cr-III removal of 99% was attained in the treatment system. The removal efficiency of 72% achieved in the settling basins was due to precipitation enhanced by lime induced pH (10.5-11) of the raw waste. Under such pH, chromium is precipitated as chromium hydroxide and remains trapped in the sludge until the pH drops below 6. A 95% chromium removal was obtained in advanced facultative pond. Such remarkable removal may largely be the result of chromium precipitation with the sulphide generated in anaerobic fermentation pit. The phenomenon was confirmed by Haas and Polprasert, 1993 in their investigative work on heavy metal removal by sulphide generated from anaerobic digestion processes. The remaining removal (52% in the secondary facultative pond) could be explained more on the basis of pH enhanced precipitation than on sulphide precipitation as metal sulphide. The pH in the secondary facultative pond could easily fluctuate between 8.9 and 10 during peak photosynthesis. A 0.31 mg/l residue chromium in the final effluent was much lower than most stringent effluent standards that set a 1 mg/l discharge limit on trivalent chromium.

Sulphate and sulphide removals

Sulphate is one of the major constituents of tannery wastes. It is largely contributed from the deliming operations using ammonium sulphate, pickling using sulphuric acid and tanning using chromium sulphate. Inorganic sulphides originate from unhairing and dewooling operations using depilatory chemicals such as sodium sulphide, sodium hydrosulphide or calcium hydrosulphide. Besides the inorganic sulphides, sulphide is also fromed biologically in the treatment system through reduction of inorganic sulphate or from decomposition of organic matter where the assimilated sulphur is reduced to hydrogen sulphide. The removal of these forms of sulphur in a biological treatment may be achieved through the following mechanisms:

- precipitation as insoluble metal sulphides or combination with other complexing substances
- volatilization as gaseous hydrogen sulphide
- biota assimilation and/or extracellular deposition of free sulphur

The variation and removal efficiencies of sulphate and sulphide are given in Figures 17-19.

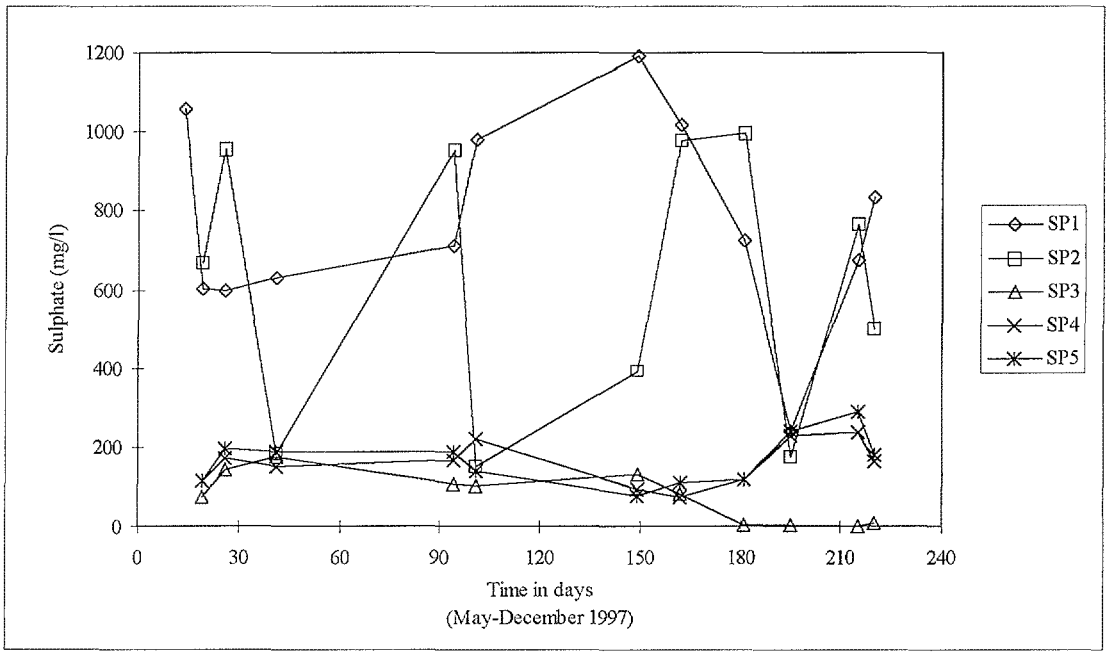


Figure 17. Sulphide variations along the reactors sampling locations.

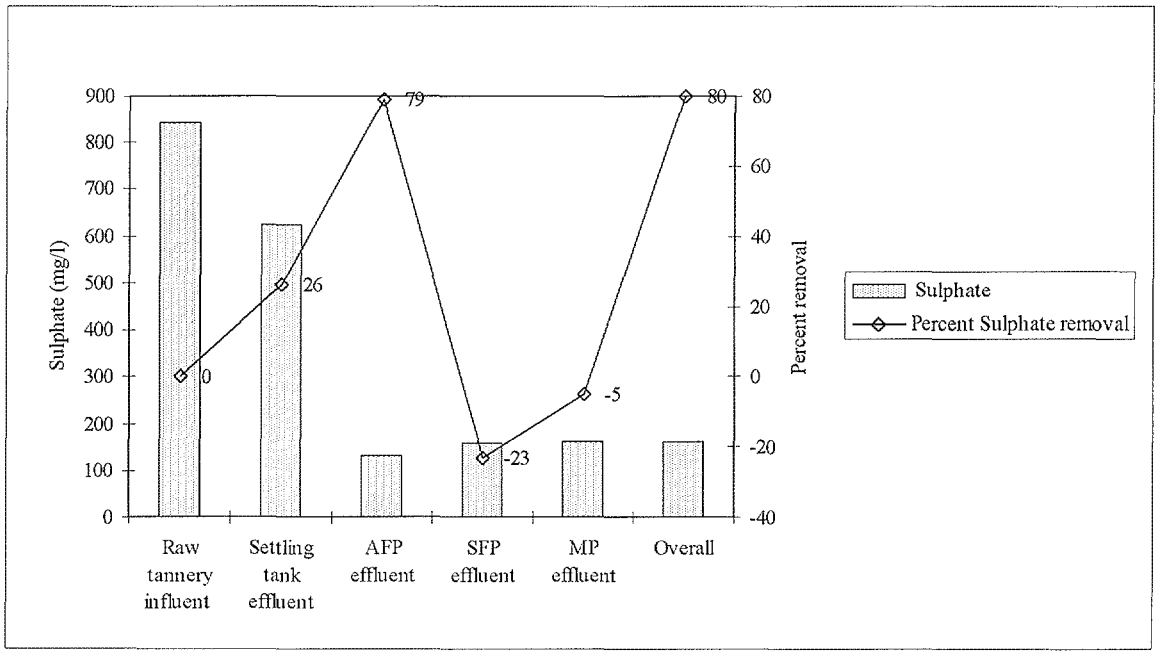


Figure 18. Sulphate removal efficiencies.

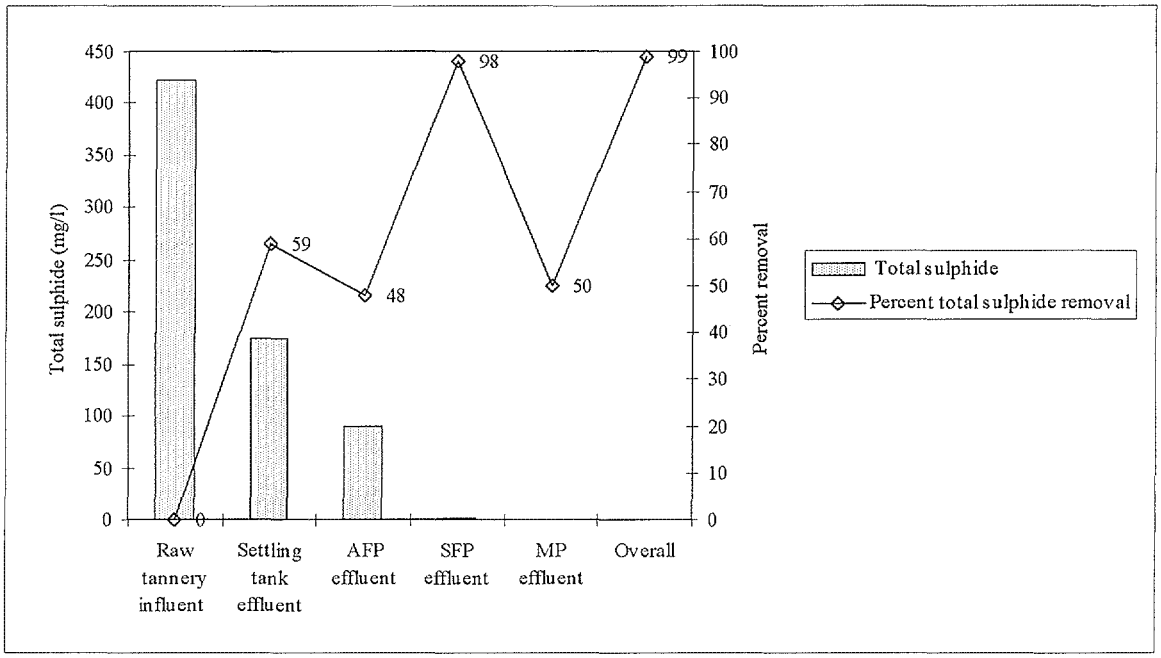


Figure 19. Sulphide removal efficiencies

The tannery waste was rich in sulphate being 840 mg/l on average. The sulphate concentration in the raw feed ranged from 1,190 to 240 mg/l and the system achieved a removal efficiency of 80%. As expected the highest sulphate removal (79%) was observed in the anaerobic fermentation pit (Figure 18). In the fermentation pit sulphate was reduced into sulphide.

The huge sulphate reduction in the fermentation pit did not, however, increase the sulphide concentration in AFP (Figure 19) instead a decline in sulphide took place from influent concentration of 174 mg/l to effluent concentration of 90 mg/l. Possible explanation for the rapid loss of sulphide is that sulphide was either oxidized into elemental sulphur by photosynthetic sulphur bacteria or immediately precipitated into the bottom sludge as metal sulphide. These are plausible explanations, because the surface of AFP was always covered with thick pale yellow scum (Plate 7) which was tested to be largely sulphur and the highest chromium removal (95%) occurred in the same reactor (Figure 16). The scum was scraped from the surface on daily basis while insoluble metal sulphide was immobilized in the retained sludge.

Emission of hydrogen sulphide could not be a possible pathway for sulphide removal, because at the pH levels exprienced in AFP (7.2-8.2) more than 90% of the sulphides are in ionic forms (HS^- & $S^{=}$). Of course, some sulphide may have been transferred from AFP to the successive ponds.

A number of sulphur bacteria were found responsible for the oxidation of sulphide first to elementary sulphur and then to sulphate. These include Thiobacillus, Thiocystis and Rhodopseudomonas of which Thiocystis were the most dominant. Photosynthetic purple bacteria were so dense, in the AFP, that they turned the reactor water reddish brown in early morning hours and red-pink in late afternoon (Plate 8). These bacteria played a major role in eliminating the obnoxious odour due to hydrogen sulphide.

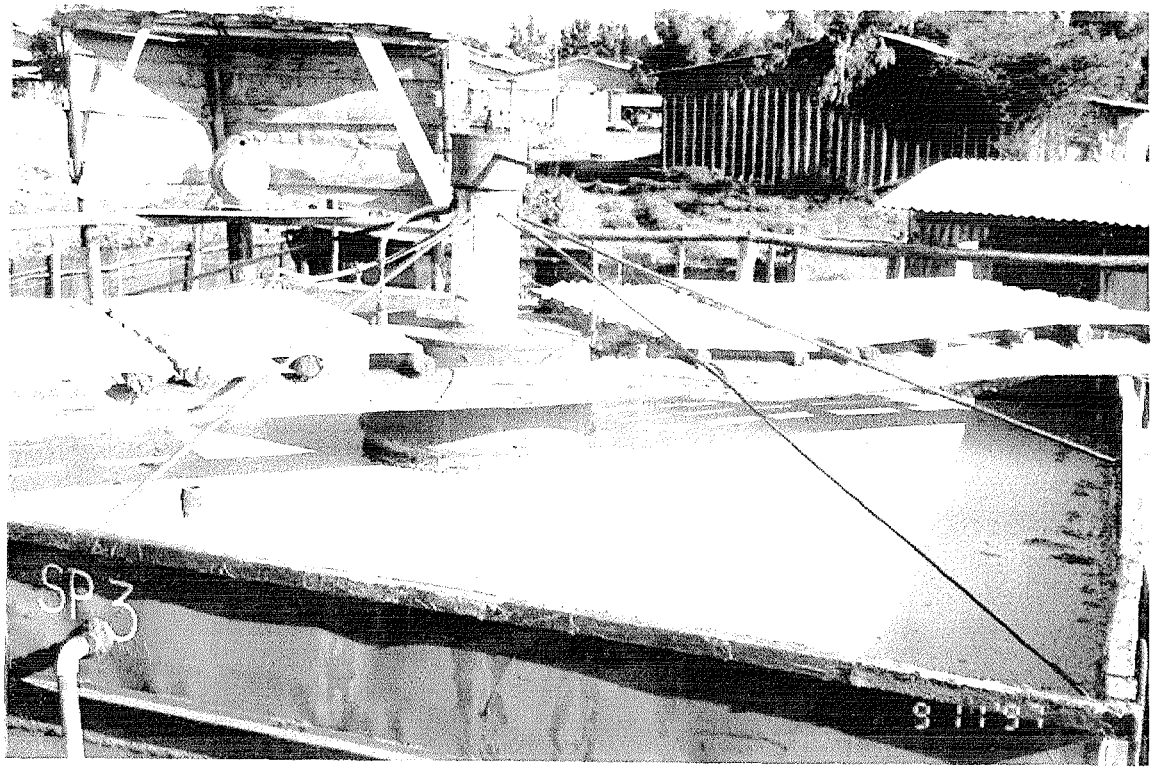


Plate 7. A white-yellow sulphur deposition on the surface of AFP at early hours of the morning.

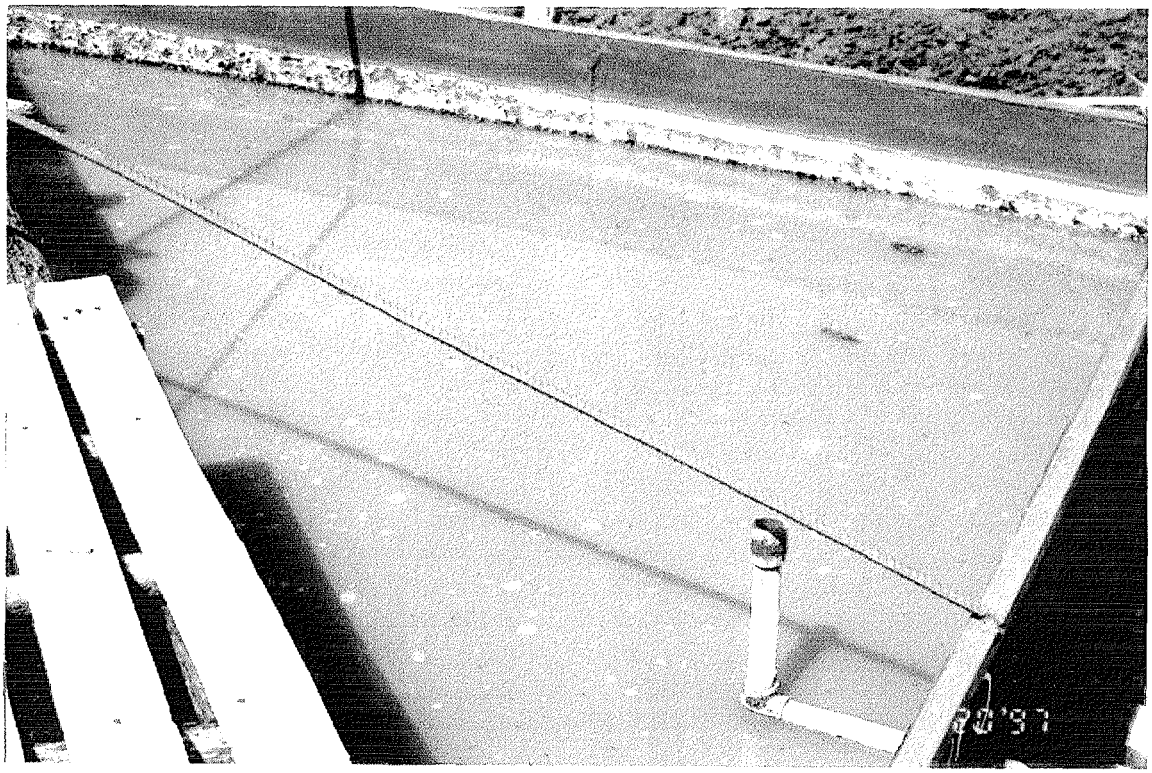


Plate 8. Proliferation of photosynthetic purple sulphur bacteria turned the surface water of AFP reddish-brown at early hours of the morning.

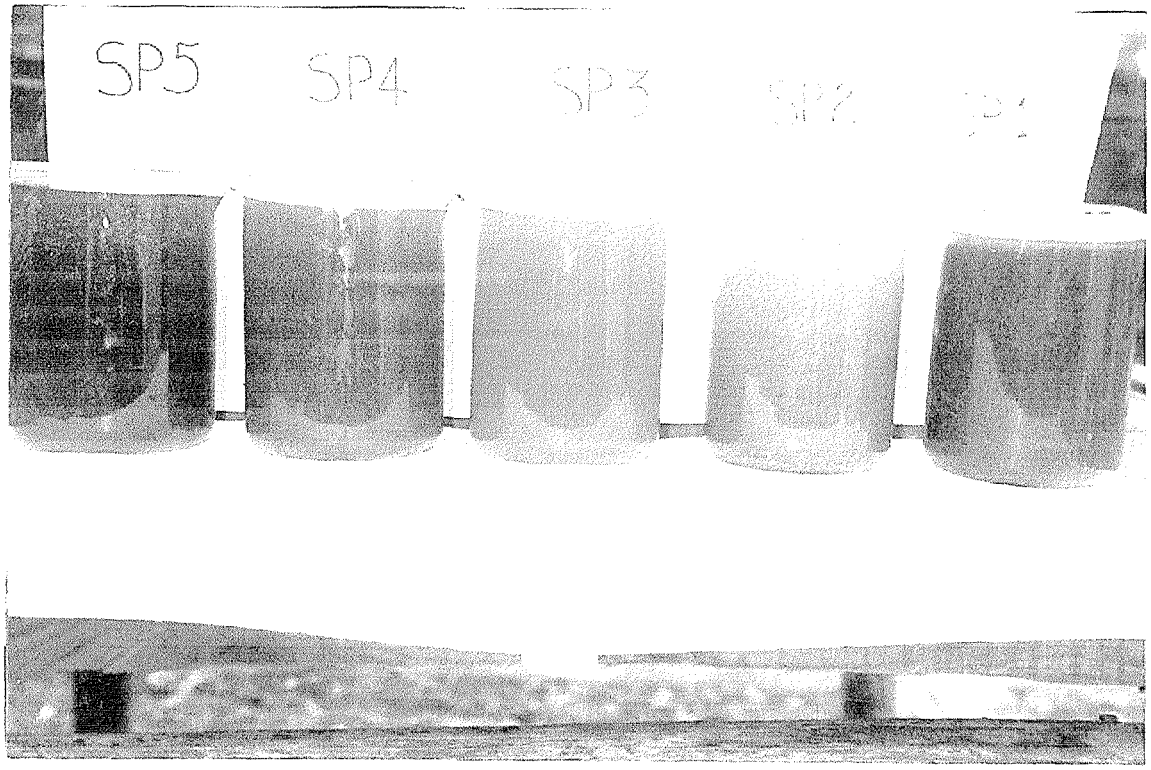


Plate 9. Coloration of influent and effluent samples. Please note the typical red-pink colour of photosynthetic purple sulphur bacteria in the effluent samples of AFP. Samples were taken early afternoon.

A very striking result is the highest removal of sulphide (98%) that took place in the secondary facultative pond (Figure 19). This huge removal of sulphide was accompanied by an increase in sulphate concentration in the same reactor. The levels of sulphate in the secondary facultative and maturation ponds were higher than that in the preceding anaerobic pond. A total of 28% increase in sulphate (23% in SFP and 5% in MP) was observed in the two algal ponds (Figure 18). A plausible explanation for this is that excess unoxidized sulphide moved from anaerobic pond into the secondary facultative and maturation ponds and oxidized there into sulphate. Oxygen levels in the two algal ponds were adequate to effect such oxidation.

Emission of Hydrogen sulphide was also monitored and the results are presented below.

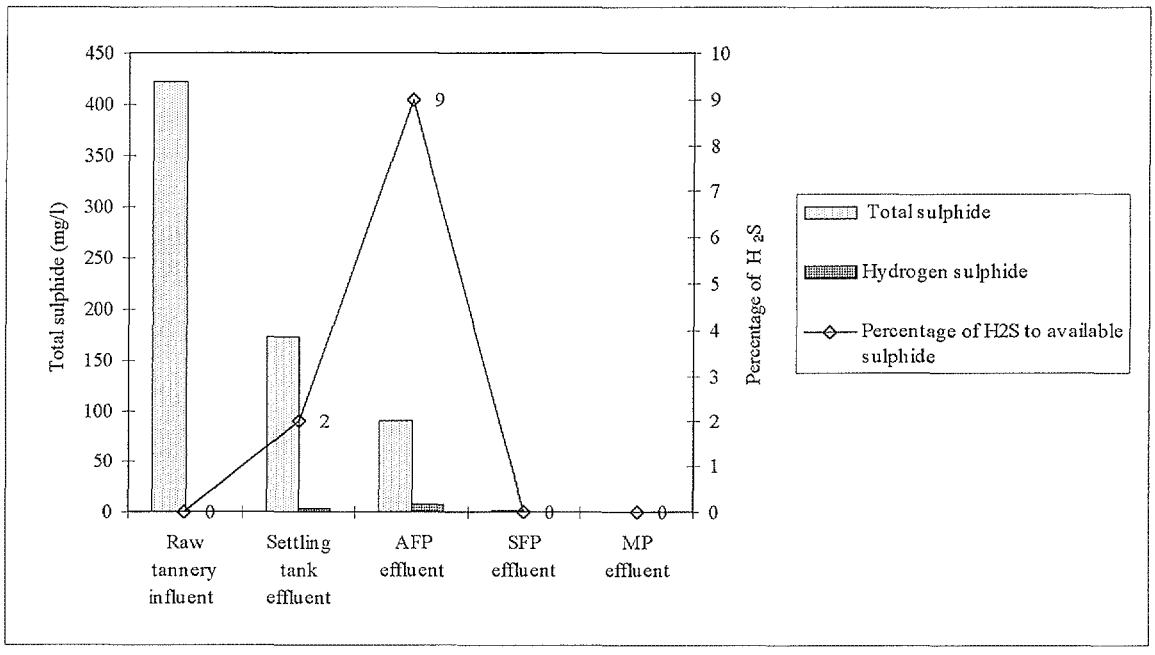


Figure 20. Proportions of H_2S to total sulphide.

As shown in Figure 20, the formation of hydrogen sulphide was insignificant to cause a real odour problem. The percentage of hydrogen sulphide to total sulphide in AFP was 9%. This is in agreement with the hydrogen sulphide normally formed at the reactors pH of 7.9-8.2.

Total phosphorus removal

Generally the use of phosphorus based chemicals are limited in leather tanning, and as such their concentrations in the effluent are low. But, in our investigations we found high concentration of phosphorus (65-20 mg/l as total P) in the tannery waste. Possible sources for such high concentrations could be raw skin washings and general cleaning activities using strong synthetic detergents and surfactants. The variation of total phosphorus and its removal efficiencies in the treatment system is give in Figures 21.

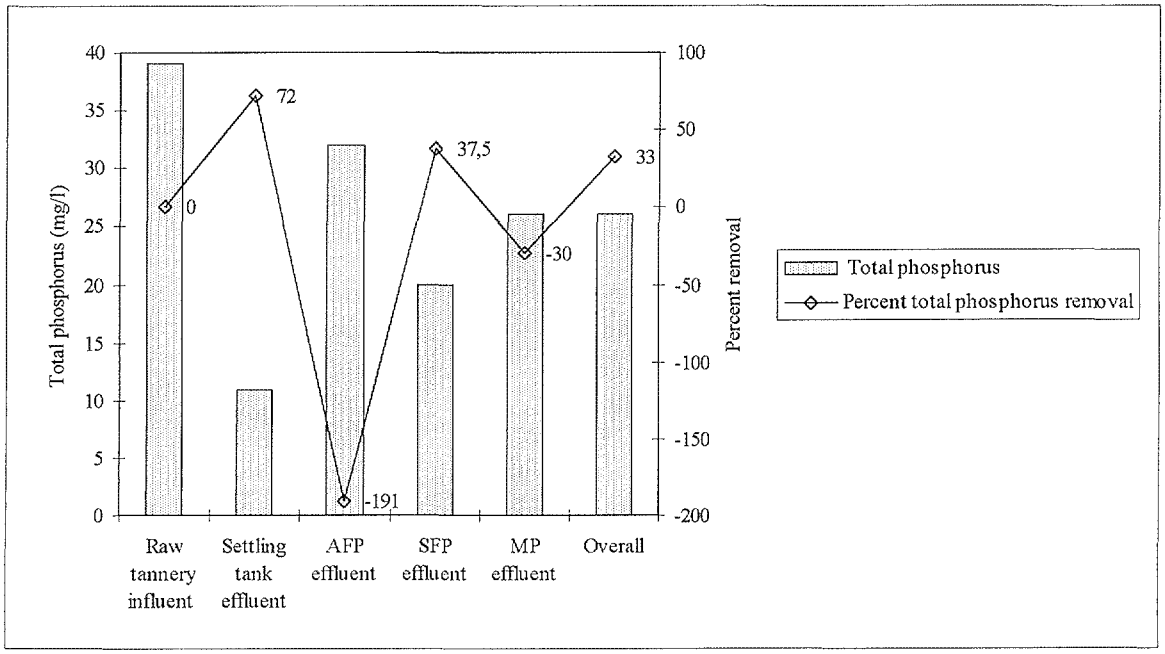


Figure 21. Total phosphorus removal.

As can be seen from Figure 21, the performance of the system in terms of total phosphorus removal was highly erratic. The highest removal (72%) took place, as expected, in the settling tanks where the pH in the tank varied between 10.5-11-5. At such high pH and in the presence of lime, soluble phosphate can precipitate as insoluble calcium hydroxyapatite, $Ca_5OH(PO_4)_3$. Adsorption to settleable solids may also account for high phosphate removal in the settling tanks. The total phosphorus concentration has more than doubled in the advanced facultative pond. This partly may be the result of hydrolysis of condensed phosphates and organically bound phosphorus to soluble phosphates. Although it is certain that phosphate is reduced to phosphine (PH_3) gas in the anaerobic pit, this process did not result in lowering the concentration of phosphate. As to why phosphate reduction did not result in lower phosphorus in the AFP, needs further investigation.

The 37% reduction obtained in secondary facultative pond is rather high and the result is comparable with the luxury uptake of micro-organisms in some efficient bioreactors, namely activated sludge processes. The overall removal efficiency of 33% is low. This is not, however, unique to this treatment system. Except for the amount that is removed by precipitation and that taken up by micro-organisms, the removal of phosphorus achieved in all conventional biological treatment methods is minimal. In general, phosphorus removal in terms of microbial uptake is low compared to nitrogen and carbon removals. Our finding which is in conformity with this generalization is shown in Figure 22.

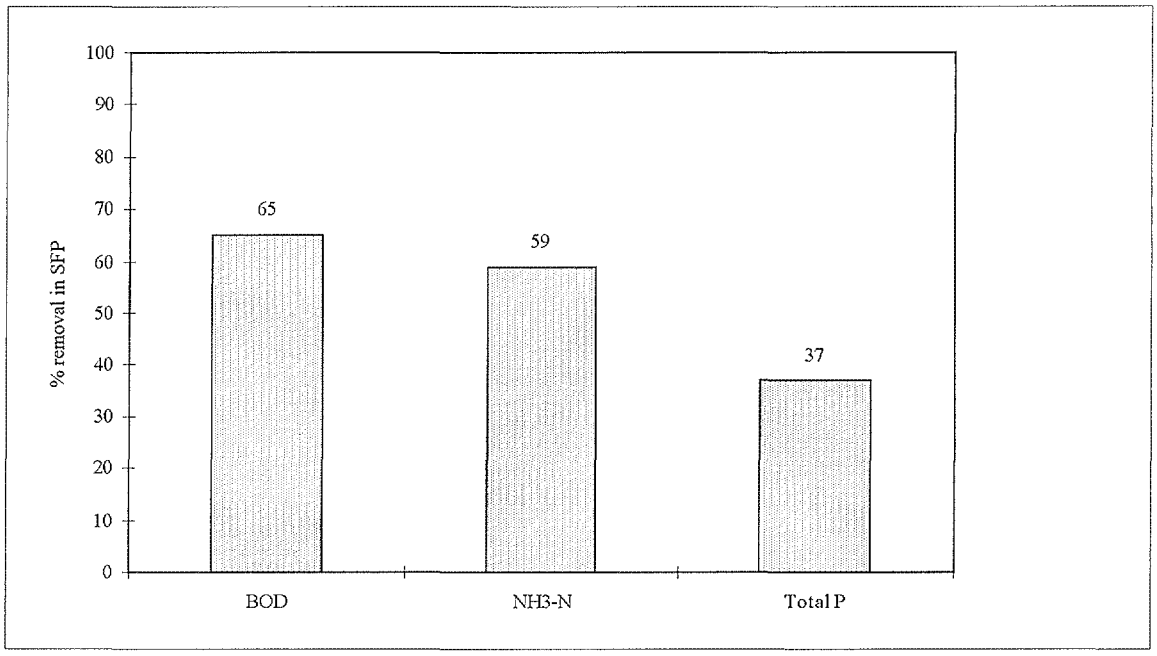


Figure 22. Comparison between microbial removals of essential nutrients in secondary facultative pond.

Chloride & sodium chloride removals

The performance of the system in terms of chloride and common salt (NaCl) removals is given in Figure 23.

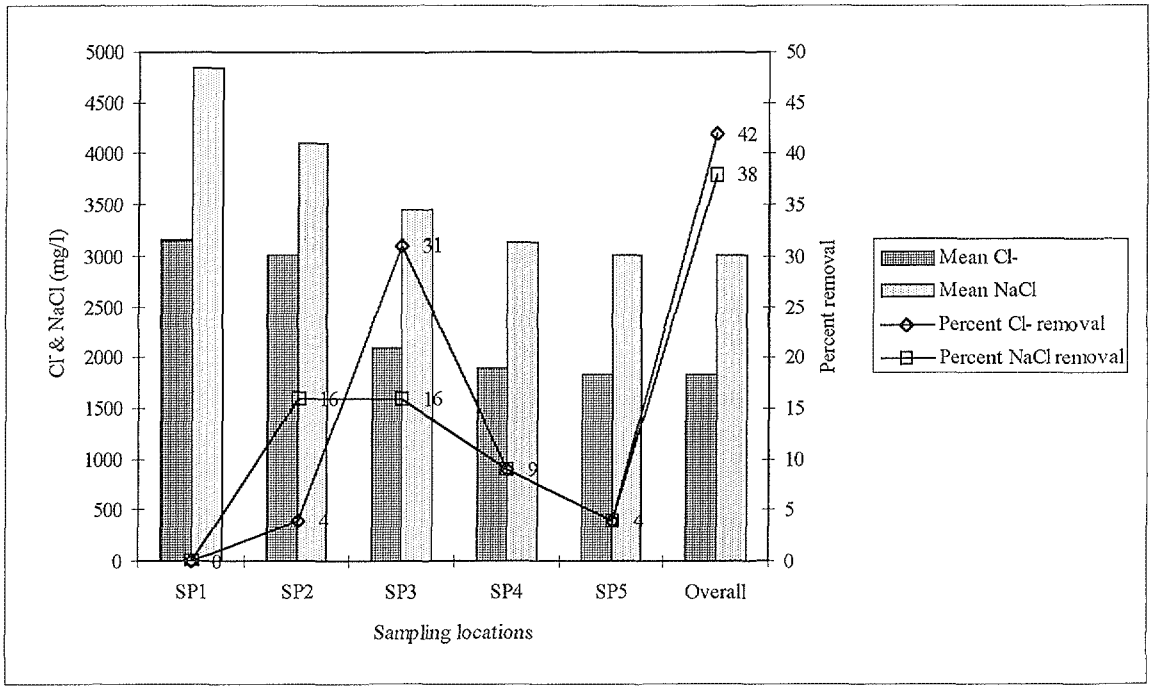


Figure 23. Removals of chloride and sodium chloride.

As expected the removals of chloride and sodium chloride are moderate with overall reductions of 42% and 38% for chloride and sodium, respectively. The variation of electrical conductivity along the reactors is given in Figure 24.

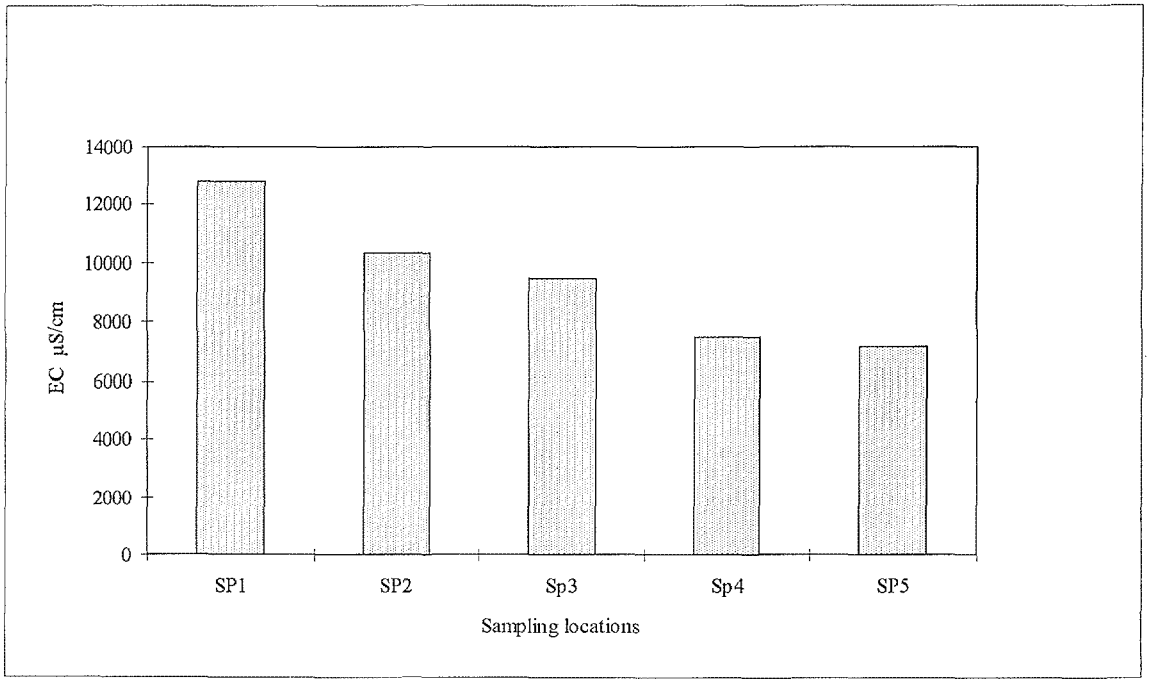


Figure 24. Variation of electrical conductivity along the reactors.

Mid-depth temperatures

Mid-depth temperature was taken on daily basis by maximum-minimum thermometer suspended at mid-depth of each reactor. The thermometers were suspended at a depth of 0.5m, 0.75m and 1.15m in the MP, SFP and AFP, respectively. Air temperature was also recorded by a maximum-minimum temperature hang at platform 2.75m above ground. The temperature variations in air and each reactor are given in Figures 25-28.

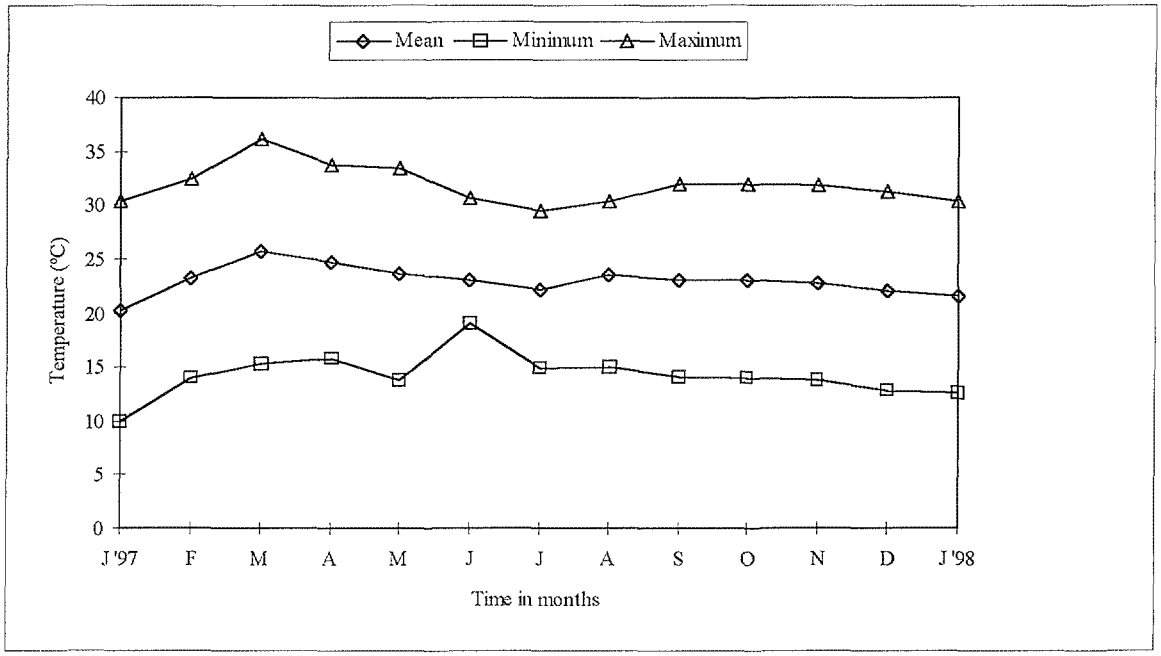


Figure 25. Air temperature variations.

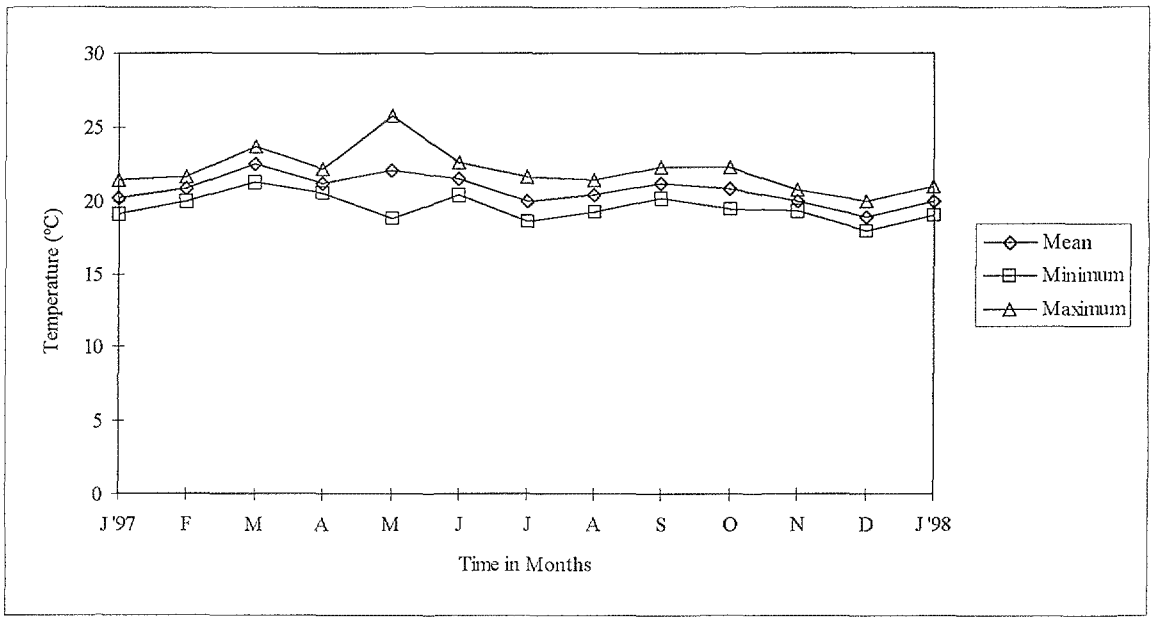


Figure 26. Mid-depth temperature variations in AFP.

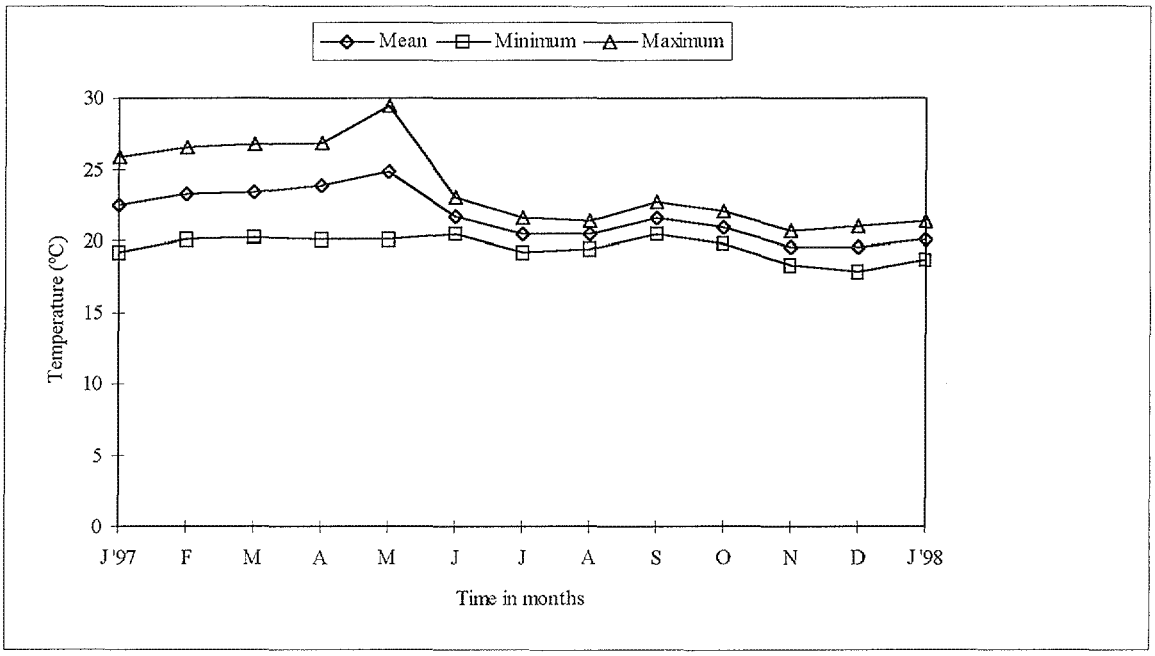


Figure 27. Mid-depth temperature variations in SFP.

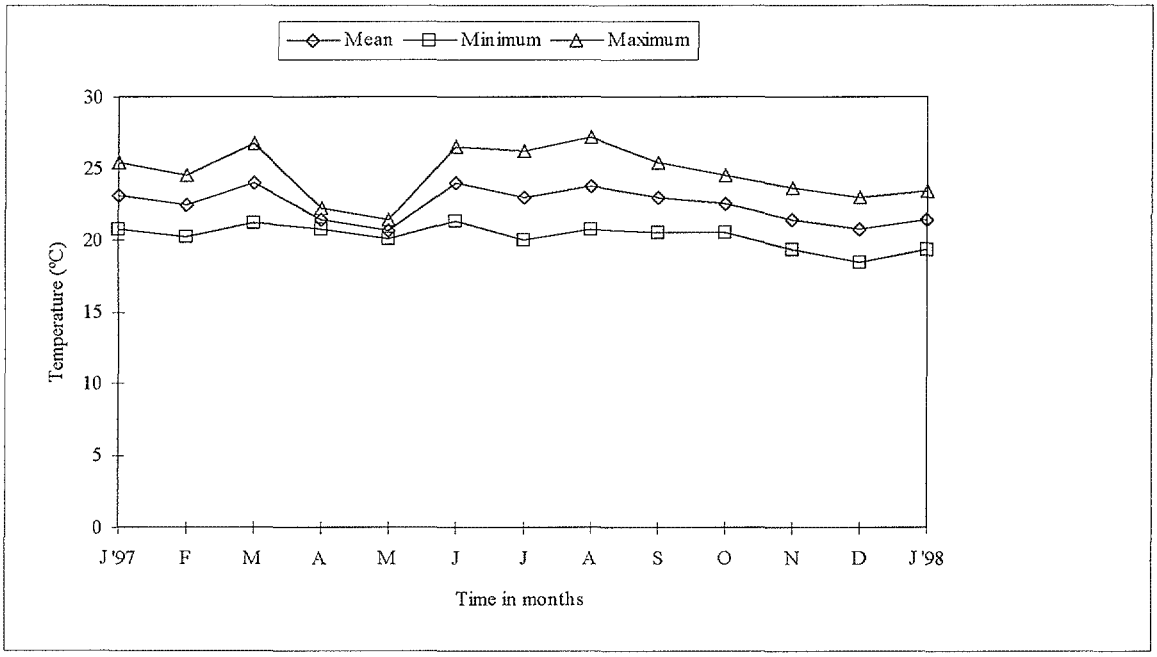


Figure 28. Mid-depth temperature variations in MP.

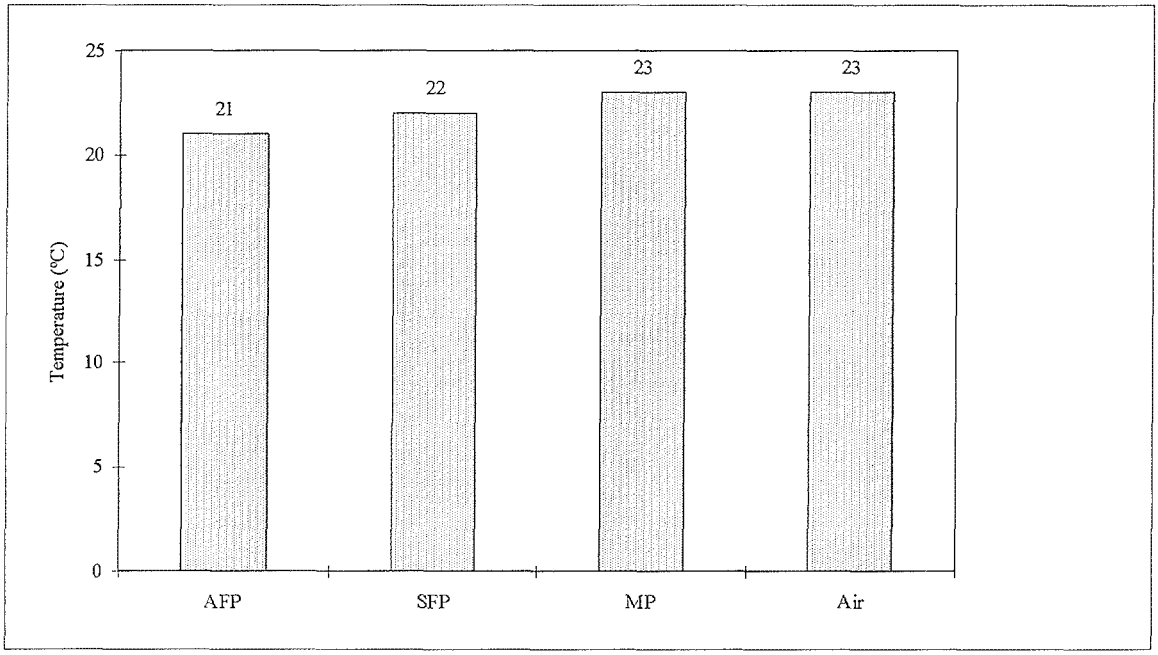


Figure 29. Comparison of mean mid-depth temperatures among the reactors.

As can be seen from Figure 29, the air was warmer by 2 °C and 1 °C than the advanced facultative pond and the secondary facultative pond, respectively. The maturation pond temperatures were observed to follow the air temperatures more closely than the other ponds. In all these temperature variations, volume is an important factor. The reactors temperatures varied inversely with their volumes, being advanced facultative pond the largest and thus the coolest. The maturation pond was so small in volume that it attained air temperature fairly quickly.

Dissolved oxygen, temperature and pH profiles

Dissolved oxygen, temperature and pH profile studies were conducted in all the reactors in order to delinate oxic and anoxic layers and check whether stratification occured. Figures 30-32 present profiles at different times during the operation.

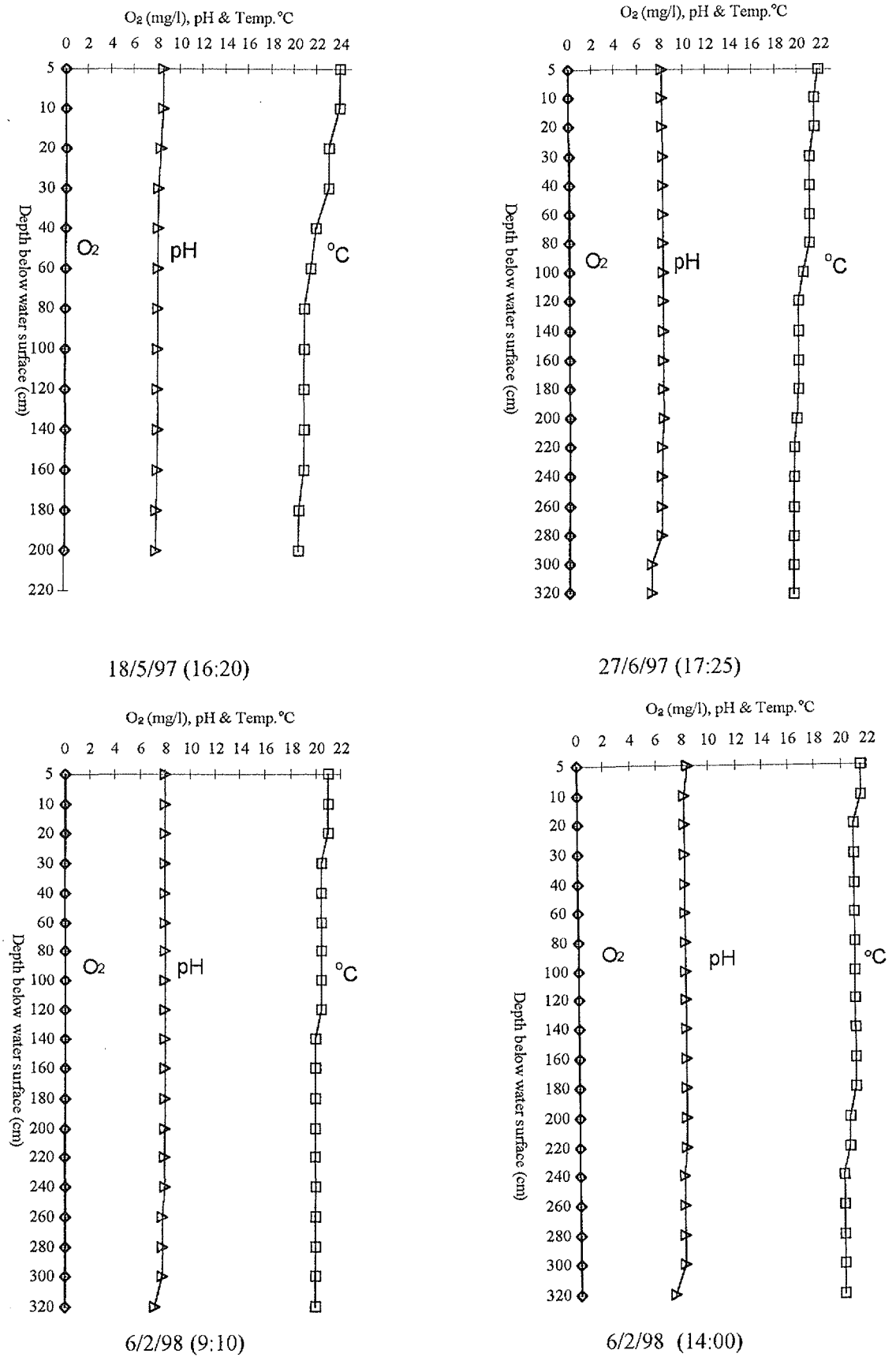
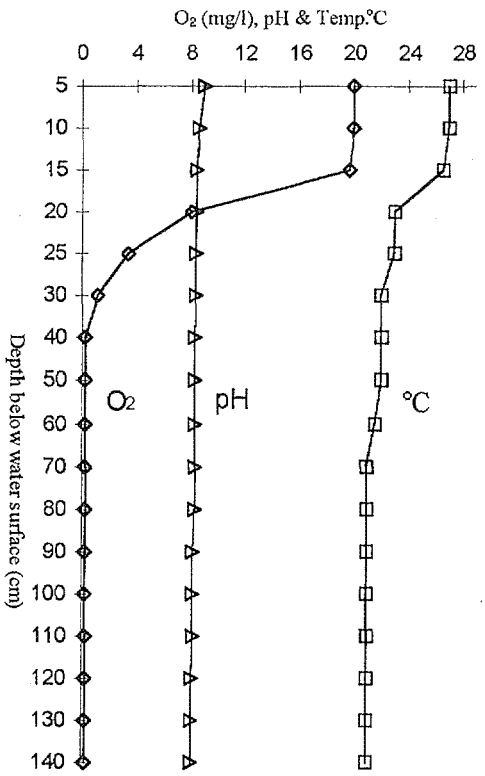
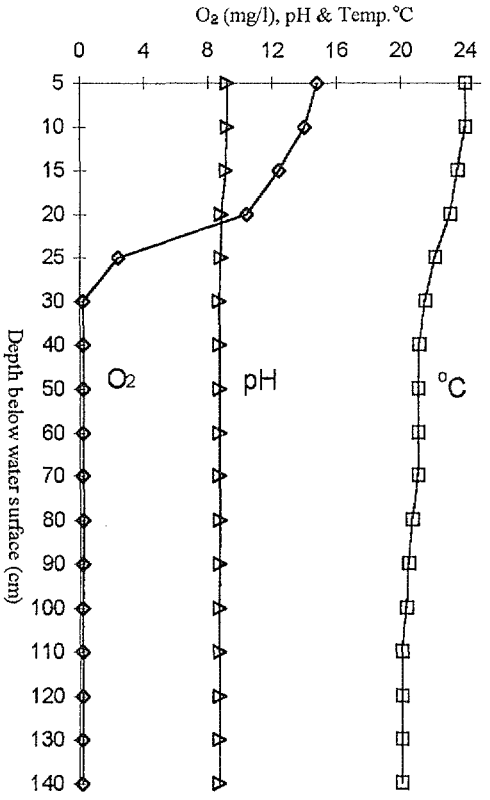


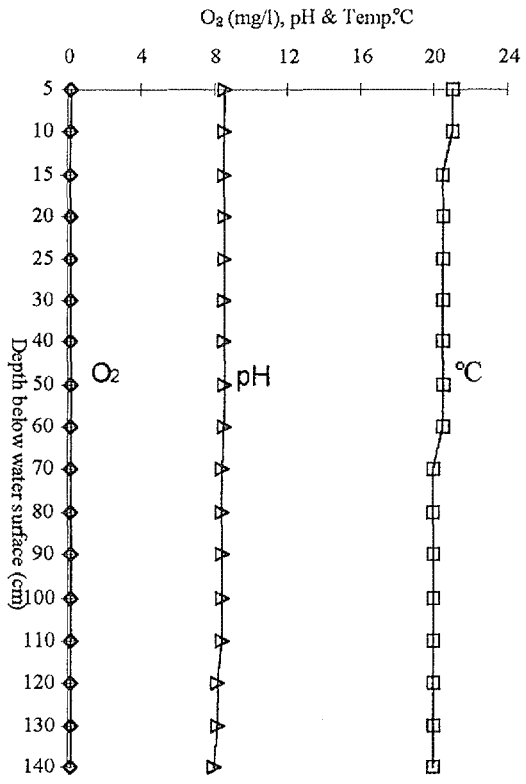
Figure 30. Vertical variation of O_2, pH and temperature in the advanced facultative pond.



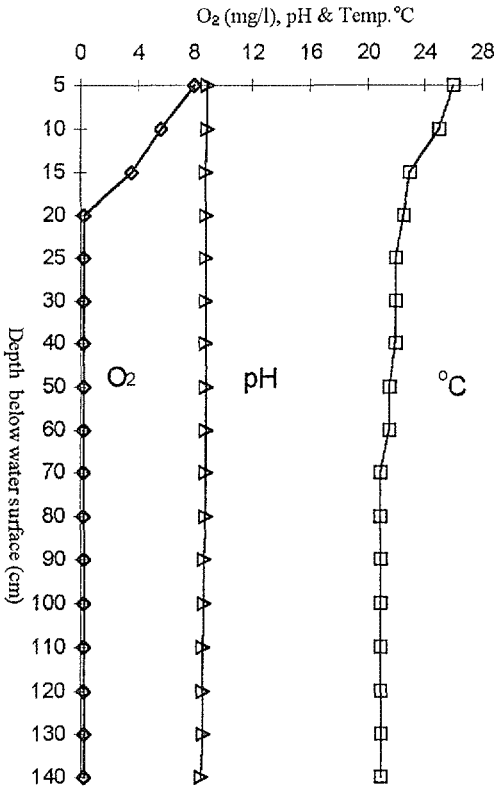
18/5/97 (15:04)



27/6/97 (18:07)



6/2/98 (10:00)



6/2/98 (14:40)

Figure 31. Vertical variation of O_2, pH and temperature in the secondary facultative pond.

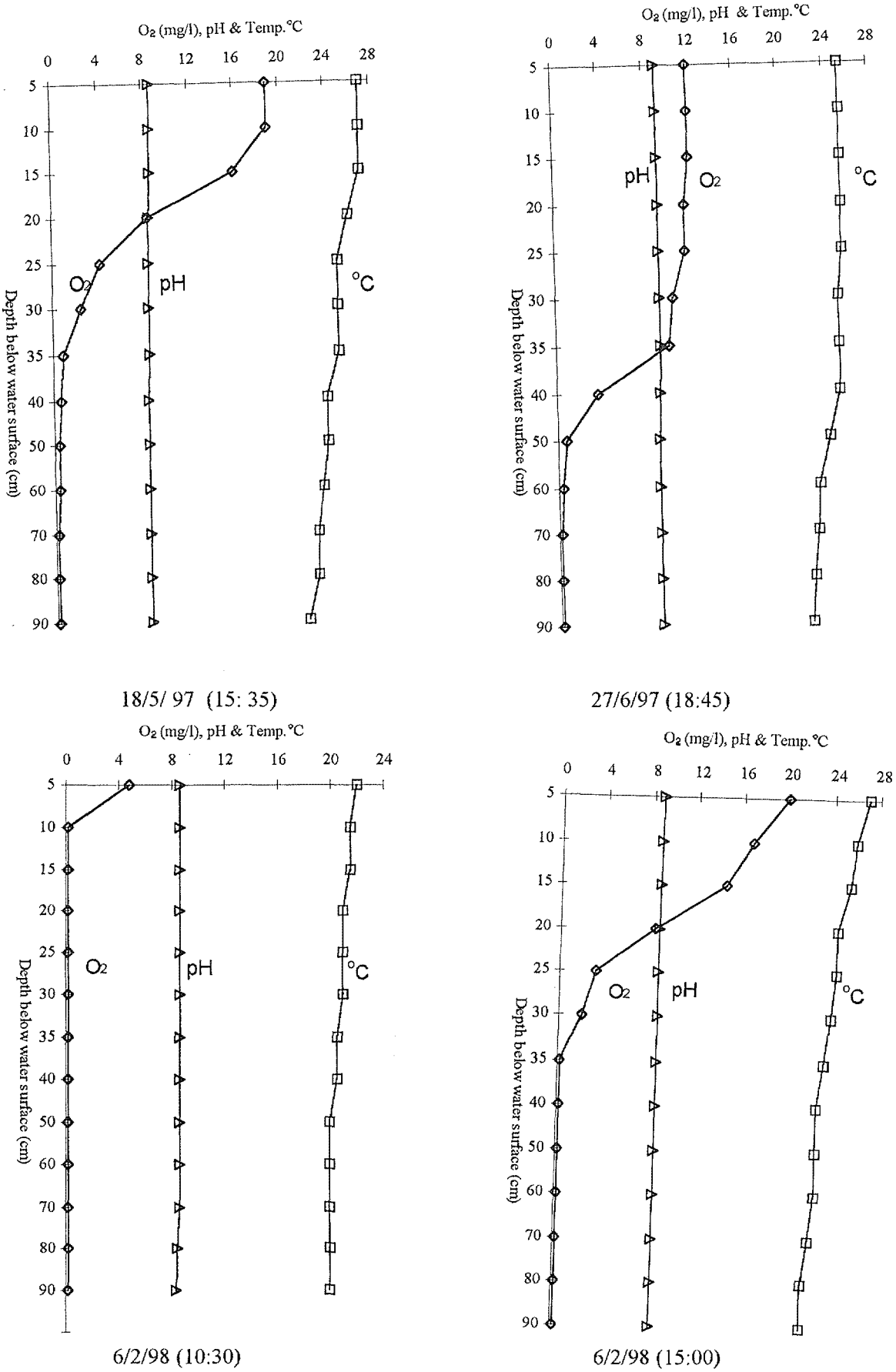


Figure 32. Vertical variation of O_2, pH and temperature in the maturation pond.

The depth variations of dissolved oxygen, pH and temperature in the advanced facultative pond were minimal, as demonstrated in Figure 30. The pond was anaerobic throughout its whole depth. The pH only varied from 8.2 at the surface to 7.2 at the bottom, which is conducive for anaerobic digestion. It is interesting to note that the pH of influent waste to the fermentation pit has dropped by 2 units (i.e., from 9.2 in the feed to 7.2 at the bottom of the fermentation pit). The pH drop, in the fermentation pit, may be the result of acidogenesis during anaerobic digestion of the waste. This near-to-neutral pH of 7.2 observed at the bottom of the fermentation pit was maintained for a depth of 40 cm above the sludge water interface. The vertical temperature in the advanced facultative pond varied only by 2 °C (i.e., 19.8 °C at the bottom to 21.8 °C at the surface) with no pronounced thermal stratification identified at any moment. This implies that a high degree of mixing predominated in this pond, which was most likely induced by the rapid evolution of bio-gas from the sludge layer. This has to be, however, ascertained by separate dispersion studies which are planned to be undertaken at the latter phase of the research.

The profile studies in the secondary facultative pond are presented in Figure 31. The profiles indicate that the pond was aerobic in its 30 cm upper water layer during much of the afternoon, when algal photosynthesis was the highest. Supersaturation was frequently observed in the secondary facultative pond. The maximum dissolved oxygen of about 20 mg/l observed at 15 hours on 18 May 1997 represent over 300% saturation. This excess dissolved oxygen produced over and above the bacterial oxidation requirement was held in the liquid and utilized in algal and bacterial respiration at night. The combined respiration almost depleted the oxygen during the night and in the morning dissolved oxygen was found to be nil (Figure 31 at 10:00 6/2/98). This phenomenon was, however, returned to normal as the photosynthetic activity picked up. The thermal behaviour of this pond was charaterized by fairly defined thermocline frequently observed during late afternoons. Although the thermocline was marked to 25 cm below the surface, the brief period this lasts did not qualify the pond to be described as thermally stratified. There was a 5 °C difference, on average, between the surface and bottom water layers. In general, pH elevation was observed in the surface water layers, with pH steadily decreasing from 9.2 at the surface to 8.2 at the bottom.

The maturation pond has been oxygenated to relatively much dipper depth, with oxypause extending to half the water depth. Its shallow water depth permits better light penetration thereby facilitating photosynthesis at greater depth. The increase in dissolved oxygen concentration in the secondary and tertiary ponds was also probably due to decreasing organic loading. The oxygen demand diminishes as the organic loading decreases. Figure 32 shows a higher pH of 9.2 extending to half the depth of the pond, possibly reflecting the extent of photosynthesis. The fairly uniform temperature variation throughout the depth signifies that the pond is thermally unstratified.

Biogas analysis

The AIWPS biological waste treatment system offered a high potential for energy in addition to its high performance and low-cost nature. This supplemental energy is derived from the biogas generated form the fermentation pit of the advanced facultative pond. This spin-off benefit makes the AIWPS method more attractive to tanneries than its counterpart conventional biological treatment methods. The biogas will find many applications in tanning industries which include but not limited to: (i) supplementary energy source for boilers, (ii) drying recovered wool and (iii) incineration of excess solids, etc. This could offer enormous saving in annual operational costs. Part of this energy may also be used to heat up the fermentation pit thereby insuring process stability and increase biogas out put during cold seasons. It was demonistrated by a number of investigators, Oswald (1964), that heating fermentation pits could result in 7 fold increase in gas evolution for every 5 °C rise in temperature. The data presented below demonstrates the biogas energy potential of the treatment system.

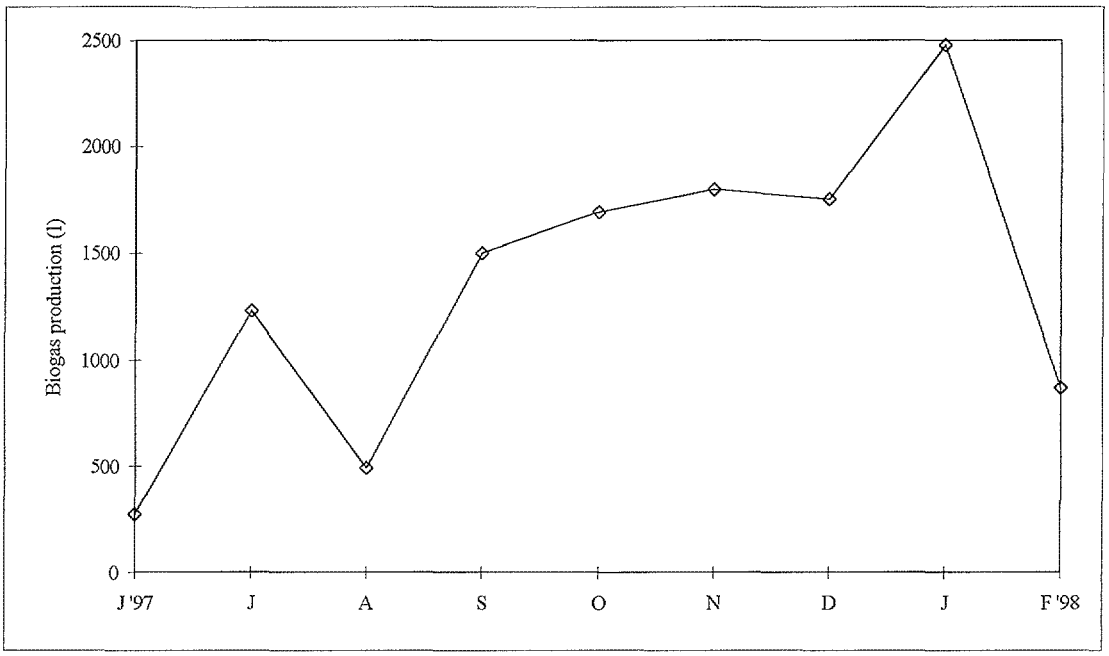


Figure 33. Variation in biogas production.

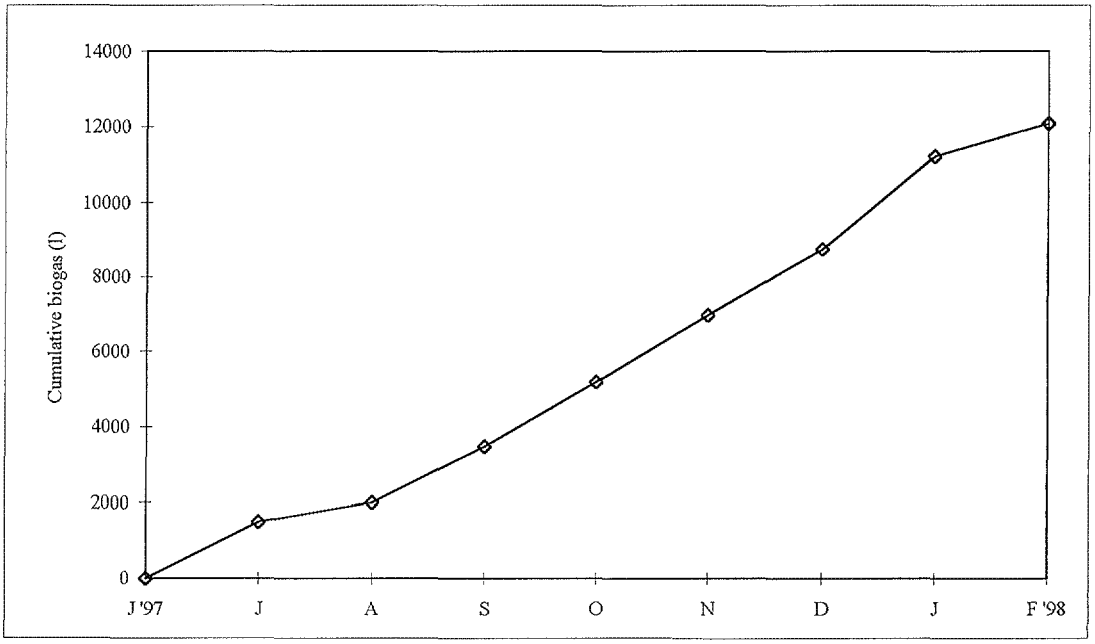


Figure 34. Cumulative biogas production.

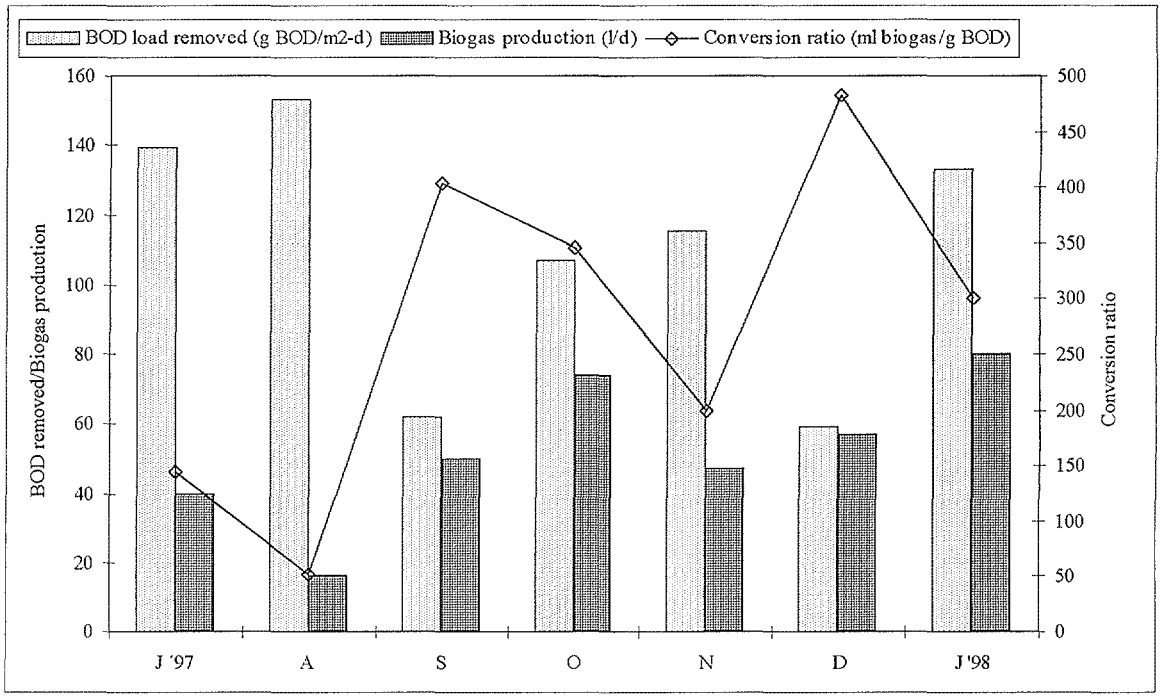


Figure 35. Biogas yield as a function of BOD load removed.

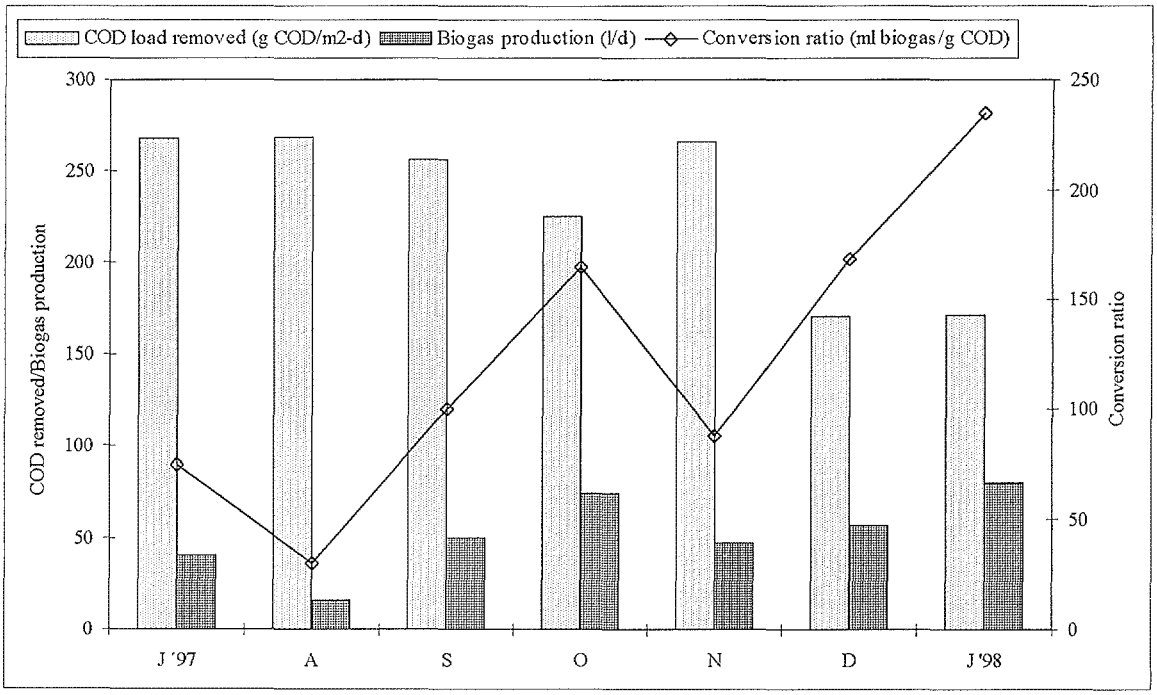


Figure 36. Biogas yield as a function of COD load removed.

As can be seen from Figures 33 and 34, the biogas generated from the fermentation pit has steadily increased from 18 liters per day in the beginning to 80 liters per day at steady state stage. The specific areal biogas production averages to 26 l/m^2-d. The biogas conversion ratio in terms of BOD removed averages 350 ml/g-BOD (Figure 35). This is comparable with the theoretical conversion ratio of 500 ml biogas/g-BOD determined from stoichiometric relationship for anaerobic digestion of glucose:

$$\underset{(180)}{C_6H_{12}O_6} \longrightarrow \underset{(132)}{3CO_2} + \underset{(48)}{3CH_4}$$

Oxidation of methane to CO_2 and H_2O:

$$\underset{(48)}{3CH_4} + \underset{(192)}{6O_2} \longrightarrow 3CO_2 + 6H_2O$$

The ratio of methane produced per kilogram of BOD converted:

$$\frac{\text{kg } CH_4}{\text{kg BOD}} = \frac{48/180}{192/180} = 0.25$$

The liter equivalent volume of 0.25 kg methane:

$$= (0.25 \text{ kg})(10^3 \text{ g/kg})\frac{1 \text{ mol } 22.4 \text{ L}}{16 \text{ g}}(10^3 \text{ L/m}^3)$$

$$= 0.35 \text{ m}^3 \text{ (At STP)}$$

Therefore, 350 ml CH_4 is produced for gram of BOD converted. The biogas production assuming 70% of CH_4 by volume:

$$= 350/0.7$$
$$= 500 \text{ ml per g BOD removed}$$

The conversion ratio of 350 ml biogas/g BOD destroyed was quite high, considering the sub-mesophilic operational temperature (19-21 °C) of the fermentation pit. Moreover, tanning wastewater can not be considered as highly digestible as glucose. The conversion ratio in terms of COD load removed was 0.165 m^3/kg COD removed.

The result is compareable to conversion ratio of 0.160 m^3/kg COD removed obtained for an anaerobic filter (Kobayashi et al.,1983) and 0.21 m^3/kg COD removed obtained for an UASB reactor (Lettinga et al.,1983). The high fermentation of methane as evidenced from high conversion ratio of biogas is a further proof for adequacy and process stability of anaerobic digestion of the waste in the fermentation pit.

The composition of biogas could not be analysed, due to lack of a gas chromatography with right type detector column. However, the flame taste (Plate 10) conducted routinely at site shows that the gas could contain 70-75% methane by volume.

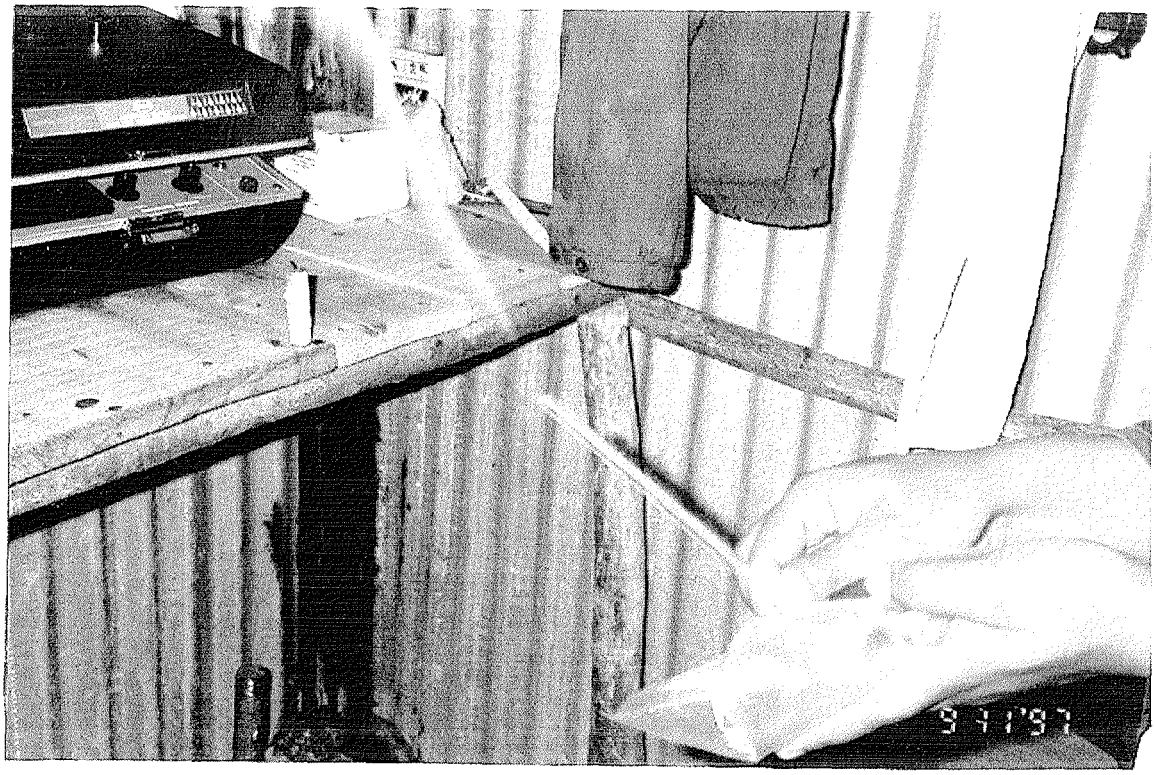


Plate 10. Routine flame test for qualitative evaluation the purity of biogas.

The research will peruse the quantitative analysis of biogas once a proper type gas chromatography is available. This is useful in assessing the energy potential of the biogas.

5.2 Biological

The biological examination of the AIWPS ecosystem is essential for the fundamental understanding of the microbial interactions and population dynamics occurring in the rectors. The examination which was carried out at regular interval during each operation phase comprised the the following studies:

- Phytoplankton
- Zooplankton and zoobenthos
- Sulphur bacteria

The biological examinations were undertaken by the Department of Biology of Addis Ababa University, and the reports are attached as Annexe 4.

Phytoplankton

Method and materials

The identification and relative abundance of the different species of micro-algae in the facultative and maturation ponds were obtained by microscopic examination of samples taken directly from the pond water column. Both fresh and Lugol's iodine and formalin fixed specimens were examined. For microscopic analysis of species composition and cell density estimates, a small volume (up to 20 ml) of phytoplankton samples preserved with Lugol's solution was allowed to settle in a counting chamber. The sedimented algal particles were counted along five horizontal transects with an inverted microscope. Five field of vision, each with a surface area of 1.5386 mm^2 were included in each transect considered in the counting procedure. Counting included the total phytoplankton communities and the number of the dominant species.

Algal biomass was measured in terms of chlorophyll a concetration. Chlorophyll a concentration is a more appropriate measure of algal biomass than algal number.

No two same counts of different algal species could give equal oxygen concentrations, as their oxygen production is rather dependant on their chlorophyll a concentrations than on their cell numbers. Two different species could contain different amounts of chlorophyll a pigments although their cells numbers are the same. For chlorophyll a determinations, duplicate 10-20 ml samples were filtered through 47 mm glass filter membrane (GF/C) with vacuum pressure of less than 10 cm Hg to prevent cell damage. The filters were folded in half, wrapped in aluminium foil and kept in freezer until later analysis. The filters were then subsequently manually grounded with a glass rod in small volume of 90% acetone. The homogenised algal material was placed in a parafilm-covered tube and centrifuged at 3000 rpm for 10 minutes. The extract was decanted into 10 ml volumetric flask and made up to the mark with 90% acetone prior to absorbance reading. Absorbance was measured at 663 and 750 nm before and after the addition of 2 drops of 1 N HCl. Before final spectrophotometric readings were taken, correction for phaeophytin a should be made. Phaeophytin a pigments are the degradation products of chlorophyll a which are photosynthetically inactive but possess nearly the same absorbance as chlorophyll a. Unless they are properly accounted for they can lead to an overestimation of the chlorophyll a concentration. When algae are dead or become inactive their chlorophyll a are soon degraded to phaeophytin a. Addition of HCl acid in the test, results in converting all chlorophyll a to phaeophytin a and when absorbance after acidification is subtracted from absorbance before acidification proper correction for phaeophytin a can be made. The concentrations of both chlorophyll a and phaeophytin a were calculated according to the following equations of the monochromatic method (Wetzel and Likens, 1979).

$$\text{Chlorophyll a } (\mu g/l) = \frac{11.3\,(E_{663b} - E_{663a})(v)}{(V)(Z)}$$

$$\text{Phaeophytin a } (\mu g/l) = \frac{17.86\left(E_{663b} - \left[2.43(E_{663b} - E_{663a})\right]\right)(v)}{(V)(Z)}$$

Where:

E_{663b} = Turbidity corrected absorption at 663 nm before acidification (i.e., $A_{663b} - A_{750b}$, where A is the absorption value)

E_{663a} = Turbidity corrected absorption at 663 nm after acidification (i.e., $A_{663a} - A_{750a}$, where A is the absorption value)

v = Volume of extract, ml

V= Volume of sample filtered, L

Z = Length of light path through cuvette, cm

Identification of algal genera and species

Algae found in the secondary facultative and maturation ponds of the AIWPS are listed in Table 9.

Table 9. Algal genera and species found in the secondary facultative and maturation ponds of AIWPS (sampling dates: 8/10/97, 27/10/97).

Algal genera and species	SFP (SP4)			MP (SP5)		
	+/-	RA 8/10/97	RA 27/10/97	+/-	RA 8/10/97	RA 27/10/97
Euglenophyta						
Euglena spirogyra Ehr	+	10	5	+	4	5
E. cf. viridis Ehr	+	3	2	+	2	3
Euglena spp.	+	14	11	+	10	10
Lepocinclis ovum (Ehr) Lemm.	-*	-	4	-*	-	2
Phacus brevicauda (Klebs) Lemm.	+	8	9	+	7	8
P. hispidula (Eichw.) Lemm.	+	7	8	+	8	9
P. longicauda (Ehr) Dujardin	+	1	1	+	1	1
P. cf. orbicularis Hubner	+	5	7	+	9	7
P. cf. pleuronectes (OFM) Duj.	+	13	10	+	11	11
Trachelomonas cf. ensifera Daday	+	9	15	+	13	14
T. volvocina Ehrenberg	+	4	12	+	6	12
Trachelomonas Spp.	+	15	13	+	2	13
Chlorophyta						
Chlamydomonas sp.	+	12	16	+	14	15
Chlorogonium cf. elongatum Dang.	+	6	6	+	5	6
Phacotus lenticularis (Ehr) Stein	+	2	3	+	3	4
Chlorella sp.		11	14	+	15	16

Key to symbols and numbers

* Was not observed in the samples brought on October 8, 1997.

SFP(SP4) = Surface and column samples collected from sampling location SP4 of secondary facultative pond.

MP(SP5) = Surface and column samples collected from sampling location SP5 of maturation pond

+ = Present

- = Absent

RA = Relative Abundance, being 1 the most abundant and 15 or 16 the least abundant

Table 10. Algal cell counts.

Species/group	SFP (SP4)	MP (SP5)
	No. x 10^8/l (8/10/97)	No. x 10^8/l (8/10/97)
Phacus longicauda	23.7	13.5
Euglena cf. viridis	2.9	3.8
Phacotus lenticularis	3.1	2.1
Euglena spp.	1.7	4.7
Others	1.1	1.0
Total	32.5	25.1

Table 11. Algal vertical distribution.

Species/group	SFP (SP4)			MP (SP5)		
	No. x 10^8/l (27/10/97)			No. x 10^8/l (27/10/97)		
	13 cm	100 cm	150 cm	12 cm	67 cm	100 cm
Phacus longicauda	1.60	0.38	0.22	2.55	0.98	0.16
Euglena cf. viridis	0.30	0.43	0.04	1.81	1.37	0.02
Lepocinclis ovum	0.20	0.06	0.07	5.93	0.15	0.01
Phacotus lenticularis	0.30	0.12	0.09	0.54	0.23	0.04
Euglena spp. + others	1.00	0.65	0.26	0.55	0.17	0.05
Total	3.40	1.64	0.68	11.38	2.90	0.28

Table 12. Algal standing crop as µg Chlorophyll a per litre.

Date	Pond	Sampling Location	Depth (cm)	Concentration (µg/l)	
				Chlorophyll a	Phaeophytin a
8/10/97	SFP	SP4	-	1410	12
,,	MP	SP5	-	1582	25
27/10/97	SFP	SP4	13	1466	545
,,	,,	,,	100	519	731
,,	,,	,,	150	90	-
,,	MP	SP5	12	3476	562
,,	,,	,,	67	888	1042
,,	,,	,,	100	203	-

As can been seen from Table 9, the most dominant algal species found in secondary facultative and maturation ponds were *Phacus longicauda* and *Euglena cf. viridis* of the phylum Euglenophyta. *Chlorella* and *Chlamydomonas* of the phylum Chlorophyta were among the least abundant algae species identified in the two ponds. In the study, no algae species of the phyla: Cynophyta (Blue-green algae) and Chrysophyta (Diatoms) were identified. Samples from both ponds appeared monoculture with *Phacus*. The apparent dominance of motile, flagellate algae such as *Phacus* and *Euglena* are something expected in the turbid and unmixed waters of the reactors. The ability of these groups of algae to move towards the surface light give them a competitive advantage over the non-motile ones such as *Chlorella* which are the least abundant in ponds.

The higher concentrations of algae (both in terms of cell numbers and chlorophyll a) observed in the maturation pond than in the secondary facultative pond (Figures 11 & 12) may have been due to less organic loading and better light penetration in the maturation pond.

Temporal variations in algal concentration, observed during 10 days (from 8/10/-27/10/97), were rather dramatic in each pond, although there were no major changes in climate or loading rates during this period. Such relatively fast changes in algal flora necessitate examinations to be performed in shorter intervals of time. The vertical distribution of chlorophyll a generally corresponded to vertical distribution pattern of cell numbers.

The average algal standing crop of 1,500 and 3,000 µg chlorophyll a /l obtained for the secondary facultative and maturation ponds, respectively were within the concentration ranges of healthy ponds which usually contain algal standing crop in the range 1000-3000 µg chlorophyll a per litre (WHO, 1987).

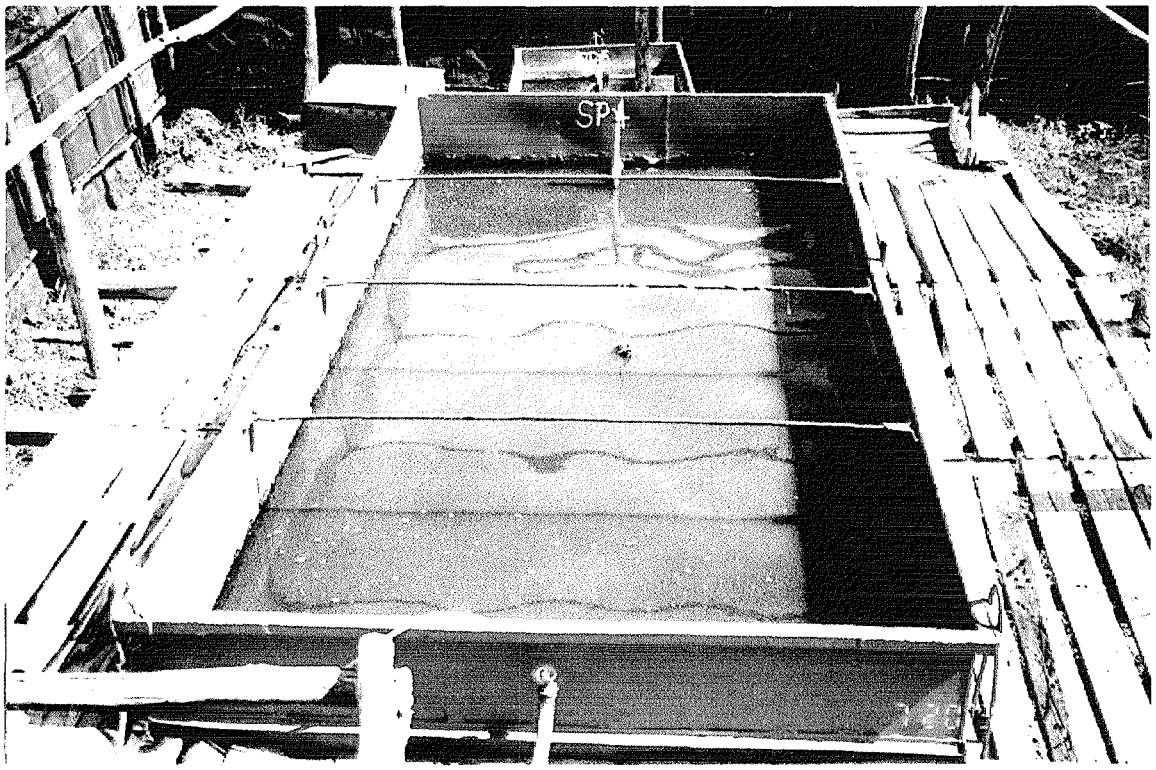


Plate 11. Healthy and stable algae bloom in the secondary facultative pond.

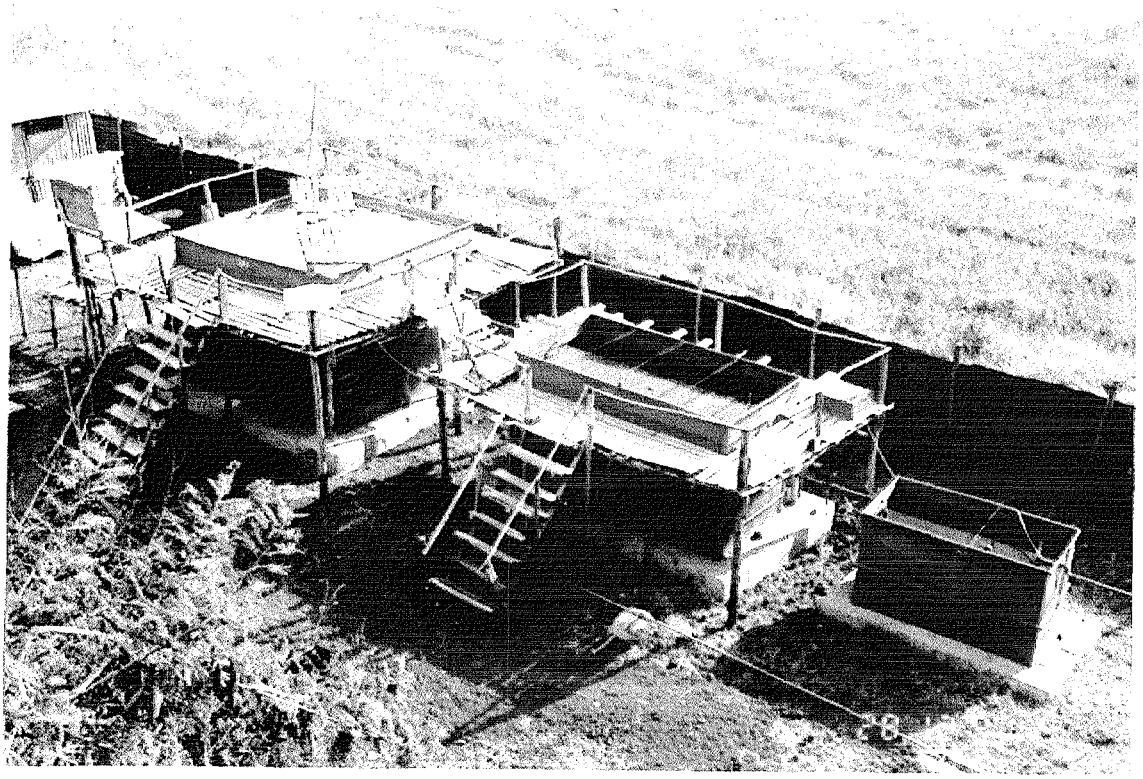


Plate 12. Appearance of surface waters of AIWPS biological reactors in early morning.

It can be seen from Plate 12, algae are just beginning to buoy or swim to the surface waters of the secondary facultative pond (2nd in the series) and maturation pond (3rd in the series) in search of light, thereby turning them green on the surface.

During the course of the biological examinations, the AFP was not thoroughly studied, particularly for algae as it is anaerobic all the time and lacks photoautotrophic algae flora. But, on 8 November 1997 a very striking thin green colour was observed overlying the dense red-pink coloration of photosynthetic sulphur bacteria, after the sulphur scum was scooped from the surface (Plate 13, below).

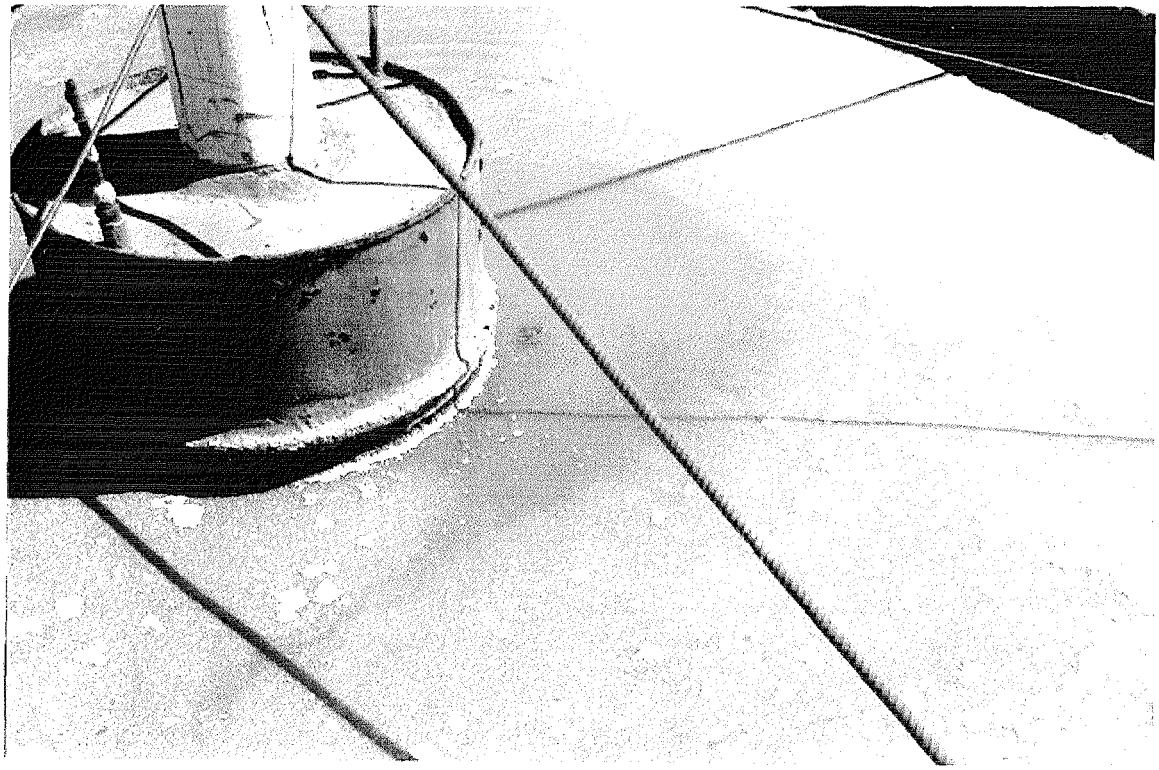


Plate 13. A thin green film overlying the pink water layer of advanced facultative pond (observed at early hours of the morning).

At first, it was thought to be due to some photosynthetic green sulphur bacteriaof the genus, *Chlorobium*. But later, evidence was sought to answer the puzzle when a separate laboratory examination conducted on photosynthetic sulphur bacteria reviled that what actually profused in the AFP were purple sulphur bacteriaof the genus *Thiocystis* instead of green sulphur bacteria. With the experimental clue at hand, further search made into literature threw some light that the thin green film observed on the surface of AFP could be due to a motile green algae of the genus, *Chlamydomonas*.

Many investigators (Alabaster et.al., 1991 and Pearson, et.al., 1987) have found *Chlamydomonas* population forming a green surface film on anaerobic ponds. *Chlamydomonas* adaptation to thrive in anaerobic ponds is explained by their ability to incorporate acetate, which is in plentiful supply in anaerobic ponds, preferentially to CO_2. This high photoheterotrophic, low autotrophic metabolism of *Chlamydomonas* contrasted with the high autotrophic metabolism of *Phacus* and *Euglena* that were dominant in the secondary facultative and maturation ponds. The low autotrophic activity of *Chlamydomonas* is also indicated by the fact that no increased dissolved oxygen levels were found in the AFP (Figure 30). This insight should, however, be verified by a separate biological examination conducted on algal flora in the AFP.

Zooplankton and zoobenthos

The study on zooplankton and zoobenthos was conducted to check wether there is a potential threat on algal flora by grazers. No elaborate procedures were required to examine zooplanktons and zoobenthos. As most of the zooplanktons were net screened from different water layers they were identified with the naked eye. Microscopic identification was relied up on when detailed examination was required. Identified groups are listed below.

Table 13. Zooplanktons/zoobenthos identified (Sampling date 8/10/97).

Sampling Location	Samplig Date	Identified groups	Feeding Role	Relative abundance
MP-SP5	8/10/97	Chironomidae Tanytarsinii	Detritivore	+
,,	,,	Culicidae Microonecta compar	,,	Rare
,,	,,	Culicidae Culex pipiens Theobaldia longiarcolata Anopheles gambiae	,, ,, ,,	+++ + Rare
,,	,,	Gerridae Gerrids sp.	Surface predator	Rare

Table 14. Zooplanktons/zoobenthos identified (Sampling date 27/10/97).

Sampling Location	Sampling Date	Identified group	Feeding Role	Total Count (No.)
SFP-SP4	27/10/97	Ostracoda Darwinula sp.	Grazer	62
,,	,,	Diptera Psychoda sp.	Detritivore	25
,,	,,	Arthropoda Culex pipiens	Detritivore	138
,,	,,	Case of Tabanidae		2
MP-SP5	27/10/97	Arthropoda Culex pipien	Detritivore	232
,,	,,	Unidentified Coleoptera		1
,,	,,	Diptera Notonectus	Predator	6
,,	,,	Diptera Micronecta compar	Bottom scraper	7
,,	,,	Diptera Psychoda larva	Detritivore	34
,,	,,	Untitled Dipteran		2

As can be seen from the list of zooplanktons and zoobenthos identified (Tables 13 & 14), no potential grazers that could endanger the algae community were found. At one moment of the early stage of the operation, the maturation pond was heavily infested with *Daphnia sp*. to such an extent that the algal biomass was near to disappearance. This phenomenon did not, however, last long as *Daphnia* was quickly consumed by efficient predators such as *Corixids* and *Notonectids.* A long term biological solution for persistent *Daphnia* infestation can be achieved by introducing planktivorous fish such as *Minnows* into the pond system. The fish should be removed immediately after the establishment of an algal bloom. A large number of *Culex pipiens* mosquito (a non-malaria vector) identified with rare or non-existent *Anopheles gambiae* (a malaria vector) was a proof that the ponds eco-system was not, as such, conducive for malaria-causing mesquites to breed.

The rapid disappearance of mosquito larvae with associated increase in numbers of *Gerrids* (water striders) was an evidence that *Gerrids* predate on mosquitoes. Propagation of larva-eating fish such as *Gumbusia* and *Peocelia* is a well known biological controlling method to cope up with any mosquito breeding of any nuisance level.

Sulphur bacteria

Sulphur bacteria played an important role in both sulphur transformation and elimination of odour due to H_2S in the AIWPS system. A study was, therefore, arranged with the Biology Department of Addis Ababa University to systematically identify them. In this section a summary of the study conducted on sulphur bacteria is given.

Sample collection

Samples were collected from different depth levels of the advanced facultative and secondary facultative ponds and brought in plastic bottles to the laboratory of the Biology Department of Addis Ababa University.

Method and materials

For microscopic examination, samples were serially diluted depending upon the population numbers to the tune of 10^{-1}-10^{-5} and gram stained using standard methods. Gram staining method is a different staining procedure using two dyes namely; crystal violet and safranin. Samples were heat-fixed for one or two minutes and stained first in crystal violet for one minute, iodine immersed for another minute and then washed in alcohol for a minute. After being washed by running water, the specimen was counter-stained by safranin for one minute. The counterstained specimen was washed with water and subsequently dried using blotting paper. Gram-stained slides were then observed under oil immersion objective (100x) with low light intensities.

The enrichment medium contains the following:

Minerals	g/l
Na_2HPO_4	7.9
KH_2PO_4	1.5
NH_4Cl	0.3
$MgSO_4$	0.1
Trace elements	5.0 ml
$Na_2S_2O_3$ (thiosulphate)	5.0
pH (4.5)	

Cultural characteristics (colour, texture, shape, size, etc.) were also recorded. The bacteria were then identified to the genus level using microscopic examination and for those who were enriched by including cultural characters.

Results and discussion

The findings of the laboratory examinations are given in Tables 15-17.

Table 15. Cell shape, colour and colony arrangement of bacteria isolated from water column samples of advanced facultative pond.

Bacteria	Cell shape	Cell arrangement	Colony colour	Trophic status
Desulfovibrio	curved rods	single	ND	SRB
Thiobacillus	rods	single	colourless	SOB
Thiosphaera	coccoid	single/pair/chain	colourless	SOB
Lamprocystis	spherical/ovoid	diplococcus/ aggregate	ND	PSB
Thiocystis	spherical	aggregate	ND	PSB
Rhodosprillium	spiral		red brown	PNSB
Rhodopseudomonas	rods	diplobacillus/ aggregate	red brown	PNSB

Key to acronyms
SRB = Sulphate reducing bacteria
SOB = Sulphide oxidizing bacteria
PSB = Purple sulphur bacteria
PNSB = Purple non-sulphur bacteria
ND = Not done

Table 16. Depth profile of bacteria counts in advanced facultative and secondary facultative ponds.

Sampling Location	Depth below water surface (cm)	Number (No./ml)		
		Cocci	Rods	Spiral/vibrioid
AFP-SP3	30	6.4×10^4	5.2×10^5	1.2×10^2
,,	130	2.1×10^4	5.1×10^4	2.1×10
,,	230	3.0×10^4	-	-
,,	330	5.1×10^6	-	2.2×10^2
SFP-SP4	50	3.8×10^5	2.6×10^4	2.1×10^3
,,	100	5.2×10^5	1.3×10^5	1.2×10^3
,,	150	2.6×10^6	6.1×10^4	3.2×10^4

Table 17. Depth profile of different bacteria in the advanced facultative pond.

Depth below water surface (cm)	Bacteria genus			
	SRB	SOB	PSB	PNSB
30	-	Thiobacillus Thiosphaera	Thiocystis	Rhodosprillum Rhodopseudomons
130	-	Thiobacillus filamentous bacteria*	Thiocystis	Rhodosprillum Rhodpseudomonas
230	-	-	Thiocystis	-
330	Desulfovibrio	filamentous bacteria*	Thiocystis Lamprocystis	-

*/ Filamentous bacteria whether they are anaerobic or aerobic were not enriched.

Table 15 shows the shape, colour and colony arrangement of the bacteria identified, while Table 16 gives the bacteria counts at different depths of the advanced facultative and secondary facultative ponds. As can be seen from Table 16, the most dominant group of bacteria were of coccoid (*Thiocystis*) and rod shaped (*Thiobacillus*) with few representatives of spiral (*Rhodosprillium*). The cocciod-shaped (*Thiocystis*) were also uniformly distributed along the depth profile. Although *lamprocystis* was recovered from the lower profile (Table 17) the dominance and ubiquitous distribution of the coccoid bacteria of the genus *Thiocystis* was shown all along the water-depth profile. This *Thiocystis* may have representatives of micraerophilic and aerobic representatives within their respective habitats.

General observations such as colour, presence of scum and evidence of gasification gave good identification of the behaviour and condition of the AIWPS biological units. Many of the colour changes observed in the AIWPS units were caused by variation in algal and bacterial population (Plate 14).

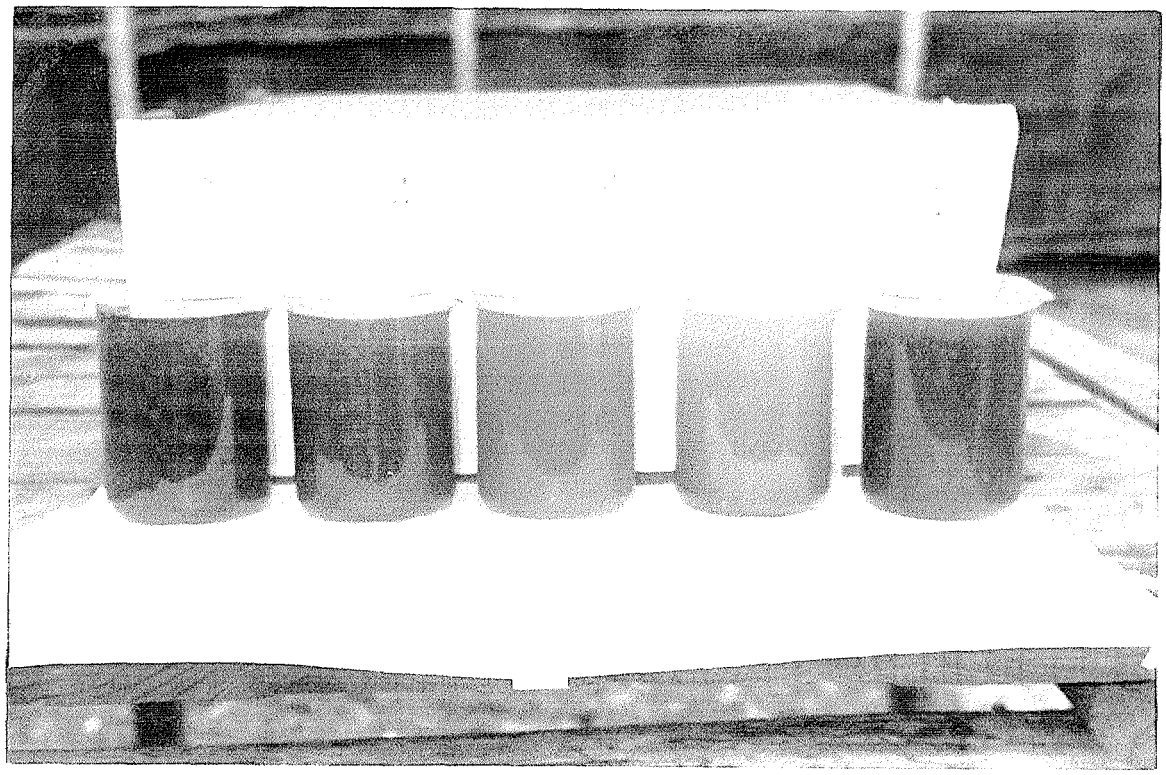


Plate 14. Colour variation among the successive AIWPS reactors.

In Plate 14, the untreated combined tannery waste (SP1) appeared dark blue, mainly due to the sulphide paste used in unhairing and chrome waste liquor resulted from the tanning operation. The grey and milky colour suspension (SP2) resulted after one day detention in the settling basins was due to the lime used in unhairing and liming operation. The red-pink appearance of the water of advanced facultative pond (SP3) was due to a consortium of photosynthetic purple bacteria identified in the biological examination (Table 17). The pink colour was largely due to ubiquitous distribution of photosynthetic sulphur bacteria of the genus *Thiocystis*. The light green (SP4) and dark green (SP5) colours of the secondary and tertiary ponds respectively were, as confirmed by the biological examination, due to the dominant algal genera of *Phacus* and *Euglena.*

As the effluent from advanced facultative pond was directly discharged into the bottom of the secondary facultative pond, pink sulphur bacteria were noticeable in lower layers of this pond.
Most of the time these bacteria occupied the anaerobic layer below the algae and were consequently barely discernible from the pond surface. But, at one moment, the pink colour reached the surface and interfered with photosynthetic process of algae by limiting the depth of light penetration. This phenomenon was reversed, in about three days, by gentle manual mixing created in the upper water layers of the pond.

The two possible reasons postulated for the disappearance of pink coloration from the pond surface were:

(i) gentle mixing might have brought algae to the surface thereby giving them a competitive advantage over the pink sulphur bacteria for available light, and

(ii) the mixing might have also augmented photosynthetic oxygenation thereby pushing the anaerobic zone along with pink sulphur bacteria down to the lower layers.

Once the algae population is re-established on the surface, the pink photosynthetic sulphur bacteria stand little chance of competing with algae due to the shading effect of the dense green surface created by flagellate algae. Thus, under normal operation conditions, the pink photosynthetic sulphur bacteria remained below the algae layer.

To verify the suppression effect of mixing on photosynthetic pink sulphur bacteria, a bubble mixed outdoor bench-scale experiment of the type shown in Plate 15 was performed.

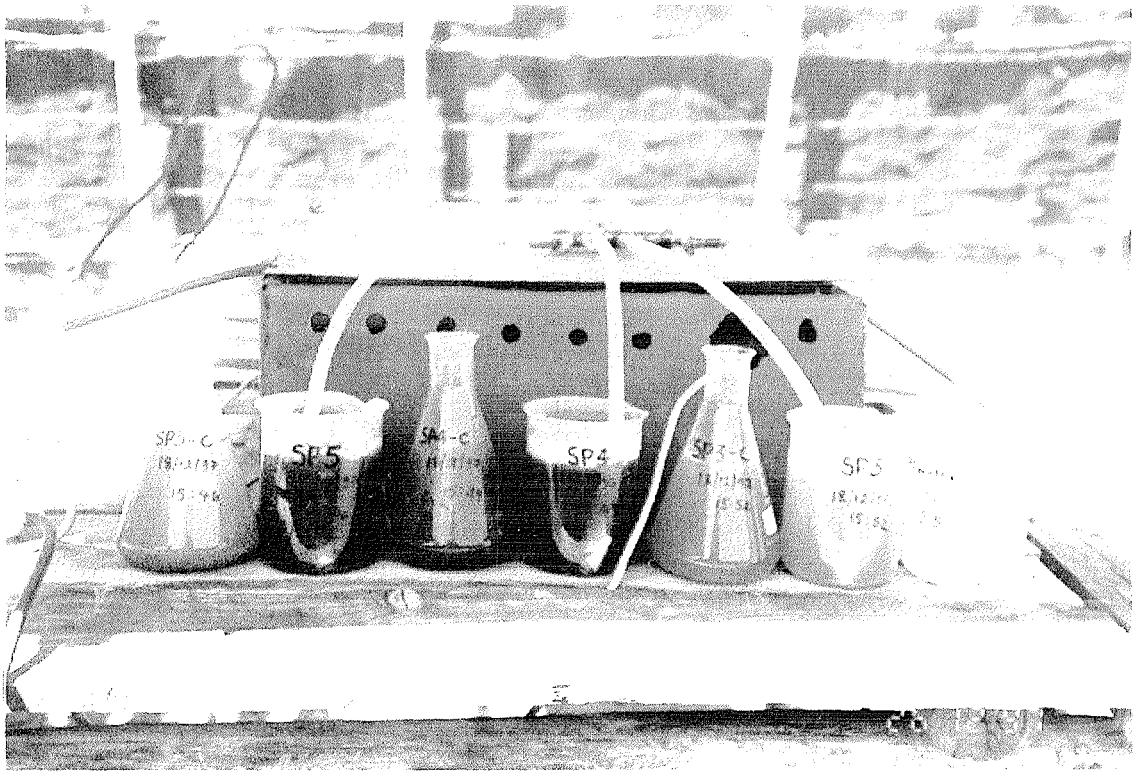


Plate 15. Outdoor experimental set-up demonstrating the effectiveness of mixing in suppressing photosynthetic pink sulphur bacteria.

In the bench scale set-up (Plate 15), pond samples drawn from the lower strata of the secondary facultative and maturation ponds were placed in the 500 ml experimental beakers (SP4 & SP5) and control flasks (SP4-C & SP5-C). A sample drawn from the dense pink surface layer of the advanced facultative pond was also placed in 500 ml experimental beaker (SP3) and control flask (SP3). Distilled water was placed in another 500 ml beaker to check the increase in oxygen concentration due to mixing. The colours of all experimental and control samples were pink before the mixing.

After three days of intermittent bubble mixing (each day only for three to four hours), the pink samples from both algal ponds turned green, confirming the re-establishment of algae flora. Although both controls (SP4-C & SP5-C) developed light green colour, the dark green colour produced by the experimental beakers (SP4 & SP5) was a further proof for mixing to contribute for the higher algal biomass. There appeared little colour change between the experimental (SP3) and control (SP3-C) samples of AFP before and after the mixing. The only change noticeable was that the experimental beaker (SP3) was slightly lighter than the control (SP3-C).

An accumulation of pink sludge at the bottoms of all experimental beakers after three days of mixing demonstrated, beyond doubt, that mixing can inactivate photosynthetic pink sulphur bacteria. Another observation is that the oxygen concentration in the beaker containing distilled water did not increase beyond and above the saturation level, indicating that oxygenation due to mixing was much smaller than photosynthetic oxygenation. So, mixing has done nothing more than bringing algae to the euphotic layer.

A host of factors are implicated in the actual destruction of photosynthetic pink sulphur bacteria in a mixing environment, the main culprits being shading due to algae, photosynthetic oxygenation, starvation and release of toxins by algae. To delineate the most responsible destruction mechanism and capitalize on it, should photosynthetic pink sulphur bacteria out compete algae, requires a more detailed and systematic investigation on all suspected factors.

5.3 Overall performance

In this section, the overall purification performances of the pilot biological reactors over the entire study period is presented below. Organic loading rates applied during the three experimental feed phases is given in Annex 5.

Biochemical Oxygen Demand

Table 18. BOD removal performance

Feed Phases	Percent removal/Loading	AIWPS Reactors			
		AFP	SFP	MP	Overall
F1	BOD removal (%)	88	65	40	98
	Surface BOD loading (kg/ha-d)	1,236	228	185	
	Volumetric BOD loading (g/m^3-d)	48	14	18	
F2	BOD removal (%)	73	34	36	87
	Surface BOD loading (kg/ha-d)	1,844	847	1,341	
	Volumetric BOD loading (g/m^3-d)	72	53	134	
F3	BOD removal (%)	82	37	32	92
	Surface BOD loading (kg/ha-d)	3,037	975	1,507	
	Volumetric BOD loading (g/m^3-d)	119	61	151	

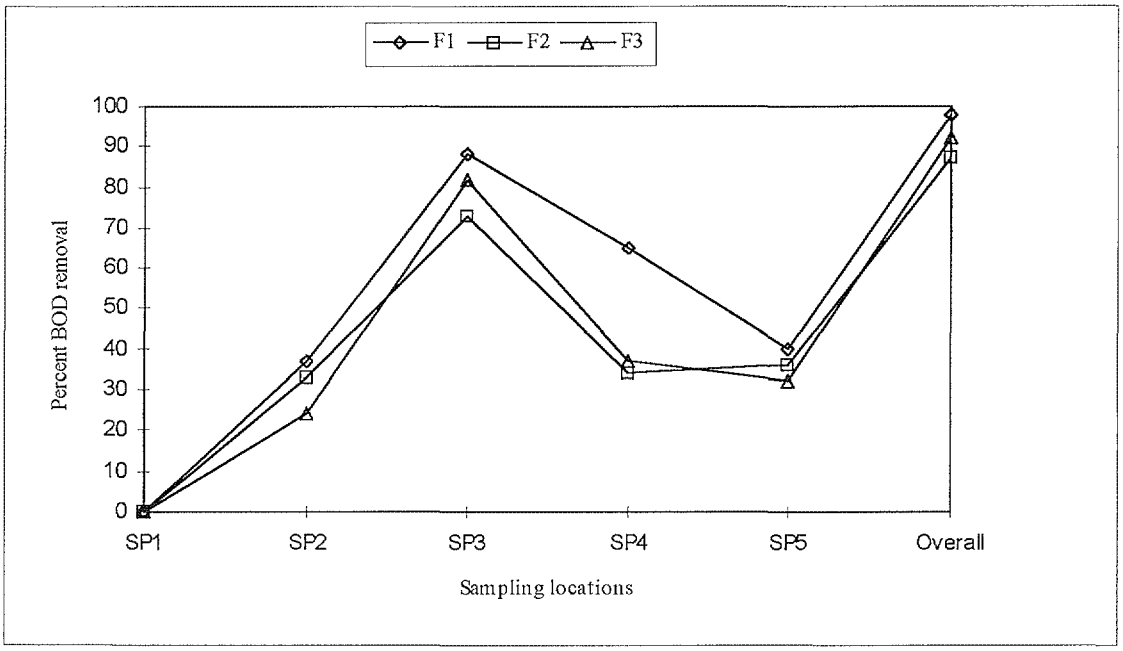


Figure 37. BOD removal efficiency during each feed phase (F1, F2 and F3).

As can be seen from Table 18 and Figure 37, the variation of BOD removal efficiency among the three feed phases is insignificant, although surface BOD loading has increased many folds over the duration of the experiment. The drop in removal efficiency (although small) as the loading increases was something expected. Besides the changes in environmental conditions, variations in unmeasured compounds of the raw effluent could play a significant role for unexpected trend observed in the second phase. It is clear from data that the aerobic/facultative reactors (SFP and MP) are more affected by increase in BOD loading than the anaerobic/facultative reactor (AFP). This is also revealed in the algological examination of water column samples from SFP and MP which resulted in less diverse algal community dominated by *Chlamydomonas fenestrata*. Chlamydomonas is the most commonly found algal species in loaded ponds (Alabaster et al. 1991). The loading did not however result in lowering the chlorophyll a and photosynthetic oxygen concentration in the two reactors.

A striking result is that the highest BOD removal (73-88%) took place in the AFP. This signifies the importance of anaerobic form of treatment prior to any aerobic waste degradation. Such configuration helps in significantly reducing the organic loads and effectively buffering the sensitive algal population in aerobic/facultative units. The maturation ponds appeared to have been subjected to a higher surface BOD loading than the preceding secondary facultative ponds, which is due to their small sizes.

Chemical Oxygen Demand

Table 19. COD removal performance

Feed Phases	Percent removal/Loading	AIWPS Reactors			
		AFP	SFP	MP	Overall
F1	COD removal (%)	85	31	21	97
	Surface COD loading (kg/ha-d)	2,524	609	983	
	Volumetric COD loading (g/m^3-d)	99	38	98	
F2	COD removal (%)	81	37	14	90
	Surface COD loading (kg/ha-d)	6,582	2,087	3,179	
	Volumetric COD loading (g/m^3-d)	2,576	130	318	
F3	COD removal (%)	60	62	8	86
	Surface COD loading (kg/ha-d)	8,279	5,808	5,308	
	Volumetric COD loading (g/m^3-d)	324	363	531	

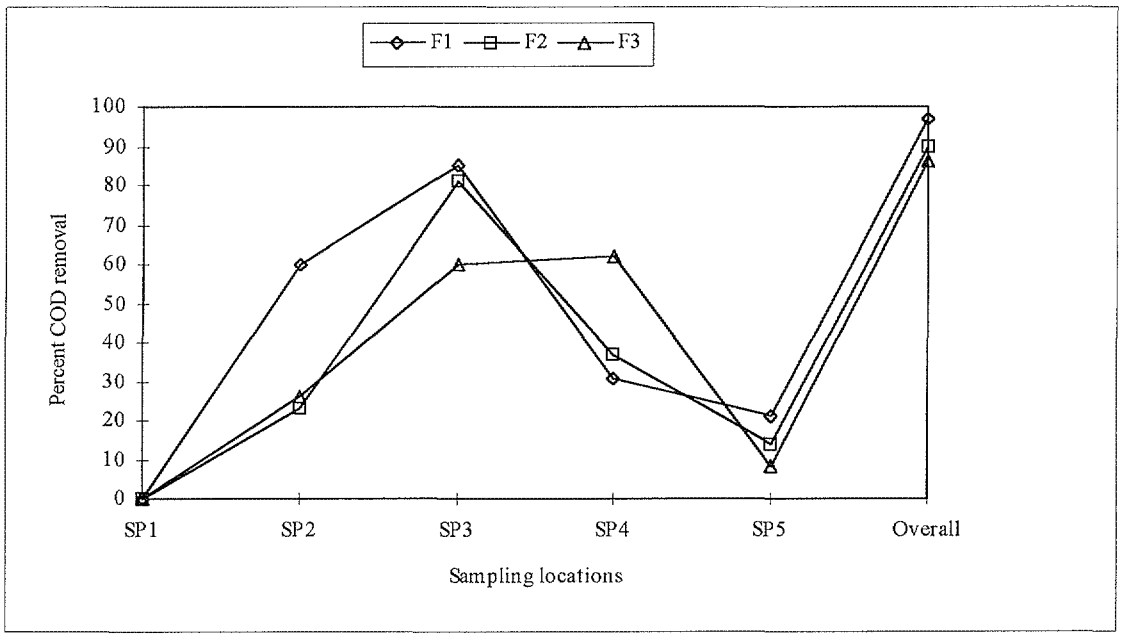


Figure 38. COD removal efficiency during each feed phase (F1, F2 and F3).

Most of the reasoning given to BOD also applies o COD, except that the overall percent COD removal over the three feed phases has shown a decreasing trend.

Solids

Table 20. Solids removal performance

Feed Phases	Solids	Percent removal in the AIWPS Reactors			
		AFP	SFP	MP	Overall
F1	Total solids	36	19	6	77
	Suspended solids	19	18	-38	92
	Dissolved solids	37	22	5	64
	Volatile solids	36	-21	-10	93
F2	Total solids	36	-3	-1.8	33
	Suspended solids	72	-57	4	58
	Dissolved solids	41	-0.62	-15	31
	Volatile solids	71	-23	13	69
F3	Total solids	38	7	-4	39
	Suspended solids	87	-11	19	88
	Dissolved solids	35	3	-5	34
	Volatile solids	77	0.53	7	79

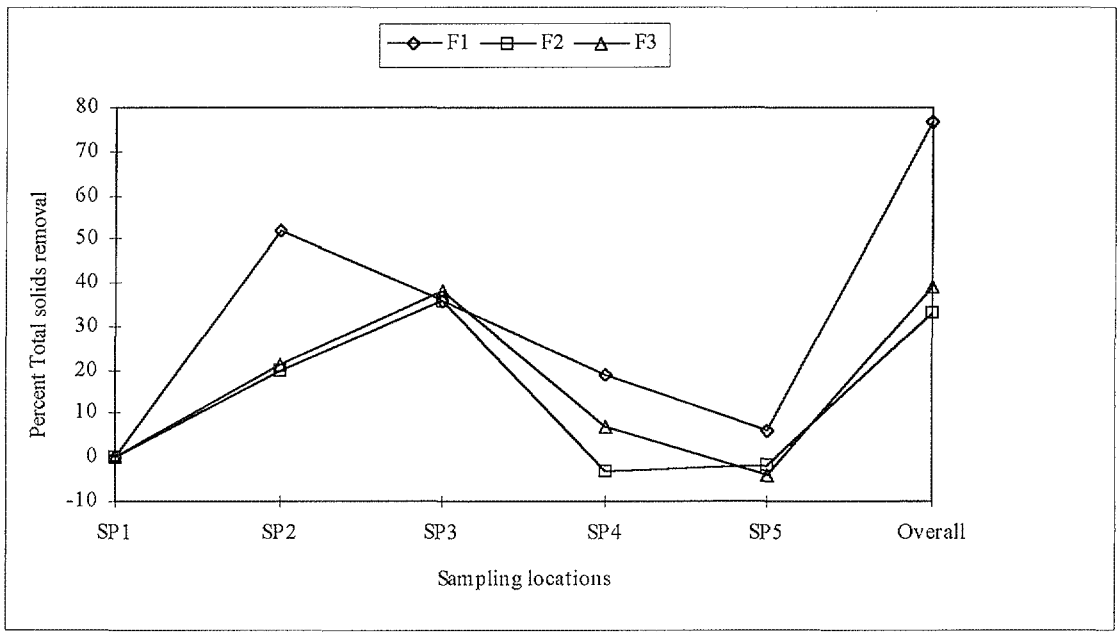


Figure 39. Total solids removal efficiency during each feed phase (F1, F2 and F3).

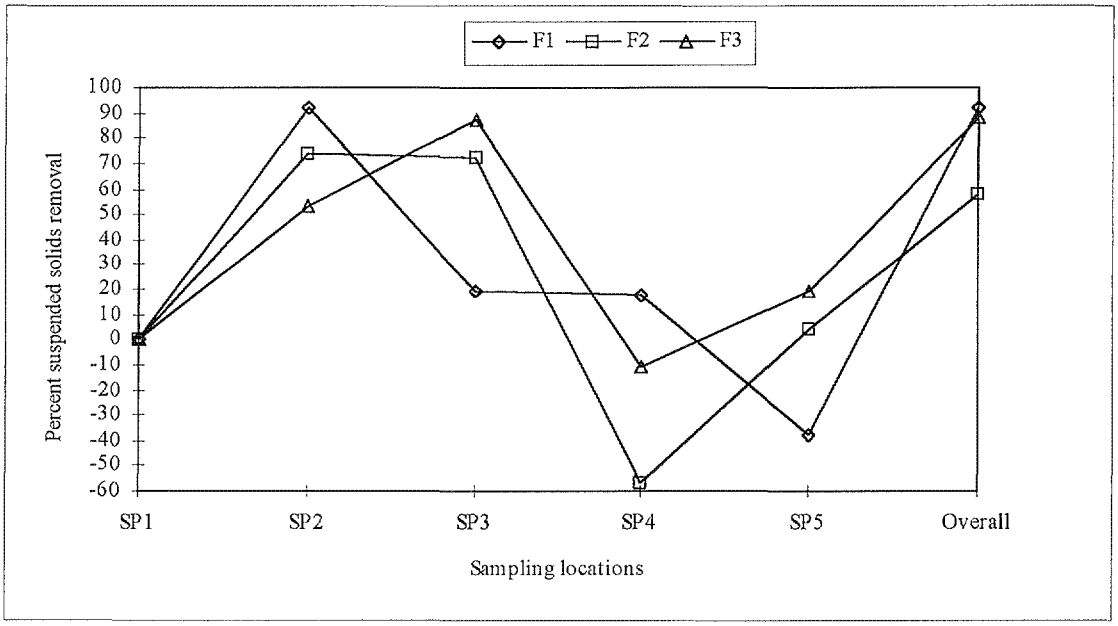


Figure 40. Suspended solids removal efficiency during each feed phase (F1, F2 and F3).

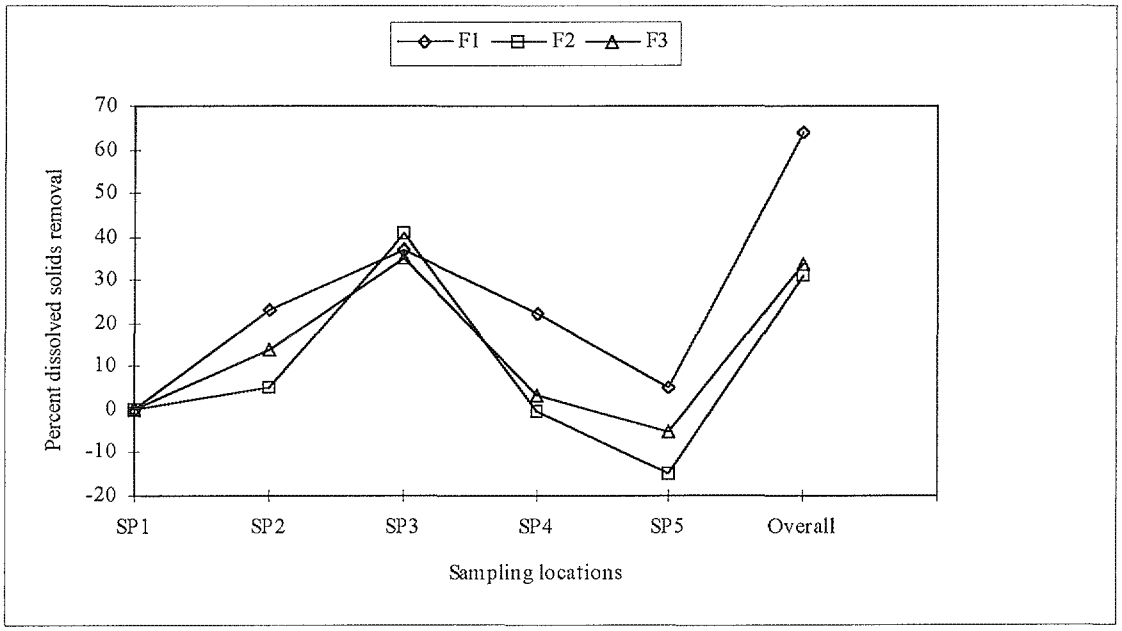


Figure 41. Dissolved solids removal efficiency during each feed phase (F1, F2 and F3).

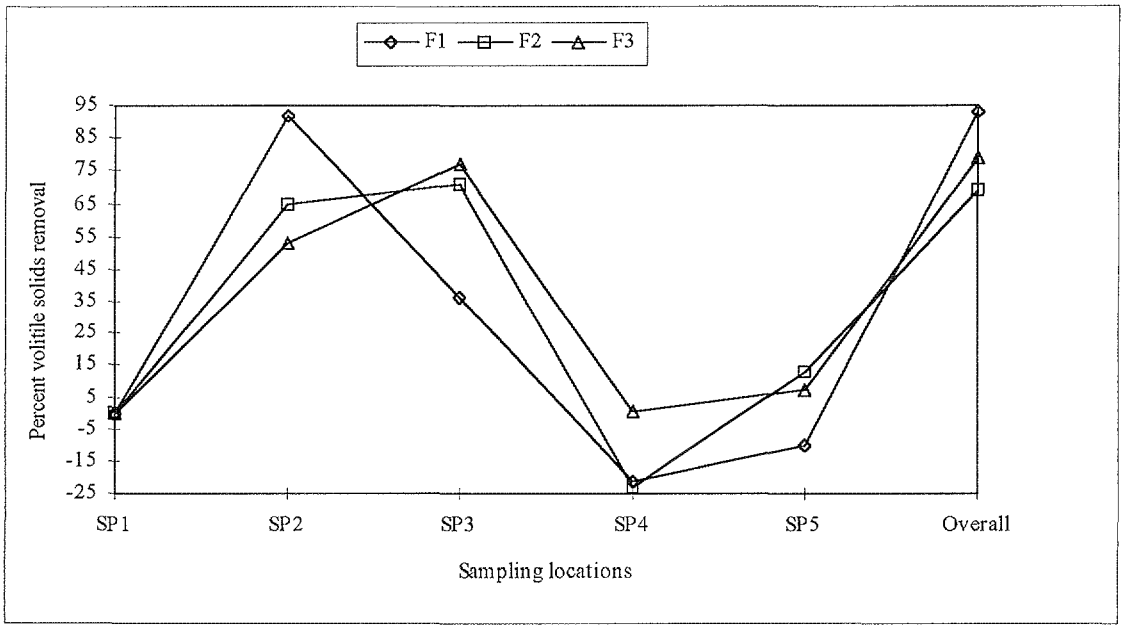


Figure 42. Volatile solids removal efficiency during each feed phase (F1, F2 and F3).

As already explained in page 32, highest removal efficiencies were observed on suspended solids and volatile solids. The overall dissolved solids removal was found to be the lowest in all the three phases. This could be due to that sodium chloride which contributed to the dissolved solids passed through the reactors unaffected.

Ammonia-nitrogen

Table 21. Mean ammonia-nitrogen concentration.

Feed Phases	Mean NH_3-N concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	137	272	137	56	40
F2	136	78	224	100	79
F3	114	137	242	120	89

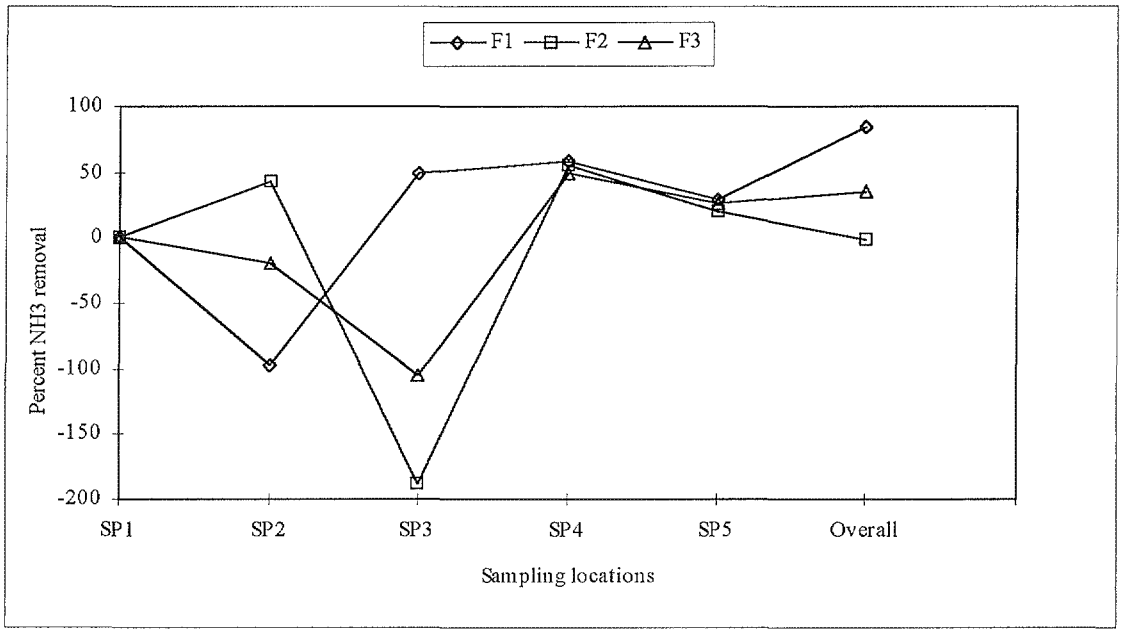


Figure 43. NH_3-N removal efficiency during each feed phase (F1, F2 and F3).

Ammonical nitrogen increased from 78 mg NH_3-N/l to 224 mg NH_3-N/l and from 137 mg NH_3-N/l to 242 NH_3-N in the AFP during feed phase 2 and feed phase 3, respectively. Intensive ammonification was the result of biological degradation of proteinaceous organic compounds under anaerobic condition in the AFP. In contrast ammonical nitrogen were reduced by a range 50-59% in the secondary facultative pond over the three experimental feed phases. A further ammonia reduction to a range 21-29% was also achieved in the maturation pond.

As already explained in page 37, the reduction in ammonia in the secondary facultative and maturation ponds was attributed to mainly assimilation in algal biomass. The organic and ammonia loading rates applied during phase 2 and phase 3 were not conducive for effecting high overall removal efficiencies. Attempts were made to get a full picture of the nitrogen transformation by analysing the Total Kjeldahl Nitrogen (TKN) and organic nitrogen of the raw and treated effluents. But, failure in the digestion apparatus prevented us from carrying out the tests.

Nitrate-nitrogen

Table 22. Mean Nitrate-nitrogen concentration.

Feed Phases	Mean NO_3-Nconcentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	Nil	Nil	Nil	2.4	2.3
F2	6	7	3	2	2
F3	27	19	4.6	2.3	2.5

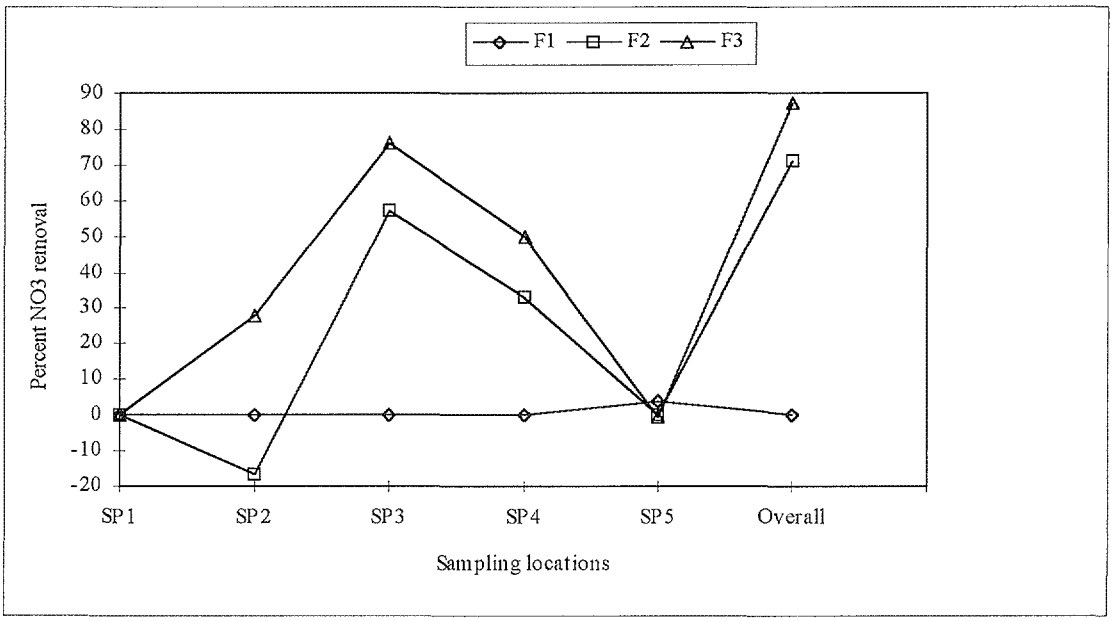


Figure 44. NO_3 removal efficiency during each feed phase (F1, F2 and F3).

The statement made in page 37 hods good for above presented nitrate data. The high overall nitrate removal efficiencies obtained during phase 2 & 3 should be viewed in relation to the relatively higher concentration of nitrate in the raw tannery waste and influent to the AIWPS reactors.

Chromium

Table 23. Mean Cr III concentration.

Feed Phases	Mean Cr III concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	46	13	0.66	0.32	0.31
F2	1.3	1.62	0.44	0.24	0.22
F3	4.2	6.7	1.37	0.94	0.93

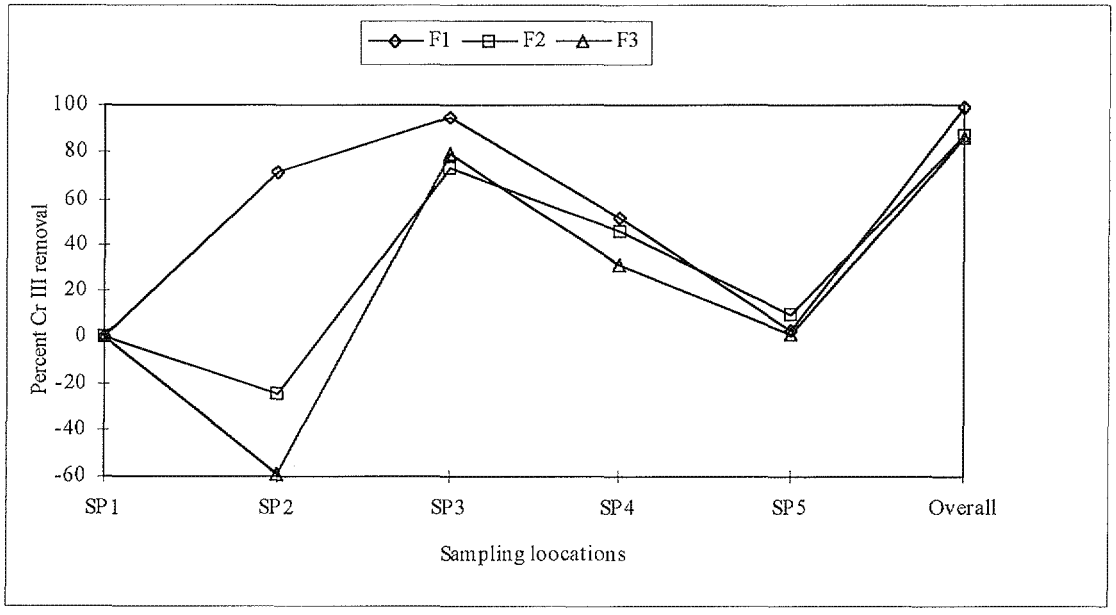


Figure 45. Cr III removal efficiency during each feed phase (F1, F2 and F3).

The increased loading in phase I and II did not as such result in significant changes in the overall Cr III removal. The overall chromium III removal efficiency were 99%, 86% and 86% during phase 1 phase 2 and phase 3, respectively.

Sulphates

Table 24. Mean SO_4^{-2} concentration.

Feed Phases	Mean SO_4^{-2} concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	841	624	129	159	161
F2	1,279	609	78	66	49
F3	842	765	54	26	18

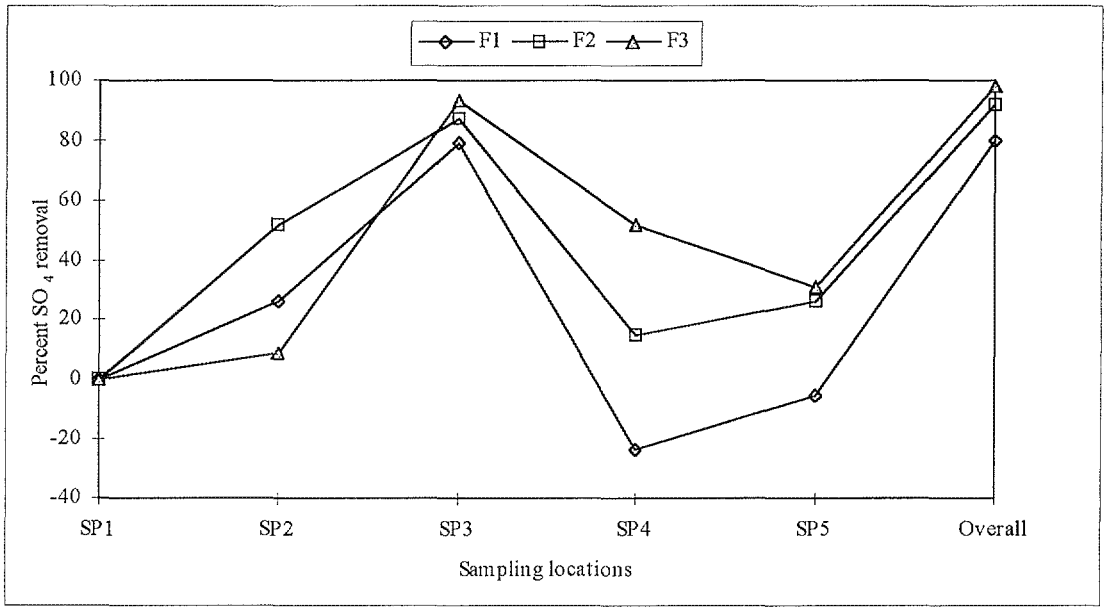


Figure 46. SO_4^{-2} removal efficiency at each experimental feed phase, F1, F2 and F3.

As expected and shown in the above data, the highest sulphate removal 79%-90% was observed in the AFP. In the fermentation pit of the AFP sulphate is reduced into sulphide. The increase in volumetric sulphate loading from 13.5 g/m^3-d in feed 1 through 21 g/m^3-d in feed 2 to 42 g/m^3-d in feed 3 did not as such result in reduction of the systems performance. Instead, sulphate removal efficiency in the AFP has increased from 79 % in phase 1 to 87 % and 93 % in phase 2 and phase 3, respectively.

The overall sulphate removal efficiency of the AIWPS system has also increased from 80 % in phase 1 to 92 % and 98 % in phase 2 and phase 3.

Sulphide

Table 25. Mean $S^{=}$ concentration.

Feed Phases	Mean $S^{=}$ concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	422	174	90	1.4	0.7
F2	494	328	115	40	13
F3	324	214	81	43	7.6

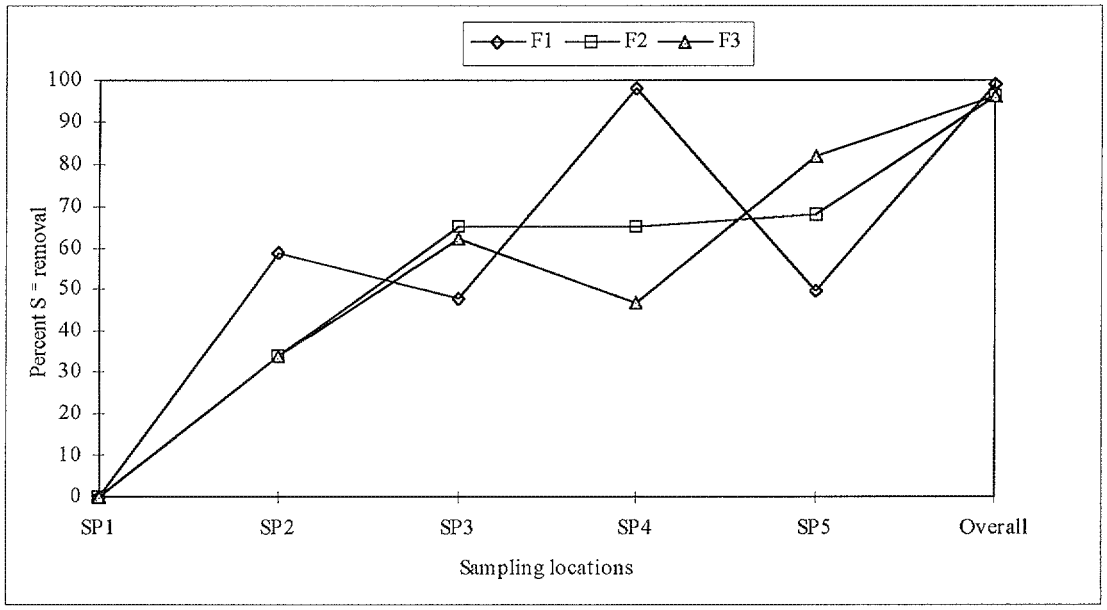


Figure 47. $S^{=}$ removal efficiency during each feed phase (F1, F2 and F3).

The overall sulphide removal efficiency remained high even at increased organic loading rates (96 % for both F2 and F3). It is also interesting to note that there was a shift in the highest sulphide removal efficiency from the secondary facultative pond (98%) during feed 1 to the maturation pond (68% and 82%) during feed 2 and feed 3.

Hydrogen sulphide

Table 26. Mean H_2S concentration.

Feed Phases	Mean H_2S concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	0	3.64	7.57	0	0
F2	0	0	8.6	1.11	0.31
F3	0	0	4.1	1.29	0.16

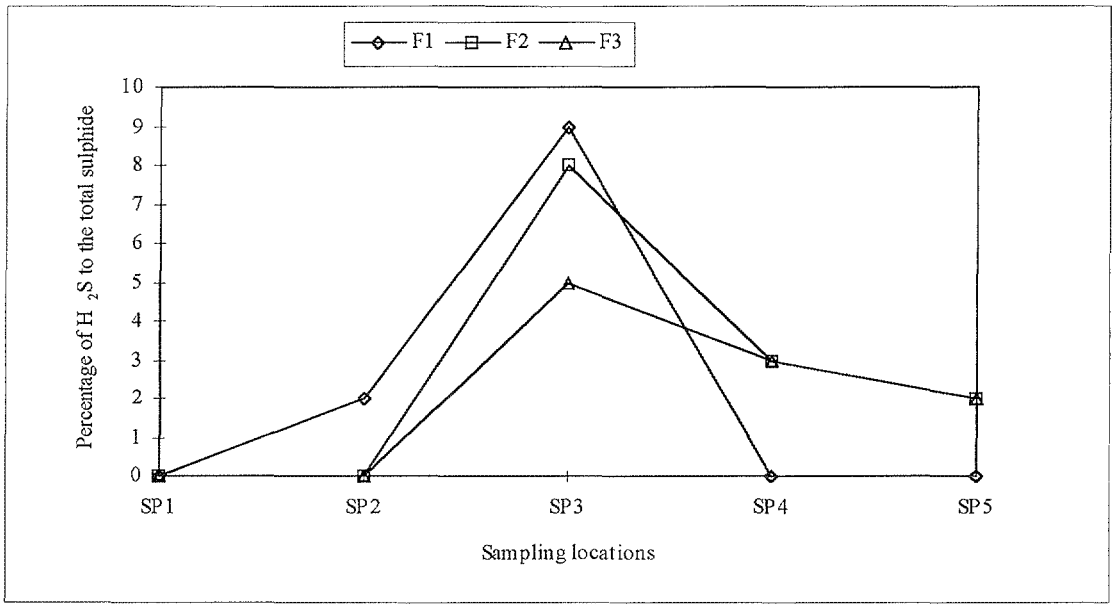


Figure 48. Proportion of H_2S to the total sulphide.

The evolution of H_2S from secondary facultative and maturation ponds, (though insignificant in quantity) was attributed to increased organic and sulphate loading rates. The emission did not, however, pose any odour problem as H_2S was less than 8% compared to the total dissolved sulphide (Figure 48.)

Phosphorus

Table 27. Mean phosphorus concentration.

Feed Phases	Mean phosphorus concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1 (P_T)	39	11	32	20	26
F2 (Ortho)	8	8	2	0.7	0.8
F3 (Ortho)	16	12	3	1.2	1.1

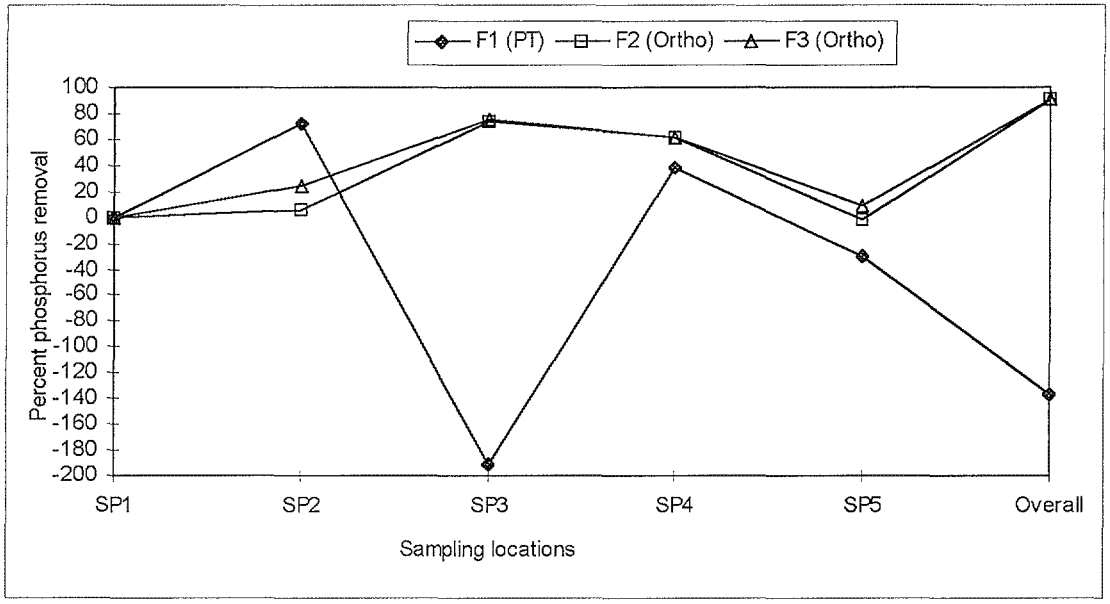


Figure 49. Phosphorus removal efficiency during each feed phase (F1, F2 and F3).

It is clear from Table 26 and Figure 49 that orthophosphate removal (90%) was higher, even at a higher organic loading, than the total phosphorus removal. This was mainly due to the fact that orthophosphates are more readily utilized by bacteria and micro-algae than the other forms of phosphorus, namely organic and polyphosphates.

Chloride

Table 28. Mean Cl^- concentration.

Feed Phases	Mean Cl^- concentration (mg/l)				
	SP1	SP2	SP3	Sp4	SP5
F1	2,938	2,482	2,092	1,900	1,824
F2	4,825	4,837	3,394	3,544	3,809
F3	4,031	4,094	2,781	2,719	2,656

The previous statement made on chloride removal on page 46, holds good for the performance of the biological reactors observed during increased loading rates. The fact that sodium chloride is not amenable to any biological treatment method was clearly demonstrated by low overall removal percentage (21-39%). It is, therefore, a good strategy to segregate salt laden effluent from the general effluent and treat it separately, perhaps in solar pond for eventual recovery of crude salt.

Electrical conductivity

Table 29. Mean EC concentration.

Feed Phases	Mean EC concentration (µS/cm)				
	SP1	SP2	SP3	Sp4	SP5
F1	12,769	10,310	9,450	7,465	7,112
F2	13,786	11,842	11,495	11,169	11,304
F3	15,449	13,320	13,034	11,741	11,781

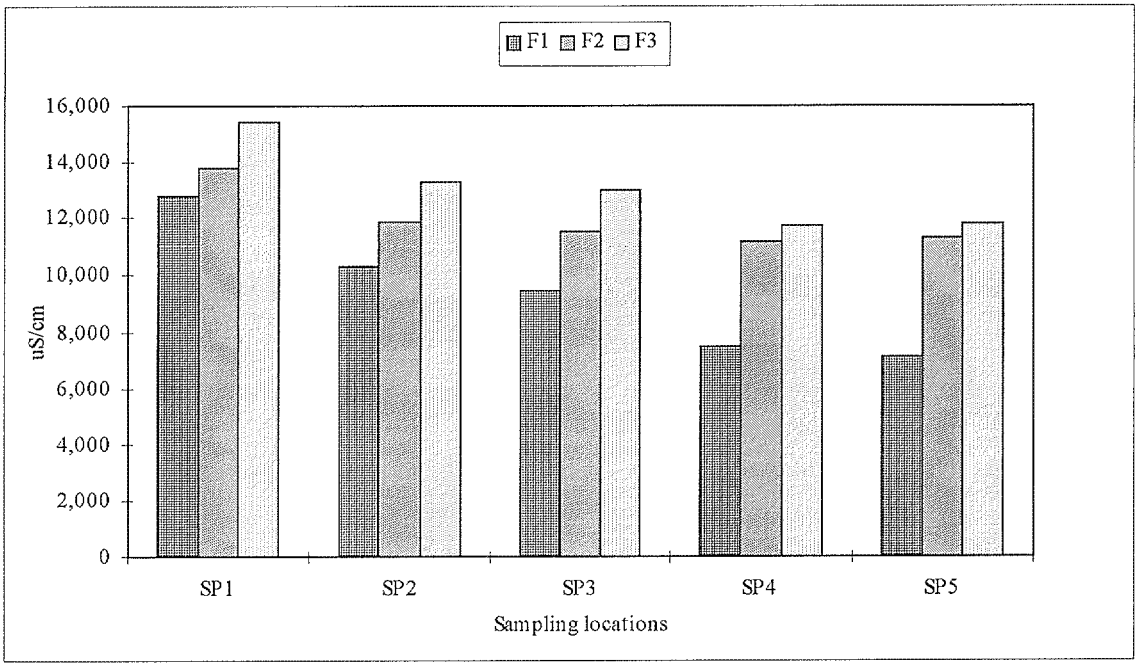


Figure 50. Electrical conductivity along the sampling locations.

A gradual build up of electrical conductivity was observed as both volumetric and organic loadings increased.

Operational temperatures

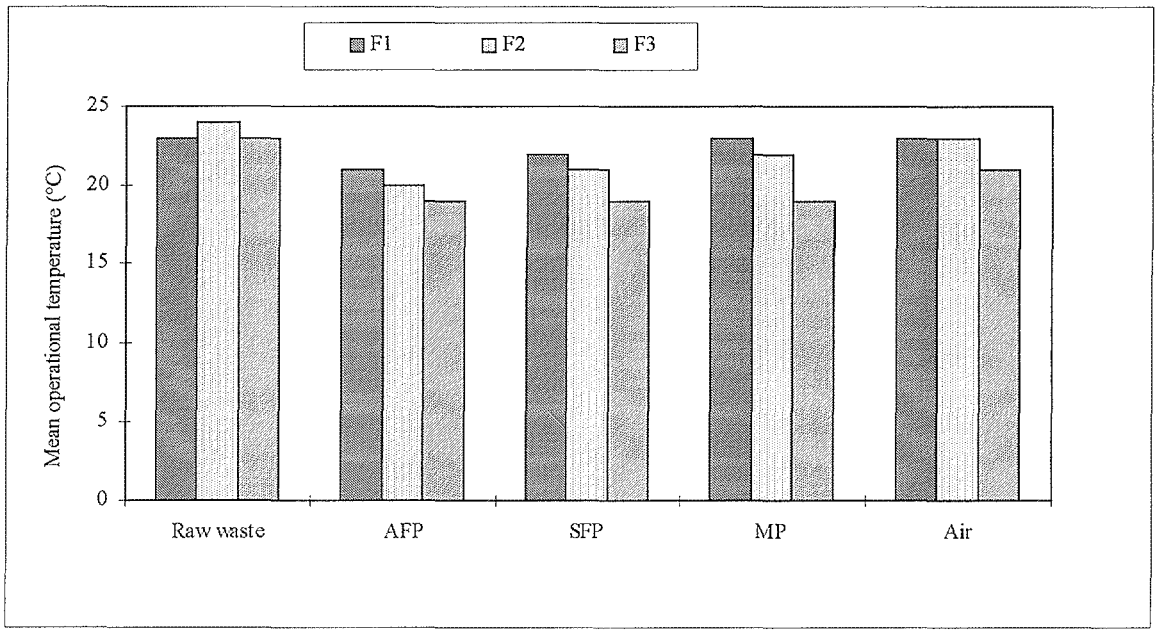


Figure 51. Operational temperature during eachfeed phase.

There was no much operational temperature variation among the three feed phases. The temperature of raw tannery waste, varied between 22 and 26 °C. The anaerobic fermentation in the AFP operated under sub-mesophilic temperatures (19-21°C), while SFP and MP operated at 22 °C and 23 °C, respectively. The mean air temperature stayed a little above 23 °C.

Diurnal variation of temperature, pH and dissolved oxygen

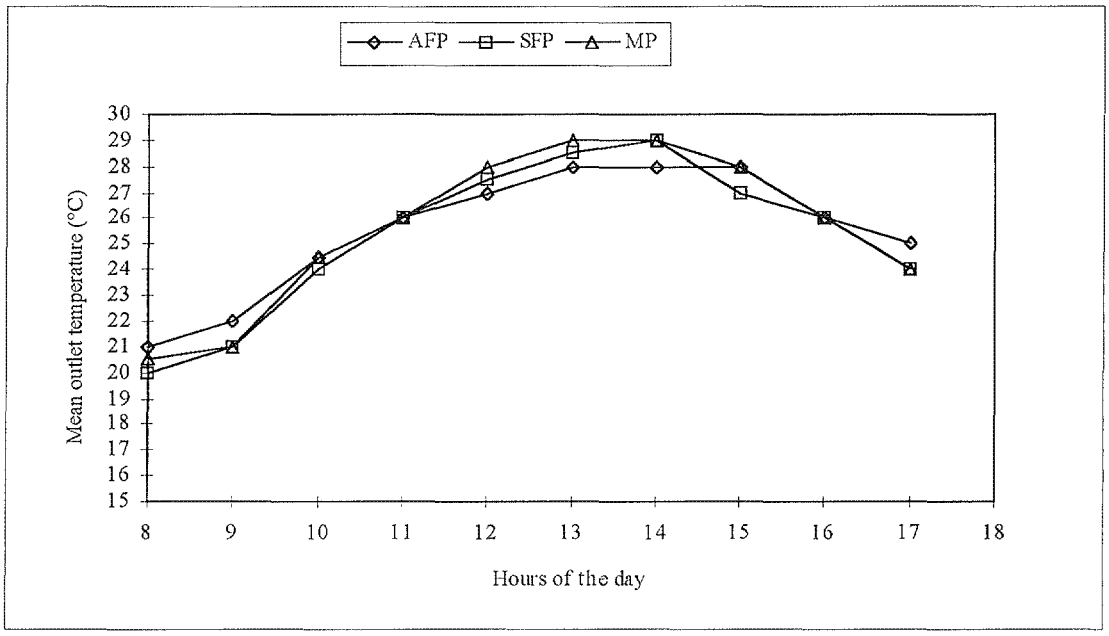


Figure 52. Diurnal variation of temperature over the pilot biological reactors during feed phase I.

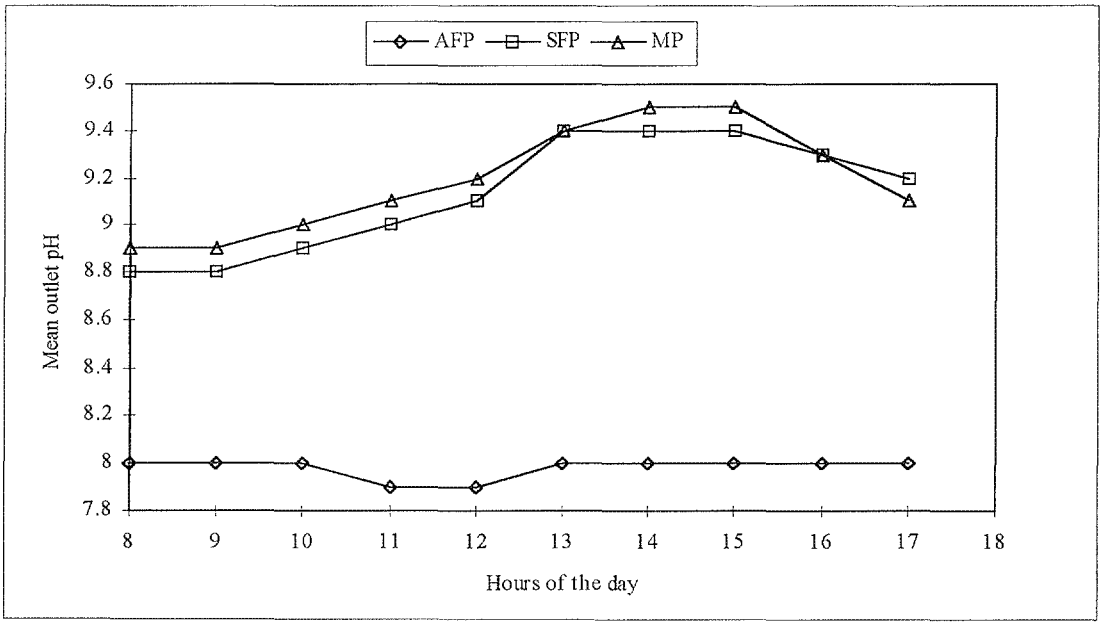


Figure 53. Diurnal variation of pH over the pilot biological reactors during feed phase I.

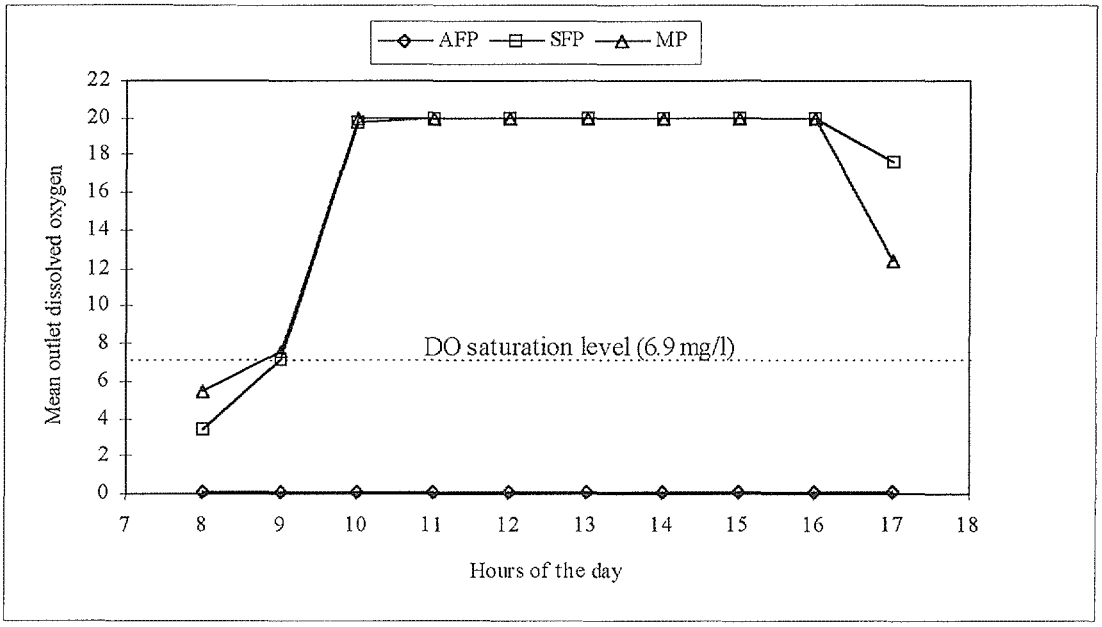


Figure 54. Diurnal variation of dissolved oxygen over the pilot biological reactors during feed phase I.

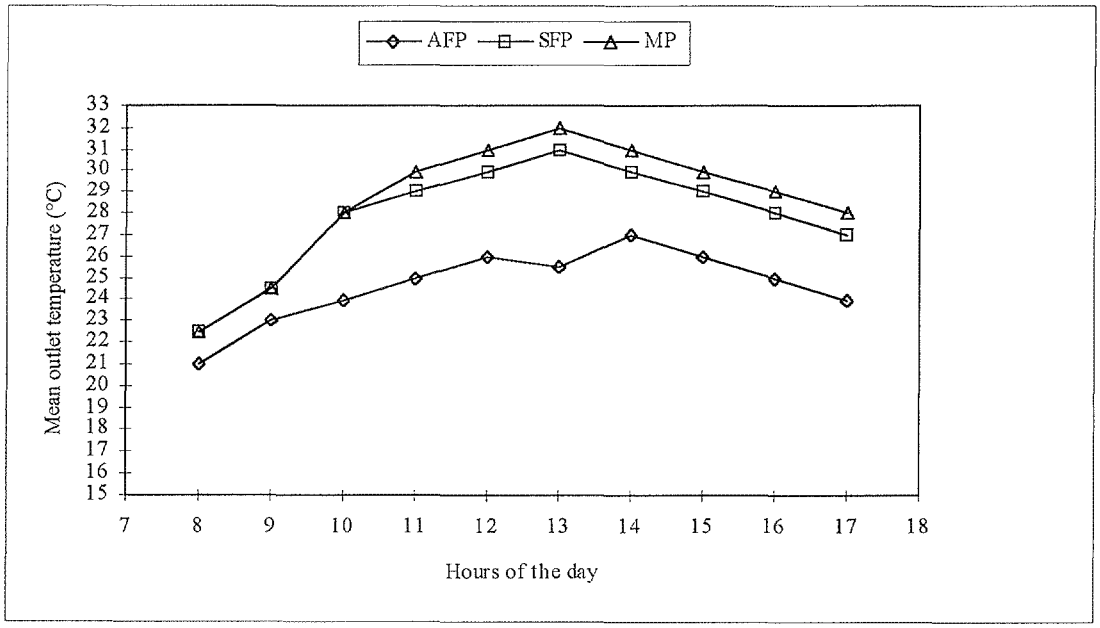


Figure 55. Diurnal variation of temperature over the pilot biological reactors during feed phase II.

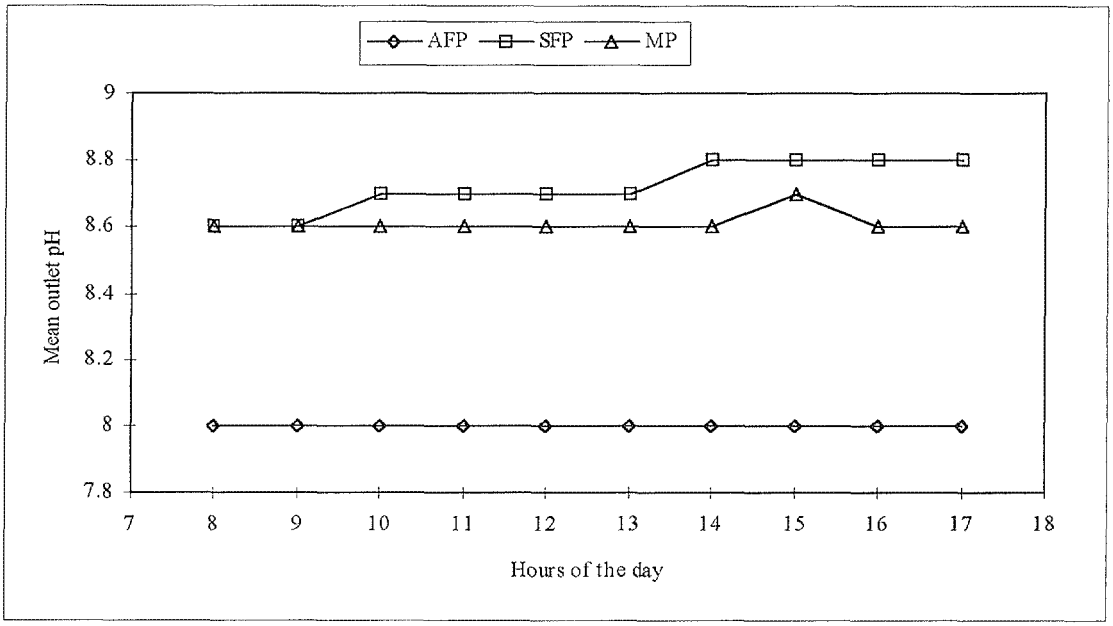


Figure 56. Diurnal variation of pH over the pilot biological reactors during feed phase II.

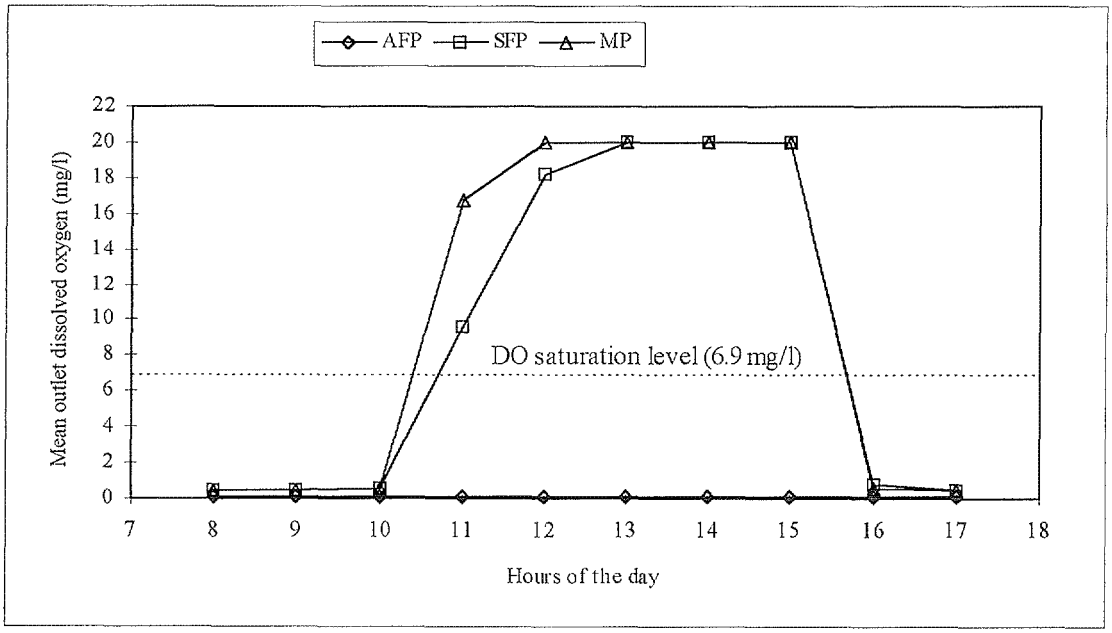


Figure 57. Diurnal variation of dissolved oxygen over the pilot biological reactors during feed phase II.

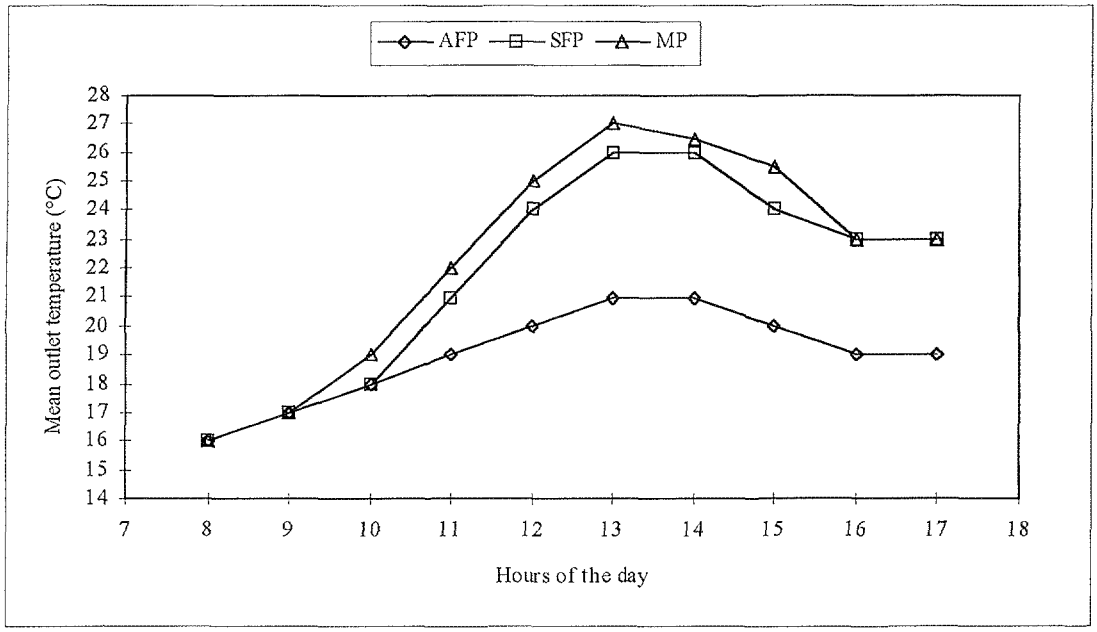


Figure 58. Diurnal variation of temperature over the pilot biological reactors during feed phase III.

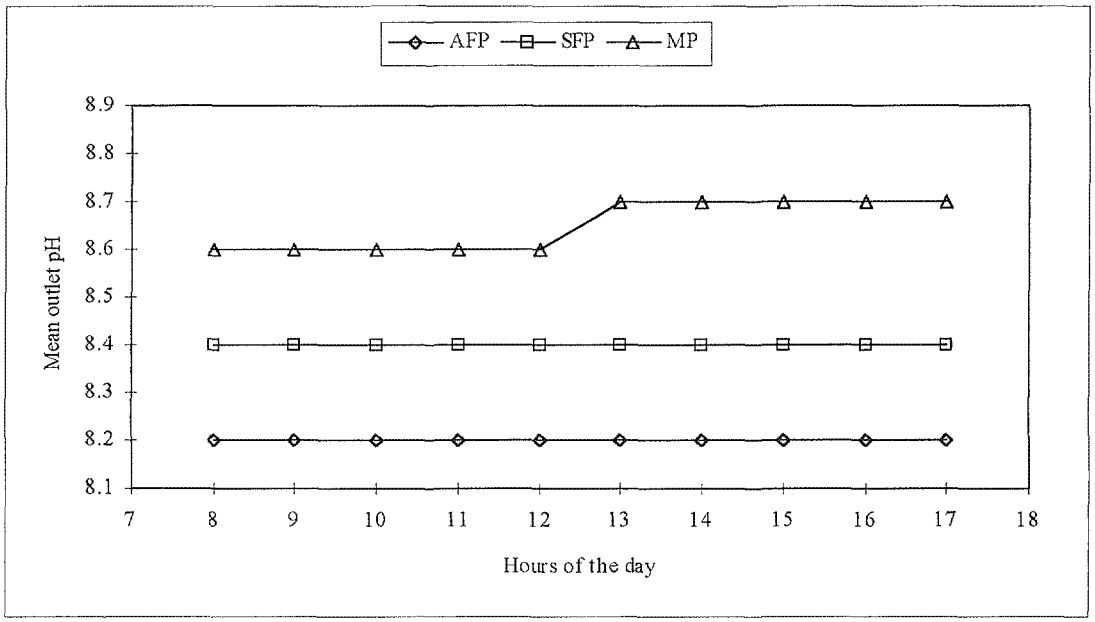


Figure 59. Diurnal variation of pH over the pilot biological reactors during feed phase III.

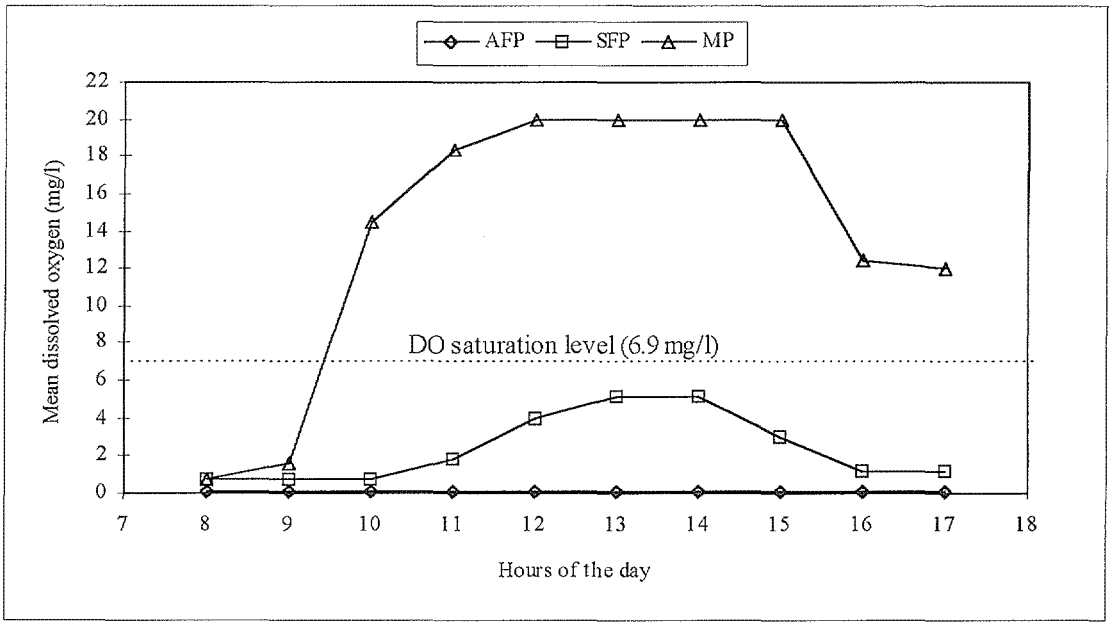


Figure 60. Diurnal variation of dissolved oxygen over the pilot biological reactors during feed phase III.

In the diurnal variation of temperature, pH and dissolved oxygen presented in Figure 50 to Figure 60, the peaks occurred between 1300 hours and 1500 hours. In the secondary facultative and maturation ponds, dissolved oxygen followed the temperature and pH variations. This is somewhat expected, because photosynthetic oxygenation will be high when solar insolation and consequently the temperature are high. When algal photosynthesis intensifies, CO_2 is obtained from dissociation of bicarbonate ions, thereby increasing the pH. Close examination of Figures 54, 57 and 60 revealed that the spread of DO curve decreased, as expected, as the organic loading increased. At the last phase of the experiment, where BOD loading has been increased by more than three folds (228 kg/ha-d during phase 1 to 975 BOD kg/ha-d during phase 3). Dissolved oxygen level in the SFP was depressed. But, the depression remained within 55-75% of the saturation level. The increased loading did not, on the contrary, affect the DO level in the MP.

Biogas production in the AFP

Table 30. Biogas production versus COD loading.

Phase	Biogas production versus COD loading								Average
Feed I	COD loading (g COD/m^3-d)	640	679	718	608	736	448	460	568
	COD load removal (g COD/d)	2,409	2,414	2,304	2,029	2,398	1,529	1,528	2,087
	Biogas production (l/d)	40	16	50	74	47	57	80	52
Feed II	COD loading (g COD/m^3-d)	1,359	1,711	1,920	1,900	549	1,093	1,257	1,540
	COD load removal (g COD/d)	4,465	5,910	6,894	1,640	-	3,402	3,625	4,859
	Biogas production (l/d)	13.6	54	9.3	17.6	25.6	9.6	-	22
Feed III	COD loading (g COD/m^3-d)	1,592	1,876	2,030	2,205	-	-	-	1,926
	COD load removal (g COD/d)	3,698	4,502	5,733	6,615	-	-	-	5,137
	Biogas production (l/d)	0.87	0.063	0	0	0	0	0	0

As can be seen from Table 28, excessive organic loading resulted in decreased biogas generation. A three-fold increase in organic loading was observed between phase I and III. Moderate loading (two and half as much higher) was also observed during phase II.

The quantity of biogas produced during anaerobic degradation is generally related to the amount of organic matter destroyed. It should be, however, borne in mind that biogas production could not continue unabated with increased organic loading. After a certain stage of loading, drastic changes in microbial speciation and their metabolic pathway could occur. These drastic changes, in turn, alter the relative dominance of one group of anaerobic bacteria over the other. Under overloading conditions (phase III), methanogenesis is generally regarded as the rate limiting step. A rate limiting step is the step with the lowest rate of reaction and affects the overall reaction. Because of this, it is logical to focus on the methanogenesis process. Among the factors generally acknowledged for retarded methanogenesis process are:

- excessive feed organic loading
- outcompetition of methane producing (MP) bacteria by sulphate reducing (SR) bacteria
- accumulation of induced and/or generated inhibitory substances

Excessive feed organic loading

As already stated, methanogenic process is very much affected by overloading condition. Unfavourable conditions associated with overloading can be explained in two ways: (i) overloading could increase accumulation of volatile acids produced by acetogenic bacteria over and above the rate decomposed by methane bacteria, and (ii) overloading could, at times, result in high volumetric loading, thereby increasing the early washout of useful group of bacteria. Washout could upset the useful microbial balance. Some investigators have, however, argued that it is the COD:$SO_4^=$ ratio which is important in explaining the effect of overloading to methanogenic process. Prasad et al. (1991) have found that at COD:$SO_4^=$ ratio above 1, methane producing bacteria (MPB) outcompeted sulphate reducing bacteria (SRB) and below 1 SRB predominated. According to such findings, the overloading must have favoured MPB over SRB. Referring to Tables 19 and 24 above, the COD:$SO_4^=$ ratios were 7.3, 12 and 7.6 for phase 1, phase 2 and phase 3, respectively. In all the three phases, the COD: $SO_4^=$ ratios were found well above 1. There was, of course, a noticeable decrease in hydraulic retention time in the fermentation pit from phase 1 to phase 3. Hydraulic retention time has been lowered from 8 days during phase 1 to 3 days during phase 3. To conclude that shortening of the hydraulic retention time has resulted in early washout of the methanogenic bacteria is generally unwarranted as it is the solids retention time that has more relevance to the phenomenon of microbial washout. There is a general consensus among investigators that methanogenic bacteria have a higher capacity of attachment to the reactor support media than sulphate reducing bacteria. So in suspended growth reactor of ours, any operation that shortened the solid retention time would certainly have some effect on methanogenic bacteria. Whatever the case, the solid retention time in the fermentation pit was certainly much higher than 3 days. As we did not do a thorough investigation on solids retention time and microbial concentration in the fermentation pit, we could not, for sure, rule out the possible effect of overloading on methanogenesis.

Competition between MPB and SRB

To maintain an anaerobic treatment system that will stabilize organic waste efficiently, the non-methanogenic and methanogenic bacteria must be in state of dynamic equilibrium. One group of non-methanogenic bacteria is the sulphate reducing bacteria (SRB). Hydrogen and acetate are the key precursors to methane formation during anaerobic waste degradation. Hydrogen and acetate may also serve as electron donors for sulphate reduction, therefore, MPB and SRP must be considered as competing for these substrates in anaerobic system where significant sulphate is present. The potential for MPB to outcompete SRB exists when the electron donor (acetate or hydrogen): sulphate ratio become high or when a buildup of sulphide occurs. Conversely, it would be expected that SRB would outcompete MPB when the electron donor: sulphate ratios were low and if sulphide concentrations were not allowed to buildup (McCartney and Oleszkiewicz, 1993)

Accumulation of induced and generated inhibitory substances

Close examination of the data given in Tables 21& 26 and media pH within AFP could give some clue as to whether there was some form of inhibition to the methanogenesis process. In all the three phases, pH varied from 7.2 at sludge water interface to 8.2 at the surface of AFP, while ammonia-nitrogen increased along with the loading (Table 21). A 76% ammonia increase was observed within AFP during phase III. This would have some effect on methanogensis the biogas production. Referring to Table 24, sulphate input to the fermentation pit, in AFP, did not show much variation. Sulphate reduction in the AFP increased as organic loading and ammonia concentration increased. A fairly constant pH observed through out the experiment might be due to the system's high buffering capacity. High alkalinity of the raw tannery waste and an internal input of alkalinity (mainly as bicarbonate) from the anaerobic degradation of the waste has prevented the pH from dropping further. From the above discussion, it is clear that both overloading operational condition and increased ammonia concentration have more adverse effect to methanogenesis than to sulphate reduction. Inhibition rating among the various sulphur compounds is: H_2S > total sulphide > sulphite > thiosulphite > sulphate (McCartney and Oleszkiewicz, 1993). According to this rating, the unionized sulphide, H_2S is the most toxic to both methanogenic and sulphate reducing bacteria.

Data in Table 26 proved that concentration of H_2S, in the AFP, did not either reach toxicity or nuisance level. Total sulphide input to AFP during phase 2 and 3 was higher than that in phase 1 (Table 25). But, due to proliferation of sulphide oxidizing bacteria sulphide did not buildup in AFP. Although biogas production has ceased at the highest organic loading (Table 28), BOD and COD removal efficiencies remained fairly high. In all the three feed phases, the highest BOD and COD removal took place in the AFP (Table 18 & 19). The maximum COD removal efficiency below which anaerobic reactors begin to serve as a primary sedimentation basin without any biological degradation is 30% (Toprak, 1995). At the highest organic loading (phase 3), COD removal efficiency was close to 60%. The system has, therefore, relied on removal mechanism other than sedimentation. The fact that anaerobic system continued to perform well in terms of organic removal while methanogenesis was upset proves that some kind of biological degradation was still active.

Biological performance

Aerobic reactors of the system, continued to perform well even under overloading conditions. Algological examination of water column samples from SFP and MP (Annex 5) showed that the most tolerant *Chlamydomonas fenestrata* continued to dominate during heavily loaded operations of phase 3. Chlamydomonas dominance was attributed to its high mobility and ability to maintain itself in the euphotic (light-rich) layer of loaded ponds. It usually forms a surface film. However, during bright sunlight these flagellate algae retreats a few centimetres from the surface. The other speciality of this group of algae is that they are by far the most tolerant to sulphide inhibition. Studies conducted by Pearson et al. (1987) on the effects of sulphides on four pond algal isolates demonstrated that Chlamydomonas was the least affected by sulphide followed by Chlorella and Scenedesmus with Euglena being the most sensitive. Alabaster et al. (1991) have also found Chlamydomonas as the most dominant algal genera in heavily loaded ponds in Kenya. Examination of the results given in Tables 25 and 26 reveals the high concentration of both sulphide and H_2S in the effluent samples of SFP and MP during operations of high organic loading. Sulphide concentration in the effluent samples of SFP during phase 3 was found 30 times higher than that during phase 1. The increase in sulphide concentration in the MP during phase 3 was 10 times as much higher. Hydrogen sulphide which was undetectable during phase 1 has also increased in the effluent samples of SFP and MP during phase 2 and phase 3 (Table 26).

The overloading did not, however, lower the chlorophyll a and photosynthetic oxygen concentration in the two reactors as the oxygenation depends more on biomass than on a particular algal species.

Table 31. Algal biomass measured as Chlorophyll a during phase 3.

Time of sampling	Reactor	Depth below SWL (cm)	Chl a (µg/l)
13:35	SFP	5	3927
13:45	,,	10	3955
13:50	,,	15	1365
13:55	,,	30	391
12:13	MP	5	2260
13:15	,,	10	703
13:20	,,	15	1285
13:35	,,	30	250

The above chlorophyll a concentration can be compared with the result obtained during phase 1 of the operation (Table 12).

Hydraulic conditions

Hydraulic flow conditions have an important influence on the performance of any biological reactor as waste characteristics, environmental conditions and physical factors of pond geometry do have. The hydraulic flow characteristics obviously have an effect on the dispersion of the waste material as well as the average detention time and, ultimately, on the organic and inorganic and pathogenic organism removal efficiency of the treatment process. To evaluate the hydraulic performance of the pilot reactors, a tracer chemical test was performed. The objectives of the tracer chemical test were:

(i) to verify the theoretical hydraulic retention time, and

(ii) to determine the hydraulic regimes governing the flow pattern in each biological reactor

There are three types of hydraulic flow conditions of which two are ideal and the other non-ideal. The two ideal flow regimes which are extreme cases are the plug and completely mixed flow conditions. In reality, no biological reactor operates in a plug or completely mixed flow condition. However, with some manipulation in the reactor geometry, inlet-outlet arrangement and manner of mixing, biological reactors could approach plug or completely mixed flow regime. Reactors with rectangular aerial geometry and having high length to width ratios and closely baffled in their longitudinal direction with minimal turbulence are generally regarded as behaving close to ideal plug flow condition. Reactors with squarish or circular geometry and subjected to intensive mixing approach the ideal completely mixing flow condition. The third flow regime, which lies in between the two ideal conditions, is the dispersed flow. Most biological reactors operate under this condition. In the actual flow condition (dispersed flow), situations such as stagnant pockets, dead spaces, short-circuiting, channeling, recycling and eddying will have adverse effects on the performance of biological reactors.

The tracer study

The tracer study was done by continually adding a known quantity of tracer chemical in the inlet of each AIWPS reactor. The tracer chemical fed into each reactor was first dissolved in 8 feed holding barrels each with a volume of 160 m^3. The solution was then continually fed into each reactor using peristaltic pump operated at feed rate designated for each experimental phase. The concentration of the chemical was then measured at the outlet of each reactor over 1 hour interval. The outlet concentration data was further analysed to prepare time-concentration curves, F-diagrams and coefficients for verifying the hydraulic flow type.

At the beginning, NaCl was used as a tracer chemical. But, its high concentration in the media water (borehole water) coupled with lack of uniformity in concentration between successive feeds resulted in unreliable data. We then switched to $CuSO_4.5H_2O$ as a tracer chemical. For this study, 56 g powder $CuSO_4.5H_2O$ was dissolved in 8 barrels to give feed concentration ranging from 10-18 mg Cu/l. Copper was analysed as per Bicinchoninate method using HACH DR 3000 spectrophotometer. As the detection limit of the instrument was 5 mg Cu/l, samples were diluted 25 times to bring their concentration within the detection limit of the instrument.

Interpretation of the results

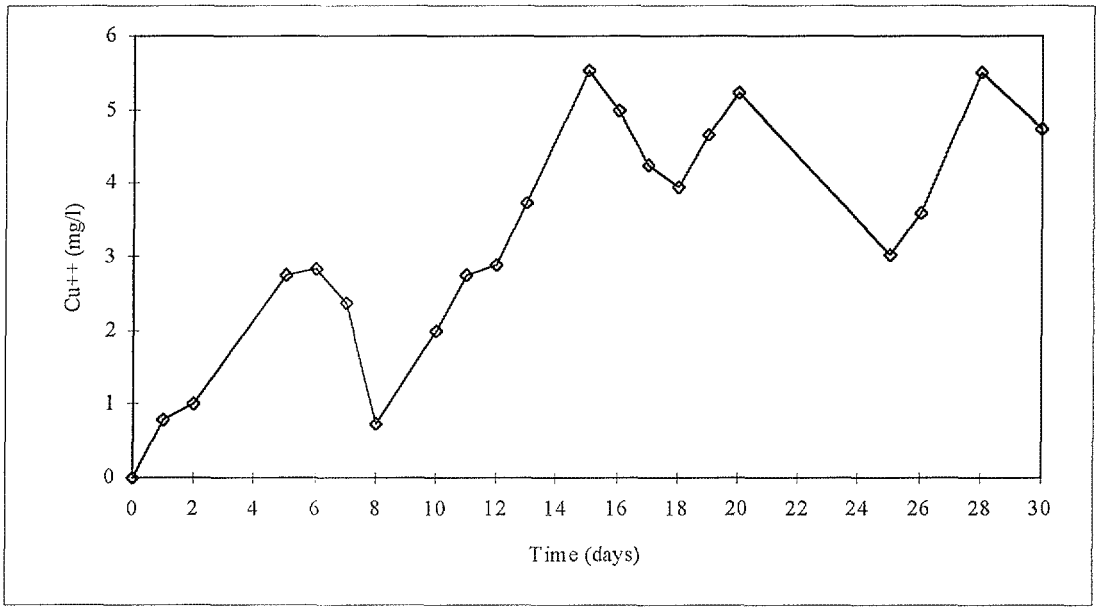


Figure 61. Time-concentration curve for continuous input of the tracer in AFP.

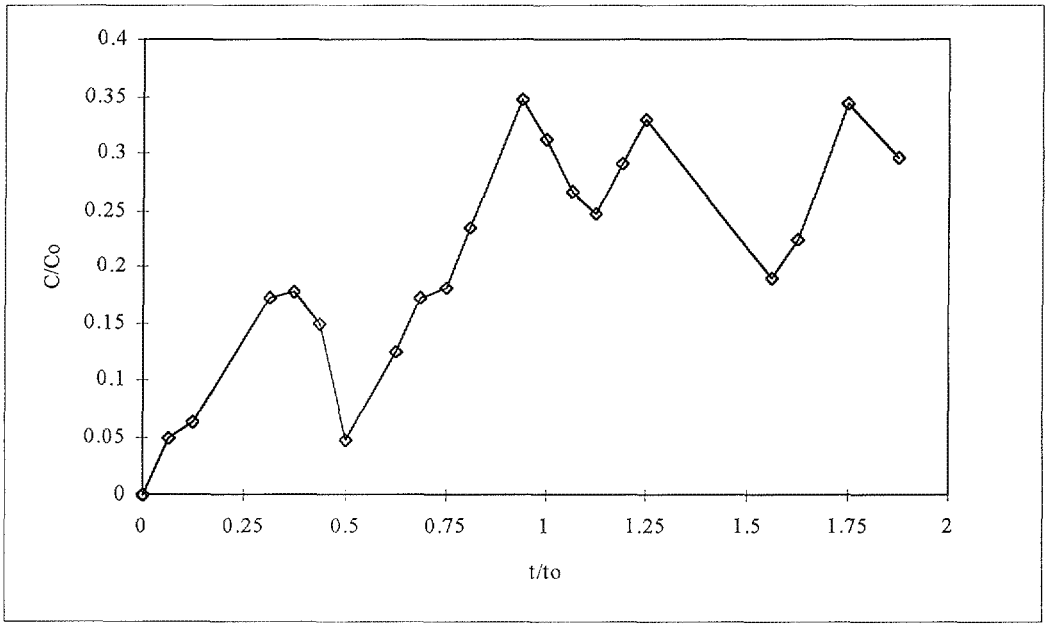


Figure 62. F curve for continuous input of tracer in AFP

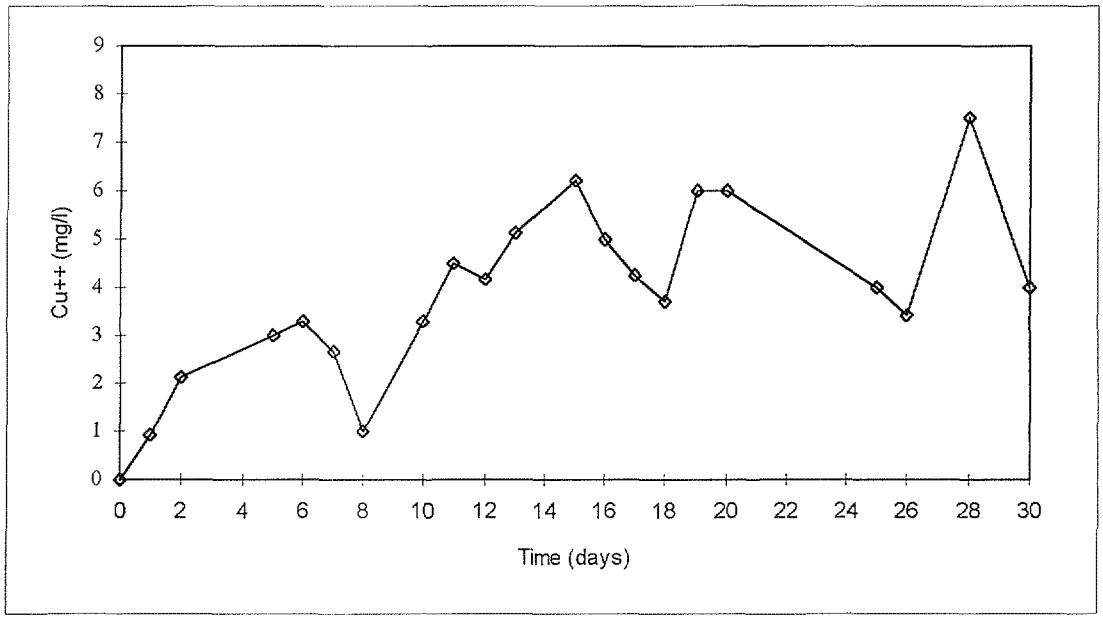


Figure 63. Time-concentration curve for continuous input of the tracer in SFP.

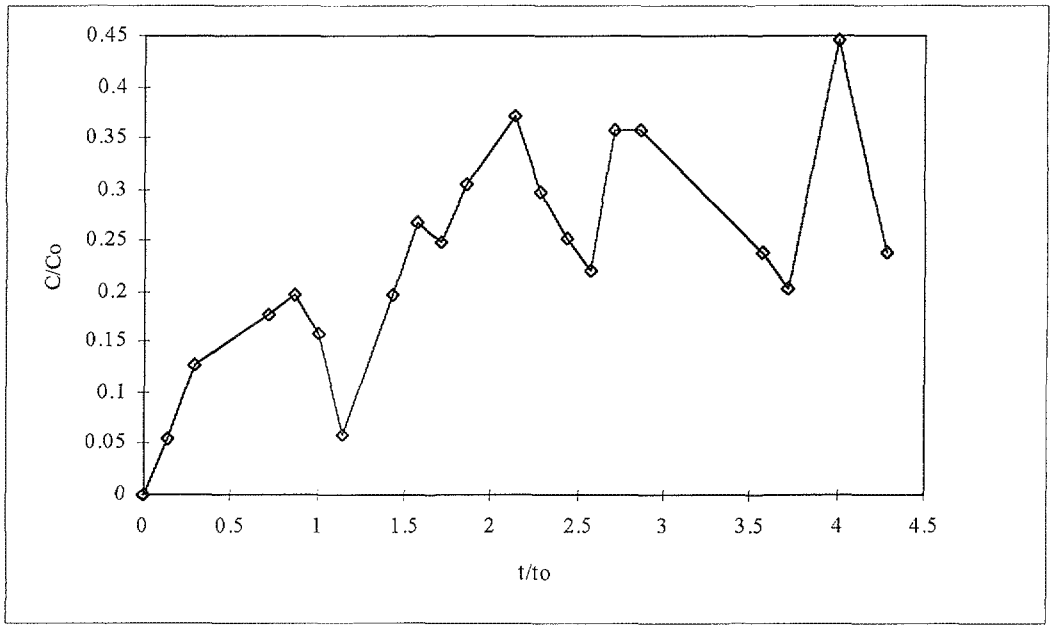


Figure 64. F curve for continuous input of tracer in SFP.

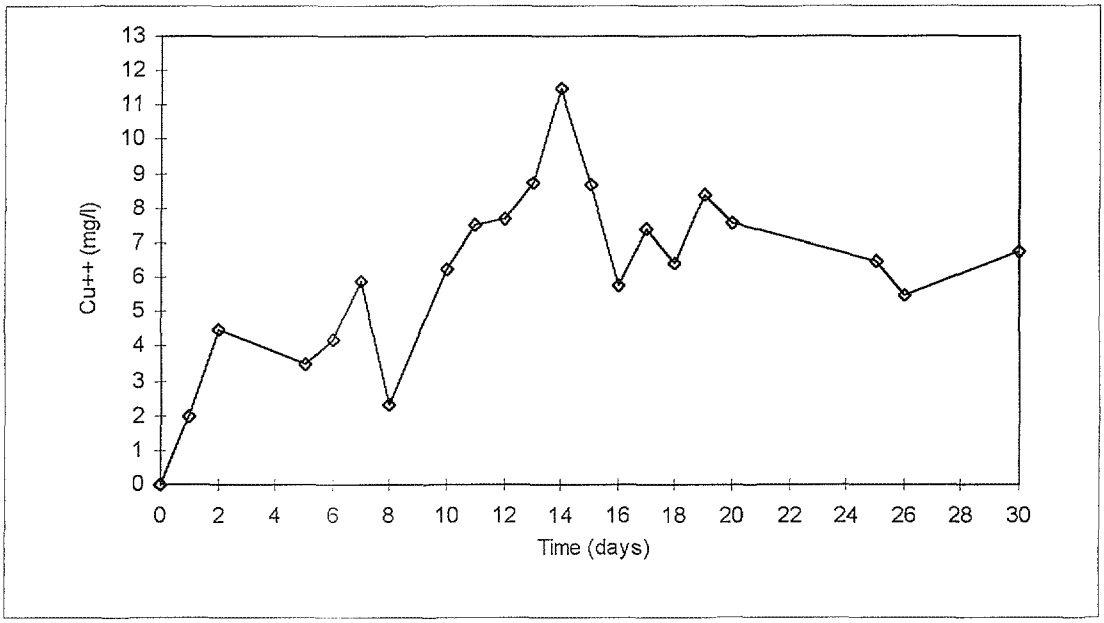


Figure 65. Time-concentration curve for continuous input of the tracer in MP.

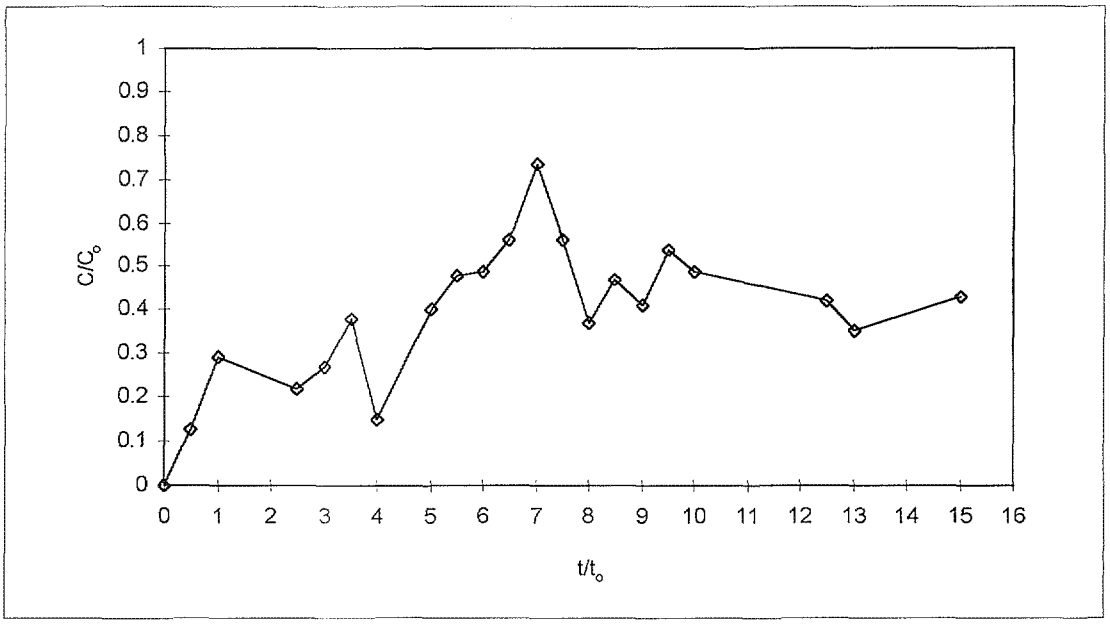


Figure 66. F curve for continuous input of tracer in MP.

From the preceding time-concentration curves and F-diagrams, it appears that the flow in the reactors have a moderate degree of dispersion close to the plug flow condition. The F-diagram gives the fraction of the tracer chemical that appears in the effluent. The dimensionless ratio of concentration C/C_o shown by the ordinate consists of:

C = actual concentration of the tracer in effluent

C_o= actual concentration of tracer continuously entering each reactor

In addition, computation was made to determine a dimensionless term, the dispersion number expressed as D/UL, where:

D = longitudinal dispersion coefficient (m^2/h)
L = mean path length of travel of typical particle in the reactor (m)
U = mean velocity of fluid (m/h)

The D/UL values vary widely from 0 to $\propto$. As dispersion is absent in ideal plug flow and, therefore, D = 0 and D/UL = 0. Conversely as dispersion is infinite in ideal complete mixing and D = $\propto$ and, therefore, D/UL = $\propto$. The D/UL values for dispersed flow regime lie in between 0 and $\propto$. Many investigators have tried to establish D/UL for many biological reactors. In general, the D/UL values range from 0.1 to 4. Reactors showing D/UL value of 0.1 possess low to moderate dispersion and reactors showing D/UL equal or grater than 4 possess high dispersion approaching the completely mixed flow condition.

Computation of dispersion numbers using the Levenspiel variance method gave, 0.12, 0.107and 0.11 for MP, SFP and AFP, respectively. It is clear from the data that the AIWPS reactors work under low dispersion. It is also obvious from the data that dispersion increases along the line from AFP to MP.

Drawbacks of the tracer study

The major practical drawback of this method of tracer study was the reduction of the concentration of $CuSO_4.5H_2O$ within the reactors as a result of formation of copper carbonate precipitate. The precipitate was formed when $CuSO_4.5H_2O$ reacted with the media alkalinity which was found to be higher than 200 mg $CaCO_3$/l. At the start, much of the copper was lost as a precipitate and this has prolonged the appearance of the chemical at the outlets there by increasing the liquid detention time. So, the method has not been found suitable for the verification of the detention time. As a whole, the above tracer result should be viewed as preliminary data and further study will be continued with a more inert tracer chemical.

Plate 16. Feed chemical preparation during the tracer study.

6 PROBLEMS ENCOUNTERED

The following are the major problems encountered during the course of the research.

Transportation problem

The original site proposed for the research was Awash tannery. Major advantage of this site was its close proximity to the laboratories in Addis Ababa. Awash tannery had to be abandoned due to lack of good will on the part of the tannery's management and its reluctance to provide the requested assistance. Modjo tannery was selected for the research after its management expressed interest in the research and confirmed in writing to provide all requested assistance. But the new site had posed unforeseen transportation problem as the tannery is located 80 kms out of Addis Ababa. This made the routine sample transportation to the laboratories in Addis Ababa extremely difficult and expensive. The research team was at last forced to rely on rented vehicle as borrowed vehicle from Ministry of Water could not be made available on a continuous basis.

Problems during plant construction and start-up

The delay in releasing the research fund from the sponsors had pushed the construction phase to the rainy season. This made the construction work difficult and posed unwanted delay in its completion. Plant star-up had to be repeated after nearly four months as the initial inoculum material did not give the required results. This has caused considerable delay in the research time table.

Lack of specialized laboratory equipment

Lack of required laboratory equipment and specialized personnel limited the research team from conducting detail studies on some parameters. Lack of gas chromatography has limited the team from conducting routine gas composition analysis. Effort made to utilize the gas chromatography of Ministry of Mines and Energy did not bear fruit as the equipment was not made operational by the department that owned it. Failure in the digestal apparatus prevented the team from conducting detail nitrogen analysis as promised.

Challenges encountered during the tracer study

The first attempt using sodium chloride as a tracer chemical did not give the expected result as the target chemical was present in the water in concentration much higher than the feed. To obviate this problem, copper sulphate was used. Although copper was minimal in the bore hole water used for the preparation of feed, it too failed to give the expected result. The problem with copper sulphate was that copper was removed within the reactors as copper carbonate precipitate, there by prolonging its appearance at the outlets. The study with copper sulphate produced a misleading result on the reactors hydraulic detention time. The repeated test has further delayed the submission of the final report to UNIDO. As the test is very important, another attempt will be made with copper sulphate after removing the natural alkalinity or using a more inert tracer chemical.

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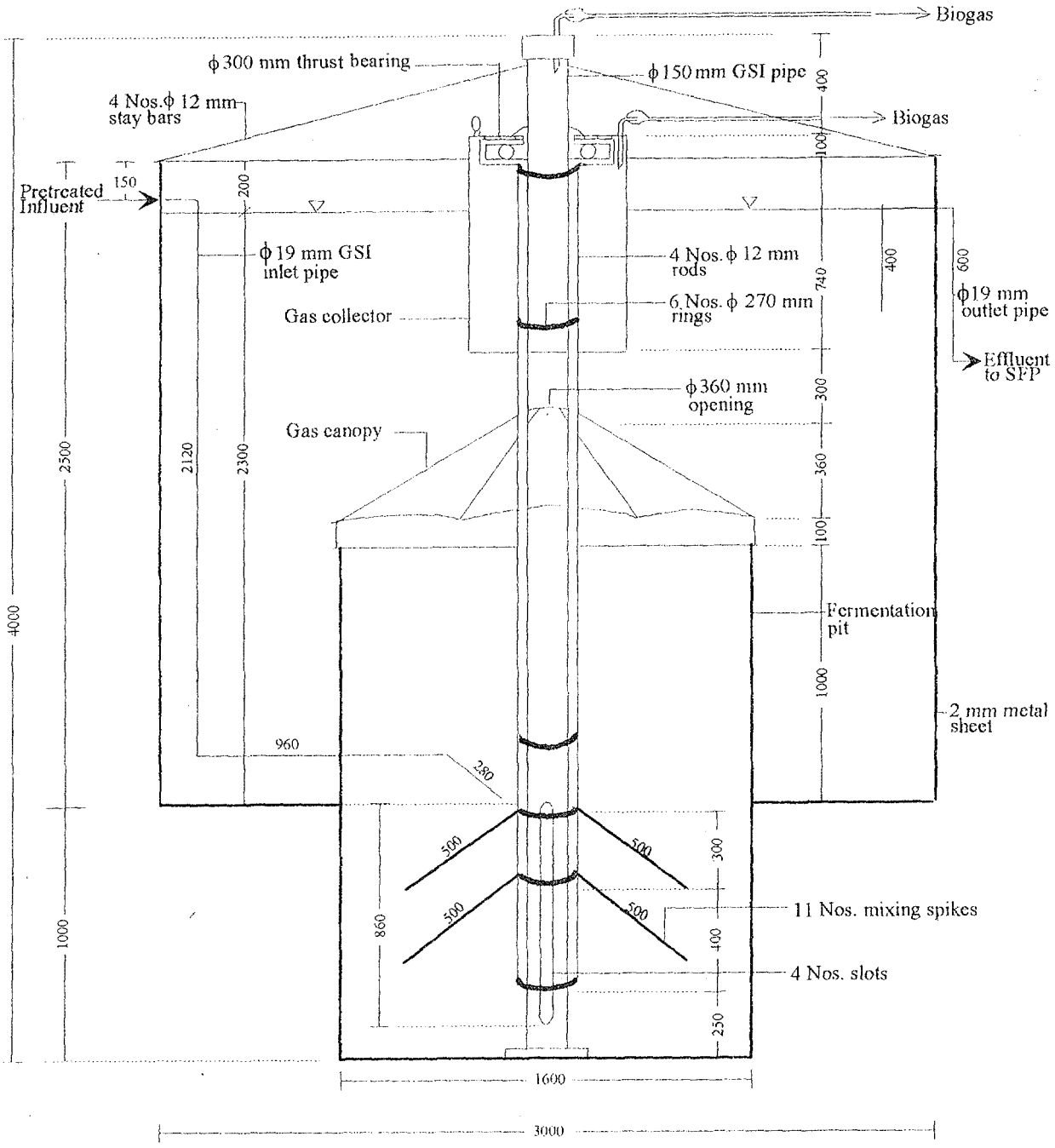
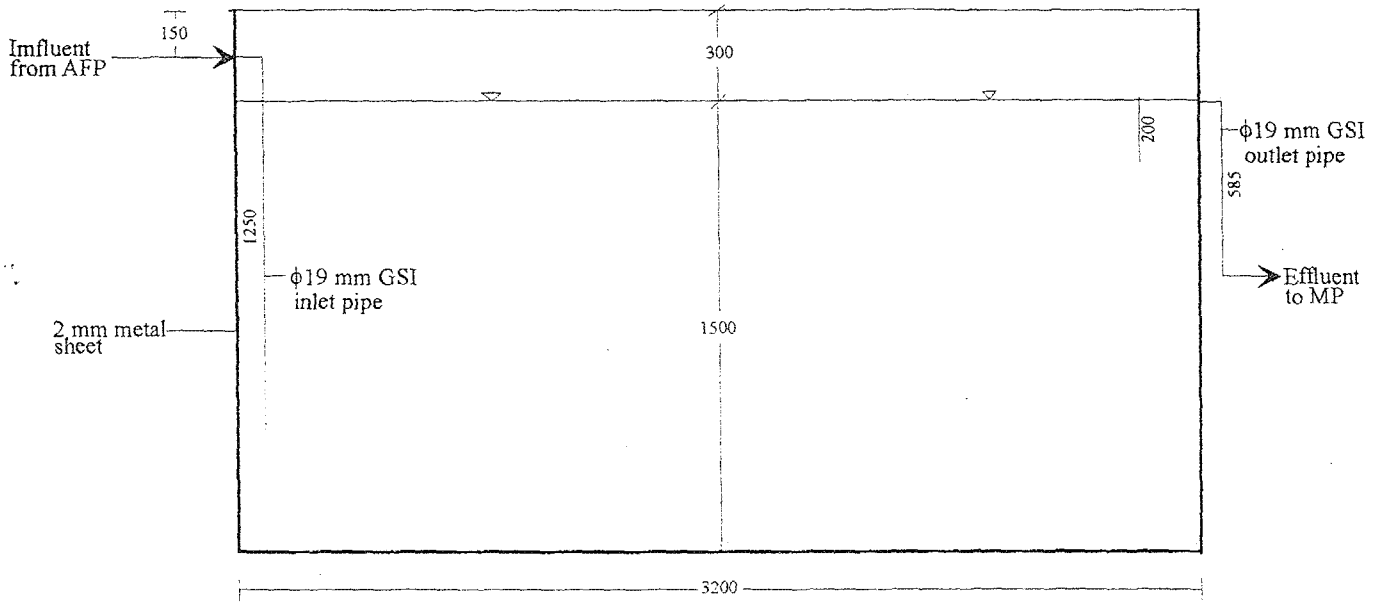
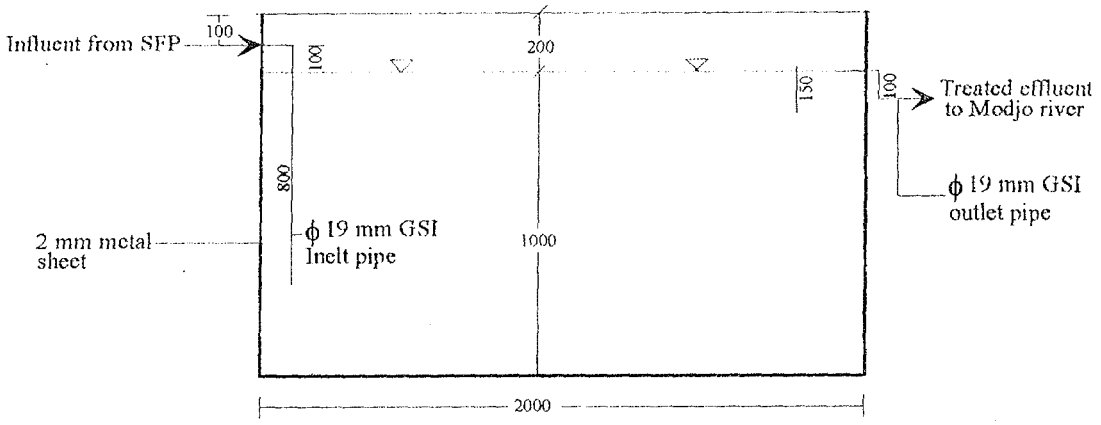
ANNEXE 1 Sectional details of AIWPS pilot plant



Project: **UNIDO CONTRACT No. 96/024/ML PROJECT DP/ETH/93/005 MODJO TANNERY, ETHIOPIA**		
Drawing title: **SECTIONAL DETAILS OF AFP**		
Scale: **1:25**	Date: **Apr. `98**	Dr. No. **1**



Project: **UNIDO CONTRACT No. 96/024/ML** **PROJECT DP/ETH/93/005** **MODJO TANNERY, ETHIOPIA**		
Drawing title: **SECTIONAL DETAILS OF SFP**		
Scale: **1:25**	Date: **Apr. `98**	Dr. No. **2**



Project: **UNIDO CONTRACT No. 96/024/ML** **PROJECT DP/ETH/93/005** **MODJO TANNERY, ETHIOPIA**		
Drawing title: **SECTIONAL DETAILS OF MP**		
Scale: **1:25**	Date: **Apr. `98**	Dr. No. **3**

ANNEXE 2 Sample submission and laboratory report

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THE FEDERAL DEMOCRATIC
REPUBLIC OF ETHIOPIA
ADDIS ABABA
WATER AND SEWERAGE
AUTHORITY

ቁጥር No. Ph-R-3
አዲስ አበባ Addis Ababa 11/12 ቀን Date 19 97
ፖ.ሣ.ቁ. P. O. Box 1505 ስልክ ቁ. Tel. No. 55 55 55 55

LABORATORY ANALYSIS REPORT

LOT No. 10

CLIENT Tannery Effluent Treatment Research Project/AIWPS/
Modjo Tannery, Modjo, Ethiopia

SAMPLE LABELS:

Site: Modjo Tannery

Sample: Tannery Effluent; Sampling Point Location No. SP1

Sample Type: Grab: Date sampled 29/11/97 ; Time sampled 8:15

Composite: - h-day comp., @ - h; - d-Week Comp.@ - d

Weekly compositing duration - to -

S.No.	Parameters	‡Method	Results	
1	**BOD_5**			
	• Filtered	5210 B	-	mg/l
	• Unfiltered	5210 B	2,662	mg/l
2	**COD**			
	• Filtered	5220 C	-	mg/l
	• Unfiltered	5220 C	12,272	mg/l
3	**Solids**			
	• Total solids	2540 B	14,120	mg/l
	• Suspended solids	2540 D	3,240	mg/l
	• Dissolved Solids	2540 C	10,880	mg/l
	• Volatile Solids	2540 E	2,020	mg/l
	• Settlable Solids	2540 F	100	ml/l
4	**Nitrogen**			
	• NH_3	4500 NH_3 C	132	mg/l
	• NO_3	4500 NH_3^{-1} E	Nil	mg/l
5	**Chromium**			
	• Cr III	3500 -Cr D	51	mg/l
	• Cr VI		Nil	mg/l
6	**Sulphate**	4500-So_4^{-2} E	241	mg/l
7	**Sulphide**			
	• Hydrogen sulphide	4500 S^{-2} F	Nil	mg/l
	• Total sulphide	4500 S^{-2} D	347	mg/l
8	**Total Phosphorus**	4500 .PE	24	mg/l
9	**Chloride**	4500-Cl^{-1} C	2,500	mg/l
10	**Electrical conductivity**	2510 B	9,540	µS/cm

Date Sample Received at Lab. 29/11/97

Date Analysed: 29/11/-7/12/97 Date Reported: 9/12/97

Analysed by (Chemist):
Signature:
Name: Zekaryas Fanta

Approved by:
Signature:
Name: Adinew Adam

‡ / Specify Method Number used from the 18th edition (1992) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, APHA, AWWA &WEF.

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REPUBLIC OF ETHIOPIA
ADDIS ABABA
WATER AND SEWERAGE
AUTHORITY

Ph-R-32

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ቀን Date

አዲስ አበባ Addis Ababa

ፖ.ሣ.ቁ P. O. Box 1505 ስልክ ቁ. Tel. No.

LABORATORY ANALYSIS REPORT LOT No. 10

CLIENT Tannery Effluent Treatment Research Project/AIWPS/
Modjo Tannery, Modjo, Ethiopia

SAMPLE LABELS:

Site: Modjo Tannery

Sample: Tannery Effluent; Sampling Point Location No. SP 2

Sample Type: Grab: Date sampled 29/11/97 ; Time sampled 8:20

Composite: - h-day comp., @ - h; - d-Week Comp.@ - d

Weekly compositing duration - to -

S.No.	Parameters	‡Method	Results	
1	**BOD_5**			
	• Filtered	5210 B	-	mg/l
	• Unfiltered	5210 B	2,489	mg/l
2	**COD**			
	• Filtered	5220 C	-	mg/l
	• Unfiltered	5220 C	5,891	mg/l
3	**Solids**			
	• Total solids	2540 B	11,855	mg/l
	• Suspended solids	2540 D	200	mg/l
	• Dissolved Solids	2540 C	11,655	mg/l
	• Volatile Solids	2540 E	200	mg/l
	• Settlable Solids	2540 F	1	ml/l
4	**Nitrogen**			
	• NH_3	4500 NH_3 C	321	mg/l
	• NO_3	4500 NO_3^{-1} E	Nil	mg/l
5	**Chromium**			
	• Cr III	3500-Cr D	15	mg/l
	• Cr VI		NIL	mg/l
6	**Sulphate**	4500 $-So_4^{-2}$ E	175	mg/l
7	**Sulphide**			
	• Hydrogen sulphide	4500-S^{-2} F	2.0	mg/l
	• Total sulphide	4500 S^{-2} D	211	mg/l
8	**Total Phosphorus**	4500 PE	4	mg/l
9	**Chloride**	4500-Cl^{-1} C	3000	mg/l
10	**Electrical conductivity**	2510 B	15, 280	µS/cm

Date Sample Received at Lab. 29/11/97

Date Analysed: 29/11- 7/12/97

Date Reported: 9/12/97

Analysed by (Chemist):
Signature: EDUPA
Name: zakaryas Fanta

Approved by:
Signature:
Name: Adinew Adam

‡ / Specify Method Number used from the 18th edition (1992) STANDARD METHODS FOR

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AUTHORITY

Ph-R-33

11/12 ቁጥር No. 97

አዲስ አበባ Addis Ababa ቀን Date 19

ፖ.ሣ.ቁ. P. O. Box 1505 ስልክ Tel. No. 55 04 55 08 55 06 55 02

LABORATORY ANALYSIS REPORT

LOT No. 10

CLIENT Tannery Effluent Treatment Research Project/AIWPS/
Modjo Tannery, Modjo, Ethiopia

SAMPLE LABELS:

Site: Modjo Tannery

Sample: Tannery Effluent; Sampling Point Location No.SP 3

Sample Type: Grab: Date sampled 29/11/97 ; Time sampled 7:15

Composite: - h-day comp., @ - h; - d-Week Comp.@ - d

Weekly compositing duration - to -

S.No.	Parameters	‡Method	Results	
1	**BOD_5**			
	• Filtered	5210 B	-	mg/l
	• Unfiltered	5210 B	415	mg/l
2	**COD**			
	• Filtered	5210 C	-	mg/l
	• Unfiltered	5210 C	1,095	mg/l
3	**Solids**			
	• Total solids	2540 -B	8,695	mg/l
	• Suspended solids	2540- D	280	mg/l
	• Dissolved Solids	2540 -C	8,415	mg/l
	• Volatile Solids	2540 -E	160	mg/l
	• Settlable Solids	2540- F	Nil	ml/l
4	**Nitrogen**			
	• NH_3	4500-NH_3 C	182	mg/l
	• NO_3	4500-NO_3^{-1} E	1	mg/l
5	**Chromium**			
	• Cr III	3500-Cr D	11	mg/l
	• Cr VI		Nil	mg/l
6	**Sulphate**	4500-SO_4^{-2} E	Nil	mg/l
7	**Sulphide**			
	• Hydrogen sulphide	4500-S^{-2} F	14	mg/l
	• Total sulphide	4500-S^{-2} D	156	mg/l
8	**Total Phosphorus**	4500- PE	3	mg/l
9	**Chloride**	4500 -Cl^{-1} C	2750	mg/l
10	**Electrical conductivity**	2510 B	12,800	µS/cm

Date Sample Received at Lab. 29/11/97

Date Analysed: 29/11/-7/12/97 Date Reported: 9/12/97

Analysed by (Chemist): Approved by:

Signature: Signature:

Name: Zakariyas Fanta Name: Adinew Adam

‡ / Specify Method Number used from the 18th edition (1992) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, APHA, AWWA &WEF.

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AUTHORITY

No.
Addis Ababa 11/12/ Date 19 97

P. O. Box 1505 Tel. No. 55 04
55 08
55 06
55 02

LABORATORY ANALYSIS REPORT

LOT No. 10

CLIENT Tannery Effluent Treatment Research Project/AIWPS/
Modjo Tannery, Modjo, Ethiopia

SAMPLE LABELS:

Site: Modjo Tannery

Sample: Tannery Effluent; Sampling Point Location No. SP 4

Sample Type: Grab: Date sampled 29/11/97 ; Time sampled 7:18

Composite: - h-day comp., @ - h; - d-Week Comp.@ - d

Weekly compositing duration - to -

S.No.	Parameters	‡Method	Results	
1	**BOD_5**			
	• Filtered	5210 B	83	mg/l
	• Unfiltered	5210 B	115	mg/l
2	**COD**			
	• Filtered	5220 C	287	mg/l
	• Unfiltered	5220 C	680	mg/l
3	**Solids**			
	• Total solids	2540 B	5,690	mg/l
	• Suspended solids	2540 D	320	mg/l
	• Dissolved Solids	2540 C	5,370	mg/l
	• Volatile Solids	2540 E	220	mg/l
	• Settlable Solids	2540 F	Nil	ml/l
4	**Nitrogen**			
	• NH_3	4500-NH_3 - C	60	mg/l
	• NO_3	4500-No_3^{-1} E	1	mg/l
5	**Chromium**			
	• Cr III	3500-Cr D	10	mg/l
	• Cr VI		Nil	mg/l
6	**Sulphate**	4500 So_4^{-2} E	227	mg/l
7	**Sulphide**			
	• Hydrogen sulphide	4500-S^{-2} F	Nil	mg/l
	• Total sulphide	4500-S^{-2} D	1	mg/l
8	**Total Phosphorus**	4500 -P E	8	mg/l
9	**Chloride**	4500-Cl^{-1} C	2,500	mg/l
10	**Electrical conductivity**	2510 B	9,030	µS/cm

Date Sample Received at Lab. 29/11/97

Date Analysed: 29/11/-7/12/97

Date Reported: 9/12/97

Analysed by (Chemist):
Signature:
Name: Zekaryas

Approved by:
Signature:
Name: Adinew Adam

‡ / Specify Method Number used from the 18th edition (1992) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, APHA, AWWA &WEF.

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AUTHORITY

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ቁጥር No. 11/12 ቀን Date 97

አዲስ አበባ Addis Ababa

ፖ.ሣ.ቁ P. O. Box 1585 ስልክ ቁ. Tel. No. 55 55 55 55

LABORATORY ANALYSIS REPORT

LOT No. 10

CLIENT Tannery Effluent Treatment Research Project/AIWPS/
Modjo Tannery, Modjo, Ethiopia

SAMPLE LABELS:

Site: Modjo Tannery

Sample: Tannery Effluent; Sampling Point Location No. SP 5

Sample Type: Grab: Date sampled 29/11/97 ; Time sampled 7:00

Composite: - h-day comp., @ - h; - d-Week Comp.@ - d

Weekly compositing duration - to -

S.No.	Parameters	‡Method	Results	
1	**BOD_5**			
	• Filtered	5210 B	31	mg/l
	• Unfiltered	5210 B	56	mg/l
2	**COD**			
	• Filtered	5220 C	272	mg/l
	• Unfiltered	5220 C	415	mg/l
3	**Solids**			
	• Total solids	2540 B	5,485	mg/l
	• Suspended solids	2540 D	480	mg/l
	• Dissolved Solids	2540 C	5,005	mg/l
	• Volatile Solids	2540 E	260	mg/l
	• Settlable Solids	2540 F	Nil	ml/l
4	**Nitrogen**			
	• NH_3	4500-NH_3 C	49	mg/l
	• NO_3	4500-No_3^{-1} E		mg/l
5	**Chromium**			
	• Cr III	3500-Cr D	9	mg/l
	• Cr VI		Nil	mg/l
6	**Sulphate**	4500-So_4^{-2} F	239	mg/l
7	**Sulphide**			
	• Hydrogen sulphide	4500-S^{-2} F	Nil	mg/l
	• Total sulphide	4500-S D	0.4	mg/l
8	**Total Phosphorus**	4500-P E	24.0	mg/l
9	**Chloride**	4500-Cl^{-1} C	2525	mg/l
10	**Electrical conductivity**	2510 B	8,750	µS/cm

Date Sample Received at Lab. 29/11/97

Date Analysed: 29/11/-7/12/97 Date Reported: 9/12/97

Analysed by (Chemist): Approved by:

Signature: Signature:

Name: Zekaryas Fanta Name: Adinew Adam

‡ / Specify Method Number used from the 18th edition (1992) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, APHA, AWWA & WEF

LOT No. 8

SAMPLE SUBMISSION FORM

CLIENT: Tannery Effluent Treatment Research /AIWPS/
Modjo Tannery, Modjo, Ethiopia

SPL No.	Grab Sample					At the time of collection				No. of Sample
	Date Collected	Time Collected	Sample Volume (ml)	Preservative Added	‡Parameters to be Analysed	Temp. (° C)	pH	DO (mg/l)	Adjusted pH	
SP1	9/10/97	8:15	3000	Nil	All	21	12	Nil	—	1
SP2	" " "	8:00	"	"	"	20	9.4	"	—	1
SP3	" " "	7:35	"	"	"	20	8.0	"	—	1
SP4	" " "	7:37	"	"	"	20	8.6	0.4	—	1
SP5	" " "	7:39	"	"	"	20	9.0	2.4	—	1
Total Number of Samples										5

Sample Received by:
Signature: _____
Name: Zekariyas Tanto
Date Sample Received: 9/10/97

Submitted by:
Signature: _____
Name: ISSAYAS TADESSE

‡ / Specify or All = BOD_5, COD, Solids (all), NH_3, NO_3^-, Cr III, Cr VI, $SO_4^=$, H_2S, Total Sulphide, Total Phosphorus, Chloride, EC.

LOT No. 8

SAMPLE SUBMISSION FORM

CLIENT: Tannery Effluent Treatment Research /AIWPS/
Modjo Tannery, Modjo, Ethiopia

SPL No.	Grab Sample					At the time of collection				No. of Sample
	Date Collected	Time Collected	Sample Volume (ml)	Preservative Added	‡Parameters to be Analysed	Temp. (° C)	pH	DO (mg/l)	Adjusted pH	
SP1	9/10/97	8:15	500	NaOH	Sulphide	21	12.0	Nil	12.0	1
SP2	"	8:00	"	"	"	20	9.4	Nil	9.4	1
SP3	"	7:35	"	"	"	20	8.0	Nil	9.0	1
SP4	"	7:37	"	"	"	20	8.6	0.4	10.0	1
SP5	"	7:39	"	"	"	20	9.0	2.4	10.0	1
Total Number of Samples										5

Sample Received by:
Signature: _____
Name: Zekariyas Tanta
Date Sample Received: 9/10/97

Submitted by:
Signature: _____
Name: ISSAYAS TADESSE

‡ / Specify or All = BOD_5, COD, Solids (all), NH_3, NO_3^-, Cr III, Cr VI, SO_4^-, H_2S, Total Sulphide, Total Phosphorus, Chloride, EC.

ANNEXE 3 Forms for on-site measurements

Form 5/Hourly Temp.,pH & DO/AIWPS/MODJO TANNERY/ETH

HOURLY TEMPERATURE, pH AND DISSOLVED OXYGEN VARIATIONS AT OUTLET LEVELS OF EACH AIWPS REACTOR

Date	Time	AFP			SFP			MP		
		Temp. (°C)	pH	DO (mg/l)	Temp. (°C)	pH	DO (mg/l)	Temp. (°C)	pH	DO (mg/l)
	6:00									
	7:00									
6/12/97	8:00	19	8.0	0.3	19.5	8.7	2.6	20	8.8	4.0
	9:00	19	8.1	0.3	20	8.7	6.0	21	8.7	3.6
	10:00	19.5	8.1	0.3	20.5	8.7	9.8	20	8.7	9.8
	11:00	21	8.1	0.3	23	8.8	13.6	23.5	8.9	13.8
	12:00	22	8.1	0.3	24	8.8	17.2	24.5	9.0	19.2
	13:00	23	8.1	0.4	26	9.0	>20	27	9.0	>20
	14:00	23	8.2	0.4	26.5	9.0	>20	28	9.1	>20
	15:00	24	8.2	0.4	26	9.0	>20	27	9.1	>20
	16:00	23	8.2	0.4	24	8.9	>20	24	8.9	16.8
	17:00	21.5	8.2	0.4	22.5	8.8	17.6	22.5	8.8	11.8
	18:00									
	6:00									
	7:00									
7/12/97	8:00	19	8.2	0.2	19	8.7	2.4	19	8.8	3.6
	9:00	19.5	8.2	11	20	8.8	6.6	20	8.8	8.2
	10:00	20	11	11	21	8.9	8.6	21	8.9	10.0
	11:00	21	11	0.4	24	9.0	15	25	9.0	15.8
	12:00	22	11	11	25	9.0	17.6	26	9.0	18.4
	13:00	24	8.0	11	27	8.9	>20	28	9.0	>20
	14:00	24	8.1	11	28	9.0	>20	29	9.1	>20
	15:00	23.5	11	11	26.5	8.8	>20	27.5	8.9	>20
	16:00	23	8.0	11	25	8.9	>20	25	8.9	18
	17:00	21	11	0.2	23	8.9	18	23	8.9	14
	18:00	19	11	11	21.5	8.7	12	21.5	8.8	9.4
	6:00									
	7:00									
8/12/97	8:00	Reading not taken due to Other works								
	9:00	20.5	8.2	0.2	21	8.8	0.8	21	8.8	3.6
	10:00	20	8.2	0.2	22	8.8	8.4	23	8.8	9.6
	11:00	21	8.2	0.3	24	8.8	15.6	25.5	8.9	16.0
	12:00	21	8.2	0.4	25	8.9	>20	27	9.0	>20
	13:00	24	8.3	0.4	27	9.0	>20	28	9.1	>20
	14:00	25	8.3	0.4	27	9.0	>20	28	9.1	>20
	15:00	24	8.3	0.4	25.5	9.0	>20	26.5	9.1	>20
	16:00	23	8.2	0.4	24	8.9	18.4	24	9.0	16.4
	17:00	22	8.2	0.3	23	8.8	13	23	8.8	10
	18:00									

pH, DO & Temp. PROFILES IN AIWPS REACTORS

Level of probe tip above reactor base = 10 cm

Reactor	Date	Time	Depth below ~~6" Casing top~~/ SWL (cm)	DO (mg/l)	Temp. (°C)	pH	Depth below SWL (cm)
OFP	18/5/97	16:21		0·10	20·5	8·00	205
				0·10	20·5	8·00	200
				0·10	20·5	8·00	190
				0·10	20·5	8·00	180
				0·10	20·5	8·10	170
				0·10	21·0	8·10	160
				0·10	21·0	8·10	150
				0·10	21·0	8·10	140
				0·10	21·0	8·10	130
				0·10	21·0	8·20	120
				0·10	21·0	8·20	110
				0·10	21·0	8·20	100
				0·10	21·0	8·20	90
				0·10	21·0	8·20	80
				0·10	21·4	8·20	70
				0·10	21·5	8·20	60
				0·10	22·0	8·20	50
				0·10	22·0	8·20	40
				0·10	23·0	8·20	30
				0·10	23·0	8·40	20
				0·10	24·0	8·60	15
				0·10	24·0	8·60	10
		16:40		0·10	24·0	8·60	5
SFP	18/5/97	15:04		0·20	21·0	8·00	140
				0·20	21·0	8·00	130
				0·20	21·0	8·10	120
				0·20	21·0	8·20	110
				0·20	21·0	8·20	100
				0·20	21·0	8·30	90
				0·20	21·0	8·30	80
				0·40	21·0	8·30	70
				0·20	21·50	8·30	60
				0·20	22·00	8·30	50
				0·20	22·00	8·40	40
				0·60	22·00	8·40	35
				1·20	22·00	8·40	30

V

pH, DO & Temp. PROFILES IN AIWPS REACTORS

Level of probe tip above reactor base = 10 cm

Reactor	Date	Time	Depth below 6" Casing top/ SWL (cm)	DO (mg/l)	Temp. (°C)	pH	Depth below SWL (cm)
SFP				3.4	23.00	8.40	25
				8.0	23.00	8.40	20
				19.6	26.50	8.60	15
				>20.0	27.00	8.80	10
	18/5/97	15:30		>20.0	27.00	9.00	5
MP	18/5/97	15:35		0.2	22.00	8.30	90
				0.2	23.00	8.30	80
				0.2	23.00	8.30	70
				0.4	23.50	8.30	60
				0.4	24.00	8.30	50
				0.6	24.00	8.30	40
				0.8	25.00	8.40	35
				2.40	25.00	8.40	30
				4.20	25.00	8.40	25
				8.40	26.00	8.50	20
				16.00	27.00	8.60	15
				19.00	27.00	8.60	10
		15:40		19.00	27.00	8.70	5

Form 4/BIOGAS/AIWPS/MODJO TANNERY/ETH

BIOGAS PRODUCTION MEASUREMENT

Gas Measuring Device: Ritter Gas Meter

Date	Time	Gas-meter Readings						Hourly Gas Production (L)
		1/100 (L)	1 (L)	10 (L)	100 (L)	1000 (L)	Total Reading (L)	
10/11/98	8:00	0.47	0	90	900	9000	9990.47	
	9:00	0.05	4	90	900	9000	9994.05	
	10:00	0.05	4	90	900	9000	9994.05	
	11:00	0.21	0	0	0	10.000	10.000.21	
	12:00	0.41	2	0	0	10.000	10.002.41	
	13:00	0.20	6	0	0	10000	10.006.20	
	14:00	0.94	9	0	0	10000	10.009.94	
	15:00	0.70	2	10	0	10000	10012.70	
	16:00	0.18	4	10	0	10000	10014.18	
	17:00	0.85	5	10	0	10000	10015.85	
11/11/98	8:00	0.75	3	60	0	10000	10063.75	
	9:00	0.41	7	60	0	10000	10067.41	
	10:00	0.44	0	70	0	10000	10070.44	
	11:00	0.76	3	70	0	10000	10073.76	
	12:00	0.84	9	70	0	10000	10079.84	
	13:00	0.60	8	80	0	10000	10082.60	
	14:00	0.44	5	80	0	10000	10085.44	
	15:00	0.69	9	80	0	10000	10089.69	
	16:00	0.83	3	90	0	10000	10093.83	
	17:00	0.70	7	90	0	10000	10097.70	
12/11/98	8:00	0.17	4	60	100	10000	10164.17	
	9:00	0.18	0	70	100	10000	10170.18	
	10:00	0.44	3	70	100	10000	10173.44	
	11:00	0.35	7	70	100	10000	10177.35	
	12:00	0.18	1	80	100	10000	10181.18	
	13:00	0.79	7	80	100	10000	10187.79	
	14:00	0.88	1	90	100	10000	10191.88	
	15:00	0.35	5	90	100	10000	10195.35	
	16:00	0.27	8	90	100	10000	10198.27	
	17:00	0.27	8	90	100	10000	10198.27	
	18:00	0.27	8	90	100	10000	10198.27	
TOTAL GAS PRODUCTION (L)								

Form 8 3

DAILY REACTOR FEED MEASUREMENT

	Eff. WD	Eff. V	MDA
F. Pit	1 m	2 m^3	2 m^2
OFP	2 m	18 m^3	9 m^2
AFP	3 m	20 m^3	9 m^2

	Eff. WD	Eff. V	MDA
SFP	1.10 m	5.6 m^3	5.12 m^2
MP	0.70 m	1.4 m^3	2.00 m^2

Date	Pump Assembly	Time Started	Time Stopped	Pumping Duration (h/d)	Inflow Feed Rate (m^3/h)	Return Feed Rate (m^3/h)	Inflow Feed Volume (m^3/d)	F. Pit θ (d)	F. Pit L (m/d)	OFP θ (d)	OFP L (m/d)	AFP θ (d)	AFP L (m/d)	SFP θ (d)	SFP L (m/d)	MP θ(d)	MP L (m/d)	Outflow Rate (m^3/d)
26/12/87	C_3/B_3-A_3	17:26	8:24															
" "	C_2/B_2-A_2	8:30	17:40	24.00	0.014	0.007	0.504	3.968	0.252	35.71	0.056	39.68	0.0756	12.20	0.090	3.263	0.214	0.419
27/12/87	C_3/B_3-A_3	17:47	7:48															
" "	C_2/B_2-A_2	7:56	17:40	23.75	0.014	0.007	0.498	4.016	0.249	36.14	0.055	4016	0.075	12.36	0.089	3.309	0.212	0.413
29/12/87	C_3/B_3-A_3	7:24	17:12															
" "	C_2/B_2-A_2	17:26	7:20	23.80	0.014	0.007	0.500	4.0	0.250	36	0.056	40	0.075	12.31	0.089	3.294	0.213	0.415
30/12/87	C_3/B_3-A_3	7:30	17:25															
" "	C_2/B_2-A_2	17:31	7:44	24.00	0.014	0.007	0.504	3.968	0.252	35.71	0.056	39.68	0.0756	12.20	0.090	3.263	0.214	0.419
31/12/87	C_3/B_3-A_3	7:50	17:10															
" "	C_2/B_2-A_2	17:18	7:37	23.65	0.014	0.007	0.497	4.024	0.249	36.32	0.0552	40.24	0.075	12.39	0.089	3.32	0.218	0.412
1/1/88	C_3/B_3-A_3	7:44	17:40															
" "	C_2/B_2-A_2	17:46	8:10	24.00	0.014	0.007	0.504	3.968	0.252	35.71	0.056	39.68	0.0756	12.29	0.090	3.263	0.214	0.419

NOTE:

1) Assumed daily evaporation rate at the research site = 5 mm/day
2) Daily evaporative loss of water from water surface of AFP = 0.045 m^3; SFP = 0.030 m^3; MP = 0.010 m^3

Form 2 /TEMP/AIWPS/MODJO TANNERY/ETH

MEAN DAILY TEMPERATURE

☒ Pond Water Mean Daily Temperature ☐ Air Mean Daily Temperature

Place Reading Taken	Temperature Readings				Mean Daily Temp. (°C)	Recorder	Remarks
	Date	Time	Min^{m} Temp. (°C)	Max^{m} Temp. (°C)			
	17/2/98	8:00	20	22.5	21.25	Atretshetu	
SFP	18/2/98	8:00	21	24	22.50	〃 〃	
	19/2/98	8:00	20	23	21.50	〃 〃	
	20/2/98	8:00	20	22.5	21.25	〃 〃	
	21/2/98	8:00	21	24	22.50	〃 〃	
	22/2/98	8:00	20	22	21	〃 〃	
	23/2/98	8:00	20	22	21	〃 〃	
	24/2/98	8:00	20	22.5	21.25	〃 〃	
	25/2/98	8:00	20.5	23	21.75	〃 〃	
	26/2/98	8:00	21	23	22	〃 〃	
	27/2/98	8:00	21	23	22	〃 〃	
	28/2/98	8:00	21.5	23.5	22.50	〃 〃	
	1/3/98	8:00	21.5	23.5	22.50	〃 〃	
	2/3/98	8:00	21.5	24	22.75	〃 〃	
	3/3/98	8:00	21.5	25	23.25	〃 〃	
	4/3/98	8:00	21.5	24.5	23.00	〃 〃	

Form 1 /FLOW/AIWPS/MODJO TANNERY/ETH

AVERAGE DAILY RAW EFFLUENT FLOW

Measuring Device: 90° Vee-notch Weir

Relationship: $Q = 1.38\ H_w^{2.5}$ Where: $Q = m^3/s$, $H_w = m$

Date	Time	1st Reading H_w (m)	2nd Reading H_w (m)	3rd Reading H_w (m)	Average Reading	
					H_{wg} (m)	Q_g (m^3/s)
15/4/97	8:02	0.110	0.120	0.125	0.1183	6.64×10^{-3}
" " "	9:02	0.110	0.112	0.0115	0.1123	5.83×10^{-3}
" " "	10:02	0.095	0.093	0.090	0.0926	3.60×10^{-3}
" " "	11:02	0.120	0.103	0.09	0.1043	4.848×10^{-3}
" " "	12:00	0.030	0.035	0.037	0.034	2.942×10^{-4}
" " "	13:02	0.054	0.05	0.053	0.052	8.51×10^{-4}
" " "	14:00	0.120	0.110	0.110	0.1133	5.96×10^{-4}
" " "	15:00	0.142	0.135	0.140	0.139	9.94×10^{-3}
" " "	16:00	0.05	0.052	0.06	0.054	9.35×10^{-4}
" " "	17:00	0.065	0.07	0.065	0.067	1.603×10^{-3}
" " "	18:00	0.032	0.033	0.035	0.033	2.73×10^{-4}
" " "	19:00					
" " "	20:00					
" " "	21:00					

Overall Average flow; Q_T (m^3/s) = $\Sigma Q_g/n$ Where: n = Number of readings = ...11....	3.219×10^{-3}
Average Daily flow; Q_{daily} (m^3/day) = Q_T . t . 60 .60 Where: t = Time of flow = ...10. (hrs) $3.219 \times 10^{-3} \times 10 \times 6 \times 60$	115.9 m^3/d

ANNEXE 4 Reports on biological examination

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PHYTOPLANKTON EXAMINATION

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For: Modjo Tannery Effluent Treatment
Research Project
Modjo, Ethiopia

Addis Ababa 15 November, 1997

Report on the analysis of Phytoplankton samples

Materials and Methods

Concentration of Chlorophyll a (chl a) as biomass indicator

Duplicate 10 or 20 ml water samples were filtered through 47mm glass fiber filter papers (GF/C) with a vacuum pressure of less than 10 cm Hg to prevent cell damage. The filters were folded in half, wrapped in aluminum foil and kept in a freezer until later analysis. The filters were subsequently manually ground with a glass rod in a small volume of 90% acetone. The homogenized algal material was placed in a parafilm-covered tube and centrifuged at 3000 rpm for 10 minutes. The extract was decanted into 10 ml volumetric flask and made up to the mark with 90 % acetone prior to absorbance reading. Absorbance was measured at 663 and 750 nm before and after the addition of 2 drops of 1N HCl. The concentrations of both chl a and phaeopigments were calculated according to the following equations of the monochromatic method (Wetzel and Likens, 1979).

$$\text{Chl a}(\mu g\ l^{-1} \text{or mg } m^{-3}) = \frac{11.3\ (E_{663b} - E_{663a})\ (v)}{(V)\ (Z)}$$

$$\text{Phaeophytin } (\mu g\ l^{-1} \text{or mg } m^{-3}) = \frac{17.86\ (E_{663b} - [2.43(E_{663b} - E_{663a})])\ (v)}{(V)\ (Z)}$$

where

E_{663b} = turbidity corrected absorption at 663 nm before acidification
$= A_{663b} - A_{750b}$, where A= the absorption value
E_{663a} = turbidity corrected absorption at 663 nm after acidification
$= A_{663a} - A_{750a}$
v = volume of extract in ml
V = volume of water filtered in liters
Z = length of light path through cuvette or cell in cm

Phytoplankton abundance

For microscopic analysis of species composition and cell density estimates, a small volume (10 or 20 ml) of phytoplankton samples preserved with Lugol's solution (American Public Health Association *et al.*, 1980) was allowed to settle in a counting chamber (combined plate chambers) with a surface area at the base of 49062.5 mm^2 for a period equivalent to a sedimentation rate of about 4 cm d^{-1} (Furet and Benson-Evans, 1982). The sedimented algal particles were counted along five horizontal transects with an inverted microscope (Leitz, Fluovert). 5 fields of vision, each with a surface area of 1.5386 mm^2, were included in each transect considered in the counting procedure.

Counting included the total phytoplankton communities and the number of the dominant species. Very small unidentifiable algal cells with more or less spherical outline were not included in the counts. To facilitate identification of algae, fresh unpreserved samples were also microscopically examined at a magnification of 1500x.

Results

Chl a concentration

Date	Pond	Depth (cm)	Concentration($\mu g\ l^{-1}$) Chl a	phaeopigments
8/10/97	SP4	-	1410	12
	SP5	-	1582	25
27/10/97	SP4	13	1466	545
		100	519	731
		150	90	-
	SP5	12	3476	562
		67	888	1042
		100	203	-

Species composition of dominant phytoplankton

+ = present -- = absent

Euglenoids	**SFP(SP4)**	**MP(SP5)**
Euglena spirogyra Ehr	+	+
E. cf. viridis Ehr	+	+
Euglena spp.	+	+
Lepocinclis ovum (Ehr) Lemm.	--*	--*
P. brevicauda (Klebs) Lemm.	+	+
P. hispidula(Eichw.) Lemm.	+	+
P. longicauda (Ehr) Dujardin	+	+
P. cf. orbicularis Hubner	+	+
P. cf. pleuronectes (OFM) Duj.	+	+
Trachelomonas cf. ensifera Daday	+	+
T. volvocina Ehrenberg	+	+
Trachelomonas spp.	+	+
Green algae		
Chlamydomonas sp	+	+
Chlorogonium cf. elongatum Dang.	+	+
Phacotus lenticularis (Ehr) Stein	+	+
Chlorella sp.	+	+

* was not observed in the samples brought on October 10, 1997.

List of species with ordinal numbers indicating their relative abundance (i.e. sp. with no. 1 being the most abundant and sp. with no. 15 or 16 being the least abundant).

Date	8/10/97		27/10/97	
Pond	SP4	SP5	SP4	SP5
Euglenoids				
Euglena spirogyra Ehr	10	4	5	5
E. cf. viridis Ehr	3	2	2	3
Euglena spp.	14	10	11	10
Lepocinclis ovum (Ehr) Lemm.	-	-	4	2
P. brevicauda (Klebs) Lemm.	8	7	9	8
P. hispidula(Eichw.) Lemm.	7	8	8	9
P. *longicauda* (Ehr) Dujardin	1	1	1	1
P. cf. orbicularis Hubner	5	9	7	7
P. cf. pleuronectes (OFM) Duj.	13	11	10	11
Trachelomonas cf. ensifera Daday	9	13	15	14
T. volvocina Ehrenberg	4	6	12	12
Trachelomonas spp.	15	2	13	13
Green algae				
Chlamydomonas sp	12	14	16	15
Chlorogonium cf. elongatum Dang.	6	5	6	6
Phacotus lenticularis (Ehr) Stein	2	3	3	4
Ch*lorella sp.*	11	15	14	16

Phytoplankton abundance

Facultative Fermentation Pond-SP4
Date----------8/10/97

Species /group	cell bumber (x 10^8 l^{-1})
Phacus longicauda	23.7241
Euglena cf. viridis	2.9336
Phacotus lenticularis	3.0612
Euglena spp.	1.6581
Others	1.0629
Total	32.4398

Maturation Pond-SP5
Date----------8/10/97

Species /group	cell number (x 10^{8} l^{-1})
Phacus longicauda	13.4771
Euglena cf. viridis	3.8265
Phacotus lenticularis	2.0833
Euglena spp.	*4.7193*
Others	1.0097
Total	25.1159

Pond----------SP4
Date----------27/10/97

Species/group	cell number (x 10^{8} l^{-1})			
depth	13 cm	100 cm	150cm	
Phacus longicauda	1.6156	0.3827	0.2232	
Euglena cf. viridis	0.3188	0.4358	0.0425	
Lepocinclis ovum	0.2338	0.0638	0.0744	
Phacotus lenticularis	0.2976	0.1169	0.0850	
Euglena spp. + others	0.9885	0.6484	0.2551	
Total	3.4543	1.6476	0.6802	5.7821

Pond----------SP5
Date----------27/10/97

Species/group	Cell number (x 10^{8} l^{-1})			
depth	12 cm	67 cm	100 cm	
Phacus longicauda	2.5512	0.9885	0.1594	
Euglena cf. viridis	1.8061	1.3711	0.0212	
Lepocinclis ovum	5.9311	0.1488	0.0106	
Phacotus lenticularis	0.5420	0.2338	0.0425	
Euglena spp.+others	0.5527	0.1700	0.0531	
Total	11.3829	2.9122	0.2868	14.5819

Results and Discussion

The chl a values clearly indicate that the bodies of water under investigation supported very high standing crops (biomass) of phytoplankton, the largest proportion of which appears to have been contributed by euglenoids, particularly the species *Phacus longicauda*, *Lepocinclis ovum* and *Euglena cf. viridis*. The concentration of phaeopigments (non-functional or degraded chl a) accounted for up to 54% of total chl A concentration. This is probably due to the ageing of the algal bloom and/or heavy grazing by zooplankton.

Bodies of water that are heavily loaded with organic matter generally support blooms of euglenoids which is probably attributable to the facultative heterotrophic nutrition of euglenoids. Because of their strong tendency towards heterotrophy , the chlorophyllous species can supplement photosynthesis by taking up organic compounds. This mode of nutrition gives the photosynthetic euglenoids a competitive advantage in bodies of water polluted with organic matter for such aquatic environments generally lack the optimal light conditions that permit sufficient photosynthetic activity to take place. The availability of certain organic compounds may explain their occurrence as all species studied require at least one vitamin and many species are incapable of utilizing nitrate as a nitrogen source.

The vertical distribution of chl a generally corresponded to the vertical distribution pattern of cell number (abundance) although the correlation between the two could be poor because of the vast differences in size and chlorophyll content per cell among species of phytoplankton. Very small nearly spherical cells (commonly designated as **monads**) were abundant in all the samples examined microscopically. These cells were not easily identifiable because of their small size. They were not included in the counts because they were too many to count. The failure to include such cells will not distort the picture of the phytoplankton biomass. In fact, the inclusion of such cells in the discussion of abundance of phytoplankton tends to mask the importance of such as other dominant species as *Phacus longicauda* since cell densities are actual cell numbers and do not take into account the cell volumes which are representative of the photosynthetic biomass.

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Client: Mojo Tannery Effluent Treatment Research Project

Analyzed by :

Date Demeke Kifle (PhD) **Signature** **Date** 15/11/97

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ZOOPLANKTON and ZOOBENTHOS EXAMINATION

By: Seyoum Mengistu (PhD)
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Addis Ababa, Ethiopia

For: Modjo Tannery Effluent Treatment
Research Project
Modjo, Ethiopia

Addis Ababa 15 November, 1997

Biological examination report
Zooplankton and zoobenthos

Reactor sample	Identified zooplankton and zoobenthos Date of collection 8/10/97 Date of analysis 20/10/97		
SFP-SP4	No sample was received for this date		
MP-SP5	Phylum Arthropoda, class Diptera, Families		Relative abundance
	Chironomidae	Tanytarsinii	+
	Corixidae	Micronecta compar	Rare
	Culicidae	Culex pipiens group	+++
		Theobaldia longiarcolata	+
		Anopheles gambiae	Rare
	Gerridae	Gerris sp.	Rare

Analyzed by : **Approved by :**

Signature: _____ **Signature** _____

Name: Sayaan Mengistu **Name :** _____

General observation:

Sampling technique: Performed by the principal investigator using nets

Results and discussion: The results indicate a dominance of mosquitoes in the maturation pond. A few chironomids (phantom midge larva) and gerrids (water striders) are also noted. It is evident that the gerrids predate on the mosquitoes and the chironomids remove detritus from the bottom. Thus no major grazers are observed, which indicates that the alga communities in the maturation ponds are not adversely affected by grazers.

Anopheles gambiae can transmit malaria, and thus its establishment in the ponds is not desirable. However, it was found in very low numbers

Report on biological examination of sample
Collection date : 27 Oct 1997
Analysed on : 8 Nov 1997

Reactor type	Identified group and abundance (Total numbers counted)
SFP – SP4	Phylum Arthropoda , class Crustacea Subclass Ostracoda Family Darwinulidae, Darwinula sp. 62
	Order Diptera, Suborder Nematocera, Family Psychodidae, Psychoda sp. 25
	Phylum Arthropoda, Family Culicidae Culex pipiens group 138
	Case of Tabanidae 2

Relative distribution with depth

SP4	
Depth 13 cm	Live Phacus growth Microcystis Culex larva
Depth 100 cm	Live Phacus growth Microcystis Ciliates Volvocids
Depth 150 cm	Microcystis Phacus Organic debris

Results and discussion

The facultative pond is dominated by detritivorous arthropods , together with a very abundant algal community composed of Phacus and Microcystis. The only possible grazers on the algae appear to be the minute Ostracod crustaceans. The other arthropods feed on the rich detritus from the decomposing algal material and organic remains. The presence of pinkish bacterial suspension in the deeper water and the domination of the algae on the surface indicate the optimal performance of the secondary facultative pond. No predatory arthropods were observed.

Analysed by: Approved by:

Signature _____ Signature _____

Name Seyoum Mengistu Name _____

Collection date : 27 Oct 1997
Analysis date : 10 Nov. 1997

Reactor sample	Identified group and relative abundance	
SP5	Phylum Arthropoda, Order Diptera Culex pipiens group	232
	Unidentified Coleoptera	1
	Order Diptera, Suborder Heteroptera Fam. Notonectidae, Notonectus	6
	Order Diptera, Suborder Heteroptera Fam. Corixidae, Micronecta compar	7
	Order Diptera, Suborder Nematocera Fam. Psychodidae, Psychoda larva	34
	Unidentified Dipteran	2
	Highly abundant Phacus material	

Depth distribution at SP5 on 27/10/97

Depth (cm)	Dominant group	
12	Phacus as green suspension	++++
67	Phacus sp.	++
100	Phacus sp. Pinkish bacterial material	Few

General discussion:

In the maturation pond, both predaceous and detritivorous dipteran arthropods were found, together with a few unidentified Coleoptera and Diptera. The algae Phacus was extremely abundant in the ponds, and in the high presence of the dipterans, it was most likely not grazed by them. It appears therefore that the dipteran community largely depend on the detrital and predatory food web, leaving the algal community largely ungrazed. Predatory arthropods are not abundant enough to seriously affect the detritivores. Grazers can emerge in the absence of predatory groups.

Depth distribution studies also indicate a large algal community at all depths, with bacterial dominance at lower depths.

More frequent monitoring of the pond ecosystem is required in order to predict the emergence and establishment of grazers such as Daphnia. This could happen in a scenario where the predator groups such as Corixids and Notonectids may totally disappear, together with drastic reduction of the detritivores. The grazers may then be advantaged in the absence of their predators and less competition from the detritvores for space and nutrients. The abundant algal biomass can then be easily grazed.

Depth distribution studies should reveal more predatory groups from the surface. The rather low number of these predatory groups should therefore be considered with caution, and more frequent sampling should be done to exclude the emergence of grazers . Obviously, such a condition will negatively affect the performance of the pond.

From the view of the performance of the maturation pond, it appears that optimal ecological conditions exist for efficient performance of the pond.

Analysed by :

Signature : _____

Name : Seyoum Mengistu

Approved by :

Signature _____

Name : _____

General comments on pond performance with respect to the dynamics of zooplankton/zoobenthos

The population dynamics of the zooplankton and zoobenthos can positively or adversely affect the performance of the facultative and maturation ponds, particularly the latter one.

As was observed earlier, zooplanktonic groups such as *Daphnia sp.* became dominant in the maturation pond to such an extent that the algal biomass was severely affected by their grazing. This could be attested by the water clarity and the disappearance of the green algal color from the maturation pond. This of course affects the activities of the bacterial and other heterotrophic communities which depend on the algal oxygen for respiration.

Such a scenario can develop under the following ecological dynamics of the pond ecosystems.

A/ Rapid disappearance of predatory species such as Corixidae, Gerridae, Notonectidae, etc, could reduce the predatory pressure on grazers such as *Daphnia* which could then flourish under this condition to bloom conditions.

B/ Decrease and disappearance of detritivorous groups can accentuate this preferred condition by reducing the competition for nutrients and space from the detritivores.

C/ Thus close monitoring should be done to follow the dynamics of predatory and detritivore groups in the maturation ponds so as to be alert about possible outbreak of grazers in the event of the decline and disappearance of the consumer communities.

Possible measures to control grazer blooms

A/ *Daphnia* grazers can easily be removed with nylon nets or removed from the pond by introducing a small number of planktivorous fish such as minnows. The latter can likewise be removed after the establishment of the algal bloom.

B/ Close monitoring can enable prediction of grazer bloom, and thus effective Preparations can be done to eliminate the grazers at their first sighting.

Analysed by: Approved by :

Signature : _____ Signature: _____

Name: Seyoum Mengistu Name : _____

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SCREENING FOR SULPHATE REDUCING AND SULPHIDE OXIDIZING BACTERIA FROM EFFLUENT OF PILOT TANNERY WASTEWATER TREATMENT PLANT IN MODJO, ETHIOPIA

By: *Fassil Assefa (Ph.D.)*
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For: *Modjo Tannery Effluent Treatment*
Research Project
Modjo Tannery

Addis Ababa **25 November, 1997**

INTRODUCTION

Sulphur-containing chemicals are one of the major chemicals widely utilized for the processing of hides and skin in the tannery industries. They are toxic when discharged as waste effluent into receiving water bodies, soil, etc. Consequently they are considered as pollutants to pause environmental hazards.

This, therefore necessitates the search for ways and means by which these chemicals are detoxified, and not the least, used for the production of useful commodities such as animal feed, biogas, etc.

Generally, waste treatment using micro-organisms in well-designed treatment plants not only provides useful products but also detoxifies toxic effluent so as to render then harmless. A requisite for the use of these industrial wastes in the integrated management of waste treatment and the production of useful commodities.

This work is meant for the preliminary evaluation of the spectra of sulphide-oxidizing and sulphate-reducing bacteria from a tannery waste treatment research facility at Modjo tannery, Ethiopia.

SAMPLE COLLECTION

Samples were collected from different depth levels of the advanced facultative and secondary facultative ponds and brought in plastic bottles to the laboratory of the Biology Department of Addis Ababa University.

METHOD AND MATERIALS

For microscopic examination, samples were serially diluted depending upon the population numbers to the tune of 10^{-1}-10^{-5} and gram stained using standard methods. Gram staining method is a different staining procedure using two dyes namely; crystal violet and safranin. Samples were heat-fixed for one or two minutes and stained first in crystal violet for one minute, iodine immersed for another minute and then washed in alcohol for a minute. After being washed by running water, the specimen was counter-stained by safranin for one minute. The counterstained specimen was washed with water and subsequently dried using blotting paper. Gram-stained slides were then observed under oil immersion objective (100x) with low light intensities.

The enrichment medium contains the following (1)

Minerals	g/l
Na_2HPO_4	7.9
KH_2PO_4	1.5
NH_4Cl	0.3
$MgSO_4$	0.1
Trace elements	5.0 ml
$Na_2S_2O_3$ (thiosulphate)	5.0

pH (4.5)

Cultural characteristics (colour, texture, shape, size, etc.) were also recorded. The

bacteria were then identified to the genus level using microscopic examination and for those who were enriched by including cultural characters (2,3 & 4).

RESULT AND DISCUSSION

The bacteria were further characterized and grouped into their respective taxa as shown in Table 1.

Table 1. Cell shape, colour and colony arrangement of bacteria isolated from water column sample of an advanced facultative pond.

Bacteria	Cell shape	Cell arrangement	Colony colour	Trophic status
Desulfovibrio	curved rods	single	ND	SRB
Thiobacillus	rods	single	colourless	SOB
Thiosphaera	coccoid	single/pair/chain	colourless	SOB
Lamprocystis	spherical/ovoid	diplococcus/ aggregate	ND	PSB
Thiocystis	spherical	aggregate	ND	PSB
Rhodosprillium	spiral		red brown	PNSB
Rhodopseudomonas	rods	diplobacillus/ aggregate	red brown	PNSB

Key to acronyms
SRB = Sulphate reducing bacteria
SOB = Sulphide oxidizing bacteria
PSB = Purple sulphur bacteria
PNSB = Purple non-sulphur bacteria
ND = Not done

Table 2. Depth profile of bacteria counts in advanced facultative and secondary facultative ponds.

Sampling Location	Depth below water surface (cm)	Number (No./ml)		
		Cocci	Rods	Spiral/vibrioid
AFP-SP3	30	6.4×10^4	5.2×10^5	1.2×10^2
,,	130	2.1×10^4	5.1×10^4	2.1×10
,,	230	3.0×10^4	-	-
,,	330	5.1×10^6	-	2.2×10^2
SFP-SP4	50	3.8×10^5	2.6×10^4	2.1×10^3
,,	100	5.2×10^5	1.3×10^5	1.2×10^3
,,	150	2.6×10^6	6.1×10^4	3.2×10^4

Table 1 shows the shape, colour and colony arrangement of the bacteria identified, while Table 2 gives the bacteria counts at different depths of the advanced facultative and secondary facultative ponds. As can be seen from Table 2, the most dominant group of bacteria were of coccoid (*Thiocystis*) and rod shaped (*Thiobacillus*) with few representatives of spiral (*Rhodosprillium*). The cocciod-shaped (*Thiocystis*) were also uniformly distributed along the depth profile.

Table 3. Depth profile of different bacteria in the advanced facultative pond.

Depth below water surface (cm)	Bacteria genus			
	SRB	SOB	PSB	PNSB
30	-	Thiobacillus Thiosphaera	Thiocystis	Rhodosprillum Rhodopseudomonas
130	-	Thiobacillus filamentous bacteria*	Thiocystis	Rhodosprillum Rhodpseudomonas
230	-	-	Thiocystis	-
330	Desulfovibrio	filamentous bacteria*	Thiocystis Lamprocystis	-

*/ Filamentous bacteria whether they are anaerobic or aerobic were not enriched.

Although *lamprocystis* was recovered from the lower profile (Table 3) the dominance and ubiquitous distribution of the coccoid bacteria of the genus *Thiocystis* was shown all along the water-depth profile. This *Thiocystis* may have representatives of micraerophilic and aerobic representatives within their respective habitats (2). This group was not culturally characterized. Some rod shaped, coccoid and filamentous bacteria were also observed at different depths which may represent the sulphide oxidizing green sulphur bacteria, *Beggiatoa* and *Thiotrix*. No enrichment was, however, done to identify them. *Rhodosprillum* and *Rhodopseudomonas* were two representatives of purple non-sulphur bacteria isolated from the upper and middle depths (30-130 cm). *Rhodosprillum* is differentiated from *Rhodopseudomonas* by its spiral cell shaper and single distribution. The latter is different in that it is rod shaped and form aggregate on a substratum. Upon enrichment, they give a red-brown cultural charter. Under anaerobic conditions in the light, all species grow as photoheterotrophs or photoautotrophs with either molecular hydrogen, or sulphide, thiosulphate or elemental sulphur as electron donor and carbon dioxide as a sole carbon source. Under microautotrophic to aerobic condition in the dark, many can grow as chemhetertrophs or chemoautotrophs. Good representation of this group along the surface and the middle layer (Table 3) is an indication of their photoautotrophic and chemoautotrophic metabolic capabilities. Generally, analysis of the depth distribution showed more bacteria at lower depths than the shallower depths (Table 2). This may be due to the near to neutral pH environment of the lower depths (pH 7.4-7.5) observed at the time of sampling.

The lower depth samples were also characterized by a pungent smell of hydrogen sulphide suggesting that the activity of anaerobic sulphate-reducing bacteria of the genus *Desulfovibrio* was very high. This region is normally dominated by anoxic water-mud interface which can serve as niche for other anaerobic bacteria. The activities of these bacteria result in the production of organic acids which may be responsible for the low pH in the bottom layers.

Other anaerobic, mesophilic methanogenic bacteria group that converts organic acids into methane may also be associated with sulphate reducers, although they were not separately analysed in this work.

The fact that the effluent plant is well represented by the photoautotrophic and chemolitoautotrophic bacteria stipulates that the system is self sufficient and productive using carbon dioxide as a carbon source, nitrogen waste as a nitrogen source, and sulphur as electron donor as in the case of sulphide oxidation or acceptor, as in the case of sulphate reduction.

One advantage of this system is that it does away with additional extraneous sources of carbon which are usually capital and energy intensive in the modulation of the C:N ratio towards the efficient effluent treatment process.

The knowledge of the interaction of all bacteria and other planktonic organisms in the system is essential for the manipulation of the waste effluent for many beneficial uses such as production of single cell protein and biogas fuel. This, however, calls for a through investigation of all trophic groups in the system including the anaerobic sulphur metabolizing and other methanogenic bacteria.

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PHYTOPLANKTON EXAMINATION

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For: Mojo Tannery Effluent Treatment Research Project
Mojo, Ethiopia

Addis Ababa **February 15, 1999**

1. Materials and Methods.

The procedures followed for taxonomic analysis, cell density estimates and chlorophyll a determination are as outlined in the earlier report submitted to Mojo Tannery Effluent Treatment Research Project on November 15, 1997.

2. RESULTS

The results of the analyses made on phytoplankton samples collected on December 27, 1998 from the two experimental ponds (Reactors), Secondary Facultative Pond(**SFP**) and Maturation Pond(**MP**), are tabulated below.

2.1. Biomass of phytoplankton measured as Chlorophyll a (Chl a) concentration

Time of sampling (PM)	**Reactor**	**Depth below SWL (cm)**	**Chl a ($\mu g\ l^{-1}$)**
13:35	SFP	5	3927
13:45	SFP	10	3955
13:50	SFP	15	1365
13:55	SFP	30	391
12:13	MP	5	2260
13:15	MP	10	703
13:20	MP	15	1285
13:35	MP	30	250

2.2. Phytoplankton abundance (no. of cells per litre of water sample).

Time of sampling	Reactor	Depth below SWL (cm)	Cell Number# (x 10^{10})
13.35	SFP	5	9.34389
13.45	SFP	10	8.55409
13.50	SFP	15	3.67552
13.55	SFP	30	0.75409
12.13	MP	5	5.98062
13.15	MP	10	1.01939
13.20	MP	15	3.13164
13.35	MP	30	0.69388

 # -The cell numbers are rounded to the nearest hundreds.

3. Discussion

As the concentration values of chlorophyll a seem to suggest, the ponds support very high standing crop(biomass) of phytoplankton, which is entirely contributed by a unicellular alga tentatively identified as *Chlamydomonas fenestrata* Whitford. The vertical distribution of both chlorophyll a and cell number of *Chlamydomonas fenestrata* in both ponds was of the typical pattern for phytoplankton with maxima in the near-surface regions. Cell number and chlorophyll a of the same alga did not, however, decrease continuously with increasing depth at the maturation pond. Such a vertical stratification of chlorophyll a and cell number is not unusual for phytoplankton communities. Explanations given to such a phenomenon included consideration of cells sinking from above and their subsequent accumulation at a depth, behavioural aggregation, differential growth rates resulting from the region of accumulations favouring high cell production rates due to high nutrient concentrations or more favourable light conditions.

The correlation between cell number and chlorophyll a concentration was low. This is to be expected in light of the considerable difference in size between cells of the same algal species. Cell densities are actual cell numbers and do not take into account the cell volumes which are representative of the photosynthetic mass. Moreover, fewer cells of the same size found at a deeper region of a water column may correspond to a larger biomass measured as chlorophyll a owing to their higher chlorophyll a content per cell as a physiological response to low light levels.

Client: Mojo Tannery Effluent Treatment Research Project

Analyzed by:

Name: Demeke Kifle(Dr.) **Signature:** _____ **Date:** Feb. 15, 1999

ANNEXE 5 Organic loads applied during the experiment

Organic surface and volumetric loads applied during the experiment.

Feed Phase	Parameters	Units	AIWPS Reactors			
			F.Pit	AFP	SFP	MP
Feed 1	Mean inflow	m^3/d	0.500	0.500	0.455	0.425
,,	Mean retention time	days	8	40	12	3
,,	Surface BOD loading	kg/ha-d	5,562	1,236	228	185
,,	Volumetric BOD loading	g/m^3-d	276	48	14	18
,,	Surface COD loading	kg/ha-d	11,360	2,524	609	983
,,	Volumetric COD loading	g/m^3-d	568	99	38	98
Feed 2	Mean inflow	m^3/d	0.8	0.8	0.755	0.725
,,	Mean retention time	days	5	27	7	2
,,	Surface BOD loading	kg/ha-d	8,300	1,844	847	1,341
,,	Volumetric BOD loading	g/m^3-d	415	72	53	134
,,	Surface COD loading	kg/ha-d	29,620	6,582	2,087	3,179
,,	Volumetric COD loading	g/m^3-d	14,810	2,576	1,304	318
Feed 3	Mean inflow	m^3/d	1.276	1.276	1.231	1.201
,,	Mean retention time	days	3	18	6	2
,,	Surface BOD loading	kg/ha-d	13,666	3,037	975	1,507
,,	Volumetric BOD loading	g/m^3-d	683	119	61	151
,,	Surface COD loading	kg/ha-d	37,259	8,279	5,808	5,308
,,	Volumetric COD loading	g/m^3-d	1,863	324	363	531