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Testing biological methods to treat rubber effluent

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A study was conducted with the aim of treating and disposing of the effluent of an indigenous rubber company rich in PO_4^{3-} and NH_4^+ and also determine the effect of the effluent on soil fertility. The basic method used for the biological treatment was aerobic digestion with glucose and magnesium amendments. Most naturally occurring aerobic heterotrophic bacteria in the rubber effluent were found to be capable of utilizing petroleum hydrocarbons as carbon and energy sources. Prominent among these bacteria were the genera of *Micrococcus, Bacillus, Staphylococcus, Aerobacter, Proteus, Corynebacterium, Streptococcus, Aeromonas* and *Pseudomonas*. The possibility of using the effluent as soil supplement was established. Oxidative digestion following amendment with glucose yielded only 53 and 40% reduction in levels of NH_4^+ and PO_4^{3-} respectively. Addition of glucose and magnessium ions resulted in 95% reduction for NH_4^+ and 47% for PO_4^{3-}, respectively. Our results indicate that rubber effluent can be disposed of (i) by controlled spread to agricultural soils as an fertilizing agent or (ii) by aerobic digestion before release in natural flowing waters.

Key words: Heterotrophic bacteria, rubber effluent, amendment, oxidative digestion.

INTRODUCTION

Inadequate access to clean water and sanitation has been identified as one of the most pervasive problem afflicting people throughout the world. Problems with water are expected to grow worse in the coming decades, with water scarcity occurring globally, even in regions currently considered water-rich such as Nigeria. Addressing these problems requires that research be conducted to identify robust new methods of purifying water at lower cost and with less energy, while at the same time minimizing the use of chemicals and the impact on the environment. Finding new ways and technologies to purify water polluted by agricultural and industrial activities is therefore a major issue that humankind is facing (Shannon et al., 2008).

The contributions of effluents from agricultural and agro-based industries to pollution in major agricultural countries of the world are well documented. John (1978) and Phang (1987) observed that effluents from palm oil and rubber industries as well as farm animal wastes were the major contributors to pollution of the Malaysian environment. In Indonesia, problems have been encountered with processing of tapioca, rubber and palm oil, the wastes of which are generally discharged, untreated (Alabaster, 1986). Phang (1987) estimated that the combined wastewater discharges from the rubber and palm oil industries in Malaysia contributed an organic load of about 0.5 million kilograms biochemical oxygen demand (BOD) per day. In this country, neither rubber nor palm oil mill effluents were found to be basically toxic to fish grown in a integrated treatment pond where algae are amended by palm oil effluents (Phang, 1987).

Rubber generally occurs in plants as microscopic particles suspended in aqueous fluid, the serum fluid, contained in specialised latex vessels and latex tubes and cells. Chemically, rubber is a polyterpene consisting of a long chain (500 – 5000) of isoprene units joined together end to end to form gaint molecules called polymenrs which are coiled up like tiny springs (Kocchar, 1986). The liquid produced by the plant (latex) is milky white in colour but the chemical composition however, varies from species to species. In addition to the to the rubber hydrocarbons, fresh latex contains various proportions of non-rubber constituents such as resins, sugars, glucosides, tannins, alkaloids, mineral salts, waxes and crystals which are capable of enhancing microbial growth (Archer et al., 1963; Dunphy et al., 1965).

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The pollutant level of rubber effluents varies with the process used to manufacture the rubber from the latex. The two main processing methods are latex concentrate and block rubber effluent. In the latex method, latex from various sources is initially collected and blended in a large latex bulding and blending tank. Coagulation is effected by the addition of coagulants, such as formic or acetic acid. In factory-scale processing it occurs in coagulation troughs, where latex is first diluted with water. Milling involves feeding slabs of coagulum to successive pairs of rollers ("sheeting battery") to produce sheets of uniform thickness. Appropriately grooved rollers imprint on each sheet "ribs", which expand the surface area for drying. After sizing, the sheets are hung on lines and dried in smokehouse at successively higher temperature ("ribbed smoked sheets"). Blemishes are then removed manually and sheets are visually graded. They are finally pressed into bales with talc to prevent adhesion (Barbin and Rodgers, 1994).

In block rubber production from latex, latex arriving from different locations is first blended in a large bulking tank. Chemicals are added to control viscosity and affect colour. The latex containing the requisite chemicals is then coagulated by adding coagulants (formic acid) in a long coagulation trough. Solid latex coagulum is then processed into crumb by either physical or chemical means. In the physical case, the coagulum is first fed though the rotating rolls of a creping machine. The crepe is then mashed into small pieces through a hammer mill and finally concerted into crumb by an extruder. Alternatively, incompatible oil is added to the latex in the coagulation trough. The material is then fed though a creper and transformed into crumb. In both cases, the crumbs finally obtained are dried by hot air. Depending on the throughput, factories may use single-layer tray driers with direct firing burners, apron driers, or chamber driers. The dry rubber is finally baled (commonly by means of a hydraulic press) and wrapped in polythene to prevent adhesion between bales in the crate. When field coagulum rather than latex is being processed into block rubber, the materials involved include foreign matter and require pre-cleaning. Field coagulum materials are first forced through a macerator (to slice up large blocks) and then passed through a series of creeping rolls. This method does not require coagulation (Barbin and Rodgers, 1994)).

The latex method is the most polluting (because of concentration processing that needs the addition of chemicals) whilst the later produces larger volume of less polluting effluent (Phang, 1987). Rubber effluent consists of latex washings and a serum containing proteins, sugars and lipids as well as inorganic and organic salts (Kulkarni, 1972; Kulkarni et al., 1973 a, b; John and Mohd, 1977). The high level of NH_4^+ and other plant nutrients makes it a good medium for algal growth.

Several systems to treat rubber effluent have been developed. The most common include anaerobic- facultative ponding, mechanical aeration, land disposal, enclosed anaerobic digestion (Zaid, 1990) and high rate algal pond (HRAP) system (Geetha et al., 1994). The common objectif of these treatments is to reduce or eliminate high levels of ammonium in the latex concentrate effluent that had been added as an anticoagulant to field latex prior to processing.

Geetha et al (1994) reported that aeration alone systems have been ineffective in removing high levels of ammoniacal nitrogen from anticoagulants used in treating raw latex in the Asia-Pacific Region. However, in other factories such as those within the present study area in Rivers State, Nigeria, no anticoagulants are added to field latex before processing, implying that aeration systems could be effective in treating rubber effluents.

In Nigeria, effluents from rubber processing are still directly discharged into rivers and streams without being treated. The aim of this study was to identify the best suited method to treat and dispose of rubber effluents from a local factory. The feasibility of using the effluent as a soil supplement was also examined.

MATERIALS AND METHODS

Sample collection - Rubber effluent samples were collected from a rubber factory in Etche about 50 kilometres from Port Harcourt, in the Niger Delta. Samples were collected from both the soaking and turbo mill tanks and at the discharge points (Figure 1). For microbiological analyses, samples were collected in 500 ml sterile bottles. Clean plastic containers rinsed several times with the sample were used for physicochemical determinations. Dissolved oxygen (DO) and biochemical oxygen demand (BOD), were measured in ground glass stoppers, incubation bottles (300 ml). BOD samples were fixed in the field with Winkler reagent (REF).

Soil samples were collected in polythene bags at depths of 10 and 20 cm at five random spots within one square metre quadrant (Dutch auger) around the rubber factory and the campus of Rivers State University of Science and Technology, Port Harcourt. The five random samples from the same quadrant were then bulked together to give one composite sample. A total of five composite soil samples were collected.

Sample analysis - Temperature, DO, conductivity and total dissolved solids (TDS) were measured in the field using a mercury-in-glass thermometer for temperature and a Horiba Model U-7 multi probe for the other parameters. pH was measured in the laboratory using a digital sensor Coleman pH meter. Analyses of effluents for ammonium and nitrate, phosphate, exchangeable cations (K^+, Ca^{2+} and Mg^{2+}), DO and BOD were carried out according to procedures outlined by APHA (1975). Available P (soluble reactive phosphorus) was determined by the Bray P-1 method (Bray and Kudz, 1945). Total organic carbon was determined by the Walkey-Black method (1934).

For microbiological analyses, the following media were used for the isolation, enumeration and identification of microorganisms from effluents, water, sediment and soil samples. Physiological saline (0.85% aqueous solution of sodium chloride), peptone water (1% aqueous solution of Bacteriological Peptone), potato dextrose agar and nutrient agar (15 g/L) were prepared as outlined by Cruickshank et al (1975). Petroleum utilising bacteria and fungi (PUB and PUF) were enumerated using a petroleum agar medium with the following composition: Agar (Oxoid), 15.0 g; NH_4Cl, 0.5 g; K_2HPO_4, 0.5 g; Na_2HPO_4, 1.5 g; 1:1 mixture of engine oil/diesel, 5 ml; distilled water, 1L; pH 7.6 (IPS, 1995). All media used for isola-

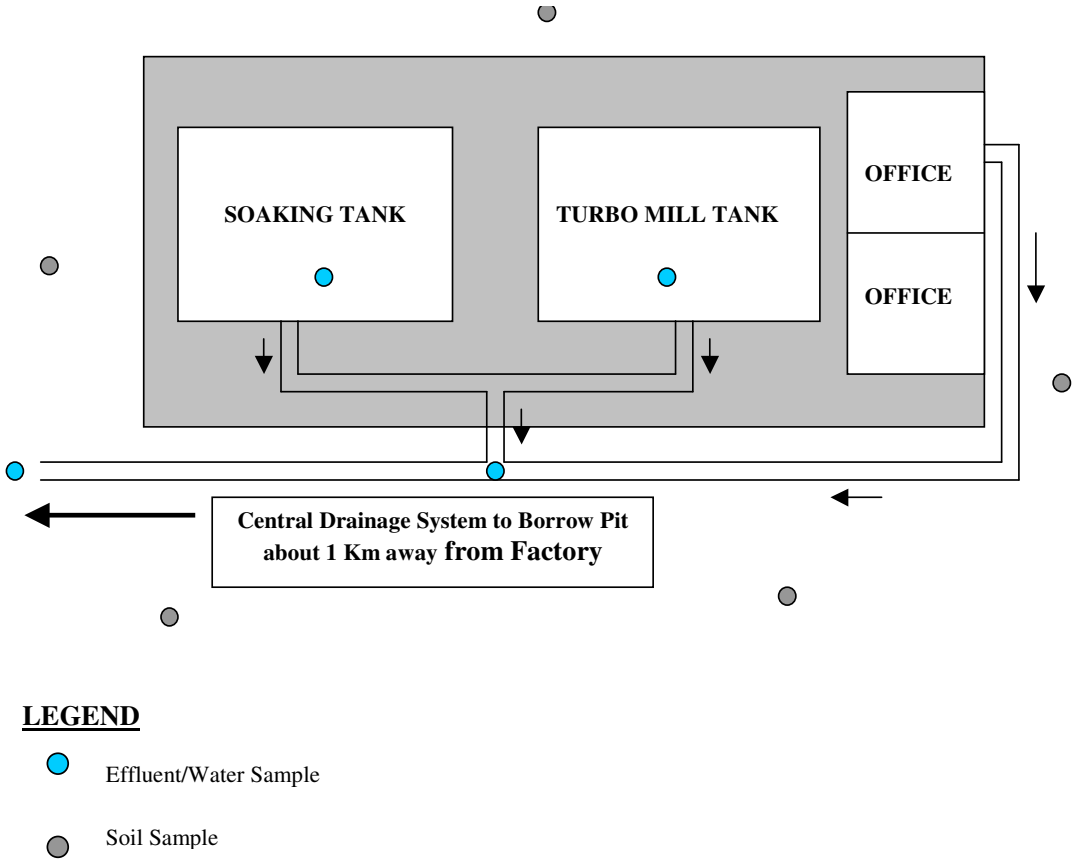

Figure 1. Schematic showing rubber factory layout and sampling points

tions and enumeration of microorganisms were sterilised by autoclaving at 121ºC for 15 min. Engine oil/diesel mixture was sterilised by membrane filtration.

The spread plate method was used for the isolation and enumeration of microorganisms. Ten-fold serial dilutions of direct effluent and soil samples and sub-samples were made with sterile physiological saline as diluent and oven-sterilised glass pipettes. 0.1 ml of appropriate dilutions were plated out in duplicates on each of four media (Nutrient Agar, Potato Dextrose Agar, Petroleum Agar and Petroleum Agar with antibiotics (1g/l of Tetracycline and Streptomycin) for petroleum utilizing fungi. Pertroleum degrading organisms were of particular interest because this region of Nigeria is prone to spills from the numerous oil exploration and exploitation activities. Exposure of these organisms to rubber hydrocarbons may be a good priming condition for later exposure of these organisms to and subsusequent degradation of petroleum hydrocarbons. All plates were incubated at 37°C. Colony counts were made after 48 h incubation for total heterotrophic bacteria while hydrocarbon degrading fungi and bacteria were enumerated after seven days. Counts were expressed as colony forming units per ml (cfu/ml for liquid samples and cfu/g for soil samples). Bacteria were identified following the methods outlined by Cowan and Steel (1979).

Amendments, 1% glucose and 0.06% $MgCl_2$, as described by Iyagba (1998), were carried out in cotton wool stoppered 2L conical flasks agitated by a magnetic stirrer at ambient temperature (30 ± 2℃). Choice of this temperature was based on need to reduce energy requirements for a largescale treatment process in a region where available power supply is very low. Also, stream tempeatures measured *in situ* were within this range. For soil samples, three kilograms of soil samples were collected using the composite sampling technique with an Auger. The soil was properly mixed together and divided equally into two plastic containers. At T_0, sub soil samples were taken from each of the containers for both microbiological and chemical analysis. Six hundred ml of the effluent was then used to flood one of the pots, the other left untreated as a control. Both pots were left under the same environmental conditions outside. Sub samples were collected from the two pots every 48 h for two weeks for both microbiological and chemical analyses as described earlier.

RESULTS AND DISCUSSION

The microbiological and physico-chemical properties of the rubber effluent and water along effluent drainage channel were analysed. Total aerobic heterotrophic bacteria in samples ranged from 2.3 to 3.5 x 10^7 cfu/ml (Table 1). The bacteria capable of utilising petroleum hydrocarbons as sole carbon source ranged from 3.2 x 10^6 to 2.5 10^7 cfu/ml. Lower counts were recorded for the fungi (2.0 x 10^4 and 5.2 x 10^5). These very high bioloads in the raw effluent and factory runoff contrast with adjacent stream waters bacterial bioloads (1.0 x 10^2 and 7.2 x 10^3 cfu/ml).

The raw effluent and factory runoff were slightly acidic (pH 6.5 - 6.9). Dissolved oxygen was not detectable in the raw effluent (completely anoxic) while the levels in the factory runoff were very low (0.8 ± 0.1 mg/l). However, the

Table 1. Microbiological and Physico-chemical Characteristics of Rubber Factory Effluent

	Colony Counts (cfu/ml)					mg/l						
Sample code	THB	PUB	TF	PUF	pH	DO	BOD	TDS	PO43-	NH4+	NO3-	Cond. µmhos/cm
Raw Effluent	2.3×10^7	2.5×10^7	3.8×10^7	5.2×10^5	6.5	0	189	550	94.3	39.3	0.07	750
DC1	3.4×10^7	3.2×10^6	2.5×10^6	2.0×10^4	6.8	0.8	295.5	530	8.7	3.45	0.04	760
DC2	3.5×10^7	4.8×10^6	3.1×10^6	2.3×10^4	6.9	0.8	295.5	528	9.1	3.3	0.05	770

DC: Discharge channel

Table 2. Microbiological and Physico-chemical Characteristics of Rubber Factory Soil Samples

	Log10 Colony Counts (cfu/g)					Meq/100g			P	%		
Sample code	**THB**	**PUB**	**TF**	**PUF**	**pH**	**Ca 2+**	**K+**	**Mg2+**	**(ppm)**	**N**	**OC**	**OM**
DS1	2.5×10^7	3.8×10^6	5.2×10^5	3.4×10^4	5.0	2.4	0.43	1.36	41.22	0.10	1.3	2.34
DS2	3.2×10^7	2.5×10^6	2.0×10^5	3.5×10^4	5.1	41	0.28	0.87	33.1	0.19	0.61	1.1
DS3	4.8×10^6	3.1×10^5	2.6×10^5	2.3×10^4	6.2	2.4	0.50	0.65	24.5	0.05	1.1	1.8
DS4	1.9×10^7	2.9×10^5	3.8×10^5	2.8×10^4	5.8	2.8	0.83	0.93	28.2	0.11	0.13	0.31
DS5	2.3×10^6	2.4×10^4	1.7×10^5	1.7×10^3	4.7	4.1	0.05	0.33	17.8	0.07	1.07	1.81

DS 1 and 2: Sediments from discharge channel; DS3: Soil sample around discharge pit; DS4: Soil sample around discharge channel; DS5: Soil sample from rubber plantation.

TDS levels were moderately high (528 - 550 mg/l). Phosphates and ammonium concentrations were more than ten folds higher in the rubber processing effluent than in the factory runoff which is composed of effluent plus other run off water (See also Table 1).

Microbial counts (CFU) were generally higher in soils around the factory (DS 1 - 4) than those within the plantation (DS5) (Table 2). This could be attributed to to leachates from rubber crumbs dumped outside the warehouses. Exchangeable cations other than calcium were lower in the plantation soils. A similar trend was observed for phosphorus. All the soil samples were acidic (pH 4.7 to 6.2) with the highest acidity occurring in the plantation soils, likely related to higher levels of humic substances from decaying vegetation.

The main bacteria associated with the untreated effluent and soils of the rubber factory were *Micrococcus, Pseudomonas, Bacillus, Proteus, Aeromonas* (Table 3). When the effluent was aerated by agitation a reduction of the number of bacterial taxa was observed. Most previous works employed methods that involved both aerobic and anaerobic systems in alternation (Zaid, 1990). In our study aeration alone did not affect the nutrient levels even though microbial population showed normal growth pattern (see Figure 3).

Amendment of rubber effluent with glucose only and glucose plus magnesium resulted in no major difference in the identity of the bacterial genera isolated. However, amendment of soils with rubber effluent significantly decreased the number of genera. By contrast, there was a rapid increase in the numbers of THB and PUB within 5-7 days of soil amendment with rubber effluent (Figure 2). Thereafter, slight drops in bacterial numbers were observed. A similar trend was observed for phosphorus. The levels of K^+ were not affected that much.

Results the rubber effluent analyses through time indicated that there was a rapid increase in numbers of THB and PUB within the first four days of aeration (Figure 3). This was accompanied by an overall decrease in phosphate concentration but not ammonium. This agrees with previous work showing that aeration systems had been ineffective in removing high levels of ammonium (Geetha et al., 1994).

Amendment with glucose only resulted in a sharp and rapid decline in the phosphorus concentrations (within the first two days, Figur 4). The decline in ammonium was lower and only happen within the first 24 h). These declines in phosphorus and ammoniumconcentrations were accompanied by increases in the numbers of THB and PUB for up to five days after amendment. Glucose amendment yielded 53 and 40% reductions for ammonium and phosphate, respectively. A similar trend was observed after amendment of the effluent with both glucose and Mg^{2+} (Figure 4). Here, however, the decrease in ammonium level continued up to 7 days after amendment. Combining the two amendments triggered

Table 3. Bacteria associated with rubber effluent and effluent-associated soil.

Raw rubber effluent		Pre-impacted soil	Post-impacted soil	Rubber effluent amendment with	
After aeration				Glucose	Glucose and Mg^{2+}
Aerobacter	*Bacillus*	*Acinetobacter*	*Acinetobacter*	*Bacillus*	*Bacillus*
Aeromonas	*Corynebacterium*	*Aeromonas*	*Aerobacter*	*Citrobacter*	*Citrobacter*
Bacillus	*Micrococcus*	*Alkaligenes*	*Alkaligenes*	*Corynebacterium*	*Corynebacterium*
Corynebacterium	*Pseudomonas*	*Bacillus*	*Bacillus*	*Micrococcus*	*Micrococcus*
Micrococus	*Staphylococcus*	*Citrobacter*	*Corynebacterium*	*Neisseria*	*Pseudomonas*
Proteus		*Corynebacterium*	*Micrococcus*	*Staphylococcus*	*Staphylococcus*
Pseudomonas		*Escherichia*	*Staphylococcus*		*Streptococcus*
Staphylococus		*Micrococcus*			
Streptococus		*Proteus*			
		Pseudomonas			
		Staphylococcus			

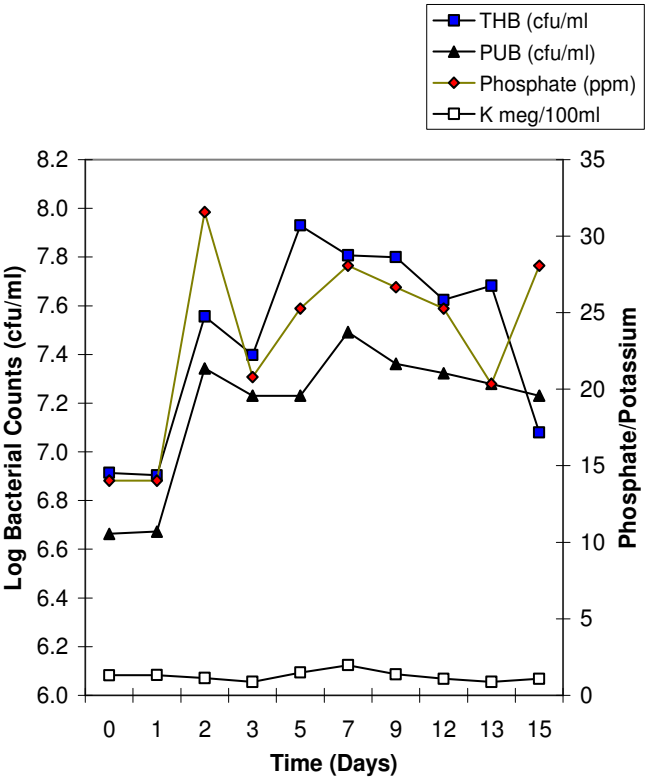

Figure 2. Effect of rubber effluent on microbial populations and some chemical parameters of pot soils.

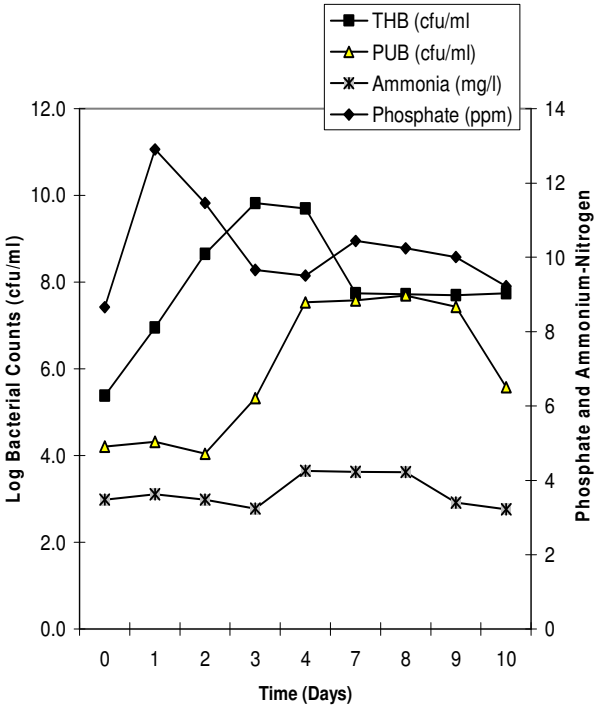

Figure 3. Effect of aeration on the microbial population and physicochemical status of effluent

then a reduction rate of 95% for ammonium and 47% for phosphate. One major role of Mg^{2+} is that it acts as a co-factor in almost all enzymes activating phosphorylation processes. The low levels of removal of phosphate could be attributable to the absence of an anaerobic cycle in our treatment system where strict anaerobes lost out in their role of phosphate metabolism. Nakamura et al. (1989) had studied the simultaneous removal of N and P in a batch activated sludge processand achieved removal rate of above 90% for both N and P. Any treatment system should be able to effectively reduce or eliminate the level of phosphorus compounds in the effluent. Failure in reducing these nutrient levels before discharging into the environment has led to accelerated eutrophication of sur-

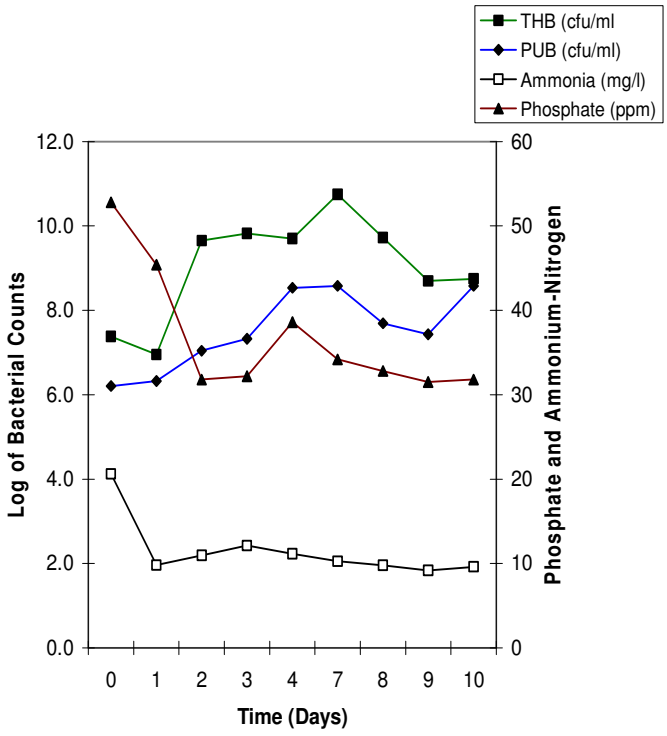

Figure 4. Effect of amendment with glucose on microbial and some chemical parameters of effluent.

face waters (Ye et al., 1988). Oswald and Gotaas (1957) proposed the biological treatment of waste waters with algae to remove nutrients and since then algal systems have been in use to treat human sewage (Shelaf et al., 1980), agro-industrial wastes (Tantichereon et al., 1990; Phang, 1990 a; b) and industrial wastes (Kaplan et al., 1986). Using the HRAP system, Geetha et al. (1994) have reported significant reductions of most of the polluting parameters (chemical oxygen demand, ammonium and phosphate). Zaid (1990) has earlier made similar reports. The present study shows that by a simple combination of aeration (by continuous agitation) and an amendment procedure in effluent holding tanks, the levels of polluting ammonium and phosphate can be reduced substantially before disposal.

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