Full Length Research Paper

# Relationship between bacterial density and chemical composition of a tropical sewage oxidation pond

P. O. Olutiola<sup>1</sup>\*, K. O. Awojobi<sup>1</sup>, O. Oyedeji<sup>1</sup>, A. D. V. Ayansina<sup>2</sup> and O. O. Cole<sup>1</sup>

<sup>1</sup>Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. <sup>2</sup>Department of Biological Sciences, Bowen University, Iwo, Osun State, Nigeria.

#### Accepted 9 August, 2010

Studies were carried out to examine the performance of the sewage oxidation pond situated in and serving the community of the Obafemi Awolowo University, Ile-Ife, Nigeria. A survey of the coliform and total bacterial populations was carried out. The sewage was also examined for biochemical oxygen demand, dissolved oxygen content as well as for nitrate, phosphate, silica and chloride contents. The mean coliform bacteria counts decreased gradually from  $69.1 \times 10^5$  per 100 ml to about  $10.1 \times 10^5$  per 100 ml as the sewage moved through the oxidation pond into the receiving stream. A similar decrease in mean biochemical oxygen demand of the sewage from 397.8 lb/acre/day to 64.2 lb/acre/day was also observed. The concentrations of nitrate, phosphate and chloride decreased from the pond influent to the pond effluent. On the other hand, both the silica and dissolved oxygen content of the sewage gradually increased from 14.1 to 19.0 mg/l and 8.1 to 13.9 mg/l respectively, across the pond to the effluent. The coliform and total bacterial counts as well as the concentrations of most of the chemicals in the receiving stream increased after being joined by the sewage oxidation pond effluent. It is therefore concluded that the receiving stream was subject to both bacteriological and chemical pollution. Building of additional oxidation ponds or addition of a primary sewage treatment to the existing system is recommended for more efficient wastewater treatment.

Key words: Bacterial density, chemical composition, oxidation pond, sewage, tropics.

# INTRODUCTION

Sewage results from the use of water in dwellings, hospitals, institutions, farms, industries and the like. Domestic sewage consists of approximately 99.9% water and the remaining 0.1% is composed mainly of organic and inorganic matter. Thus sewage may be very simply described as stream of flowing water flowing through homes, factories and businesses of a community into which waste materials are discharged. However, the same sewage, if properly treated, can again serve for drinking and the many other uses of everyday living.

Sewage oxidation ponds are scientifically constructed lagoons with a depth of 3 - 5 ft, in which sunlight, oxygen, algae, bacteria, protozoa and other organisms interact to restore water to a quality that is safe for human consumption (Suddiqi and Honda, 1971; Taber and Taber, 1976). Sewage oxidation ponds are one of the lower cost methods for treating the sewage emanating from small communities and this is achieved at minimum maintenance and operational requirements (Arar, 1988). However, space requirement and the nuisance and hazardous conditions it creates, can limit its applicability. It is however widely used in the tropics and sub-tropics because the climatic conditions are conducive to a high level of pond performance.

Sunlight is an important factor in the significant inactivation or removal of pollution indicator organisms in sewage oxidation ponds (Saqqar and Prescod, 1992; Davies-Colley et al., 1999; Ainon and Chuan, 2002; Sinton et al., 2002). The ultraviolet B portion of solar radiation from sunlight is bactericidal, causing direct deoxyribonucleic acid damage. At high intensities, photooxidation becomes more important acting through photosensitizers to damage organelles principally the

<sup>\*</sup>Corresponding author. E-mail: ooyedeji@oauife.edu.ng. Tel: 234 8033745381.

cytoplasmic membrane (Harm, 1980; Jagger, 1985; Davies-Colley et al., 1999).

Microorganisms also constitute essential agents of the sewage purification system, and because of their metabolic activities, changes are brought about to the physical and chemical composition of the sewage, as well as to the population of the microorganisms themselves (Imhoff and Fair, 1956). In oxidation ponds, organic matter is broken down by aerobic bacteria into simple, stable inorganic materials such as carbon dioxide, water, sulphates and phosphates. Algae utilize these compounds to produce complex organic materials that make up algal cells. During this process algal cells generate oxygen which is utilized by bacteria (Bian and Li, 1992). In essence all these lead to the efficient mineralization of organic matter, that is, lower biochemical oxygen demand and inactivation of pathogenic bacteria, yeasts and viruses (Taber and Taber, 1976). The use of sewage oxidation ponds has therefore been considered as the ideal way of improving effluent quality by means of natural processes (Mtethiwa et al., 2007). Apart from pathogenic microorganisms, pollutant categories of significance in this standard water treatment procedure include organic chemicals, heavy metals, phosphates and nitrates (Botkin and Keller, 2003).

This paper describes the relationship between the bacterial density and the biochemical oxygen demand, dissolved oxygen content, nitrate, phosphate, silica or chloride content of the sewage from the time of entering an oxidation pond to the time of joining a natural stream. The Obafemi Awolowo University has two sewage oxidetion ponds, A and B. The present work was carried out on pond A. The oxidation pond B was not in use throughout the period of this study.

## MATERIALS AND METHODS

#### Study area

The Obafemi Awolowo University has two oxidation ponds A and B lying side by side with a wide dyke separating them. They were constructed in 1967 but became operational in 1968. They are located in the Southwestern part of the University and drain wastewater and sewage from the students' hostels, academic area and staff residence (population: about 27,000). Each of the ponds is  $150 \times 32 \times 1.5$  m and only one of these receives wastewater influent at a time. The wastewater and sludge are retained in the pond for about two weeks during which algae, bacteria and other organisms act on them by mineralizing the organic matter content. The wastes are conveyed to the ponds through sewers made of concrete pipes and there are manholes sited at some points along the channels to change the direction of the sewers.

## Sampling

Water samples were collected from five sites: raw sewage in-flow to the oxidation pond, the oxidation pond itself, outflow from the pond, the effluent receiving stream (Opa river) and the pond effluent/ stream mixture, over a 4-month period (late June to middle September). Samples were collected by filling 500 ml sterile brown bottles, iced and processed within 12 h of collection. Collection and examination of samples were as described in the standard methods for the examination of water and wastewater (American Public Health Association, 1995).

### Temperature and pH

The temperature was measured with a thermometer while the pH was measured with a portable pH meter (E.I.L., Model 30C, Gallenkamp) at the study sites.

#### Total bacteria count

The pour plate technique according to Anon (1994) was used. The medium was plate count agar. One milliliter of appropriate serial dilutions (diluent = buffered peptone water) were used. Plates were incubated at 37 °C for 24 h. Only plates containing between 30 and 300 colonies were counted. Preliminary experiments in which the plates were equally incubated at 20 °C for 72 h yielded fewer total bacteria count.

#### **Coliform counts**

All samples were tested for total coliform population by the most probable number (MPN) technique (American Public Health Association, 1995) using MacConkey broth (Baker, 1967) for the preumptive test and eosin methylene blue (EMB) agar plates for the confirmed test; MacConkey broth for the completed test (Buchanan and Gibbons, 1974) and *Escherichia coli* EC broth for the faecal coliform test (Baker, 1967). Composition and preparation of all media, unless otherwise stated, were as previously described (American Public Health Association, 1995).

#### Faecal enterococci test

Tests for faecal enterococci were as described by Bacteriological Examination of Water Supplies (1982) and American Public Health Association (1995) using m-Enterococcus agar for presumptive and bile aesculin azide agar for confirmatory tests.

#### Clostridium perfringens test

Tests for presence of *Clostridium perfringens* was as described by Bacteriological Examination of Water Supplies (1970) and Freame and Fitzpatrick (1972) using differential reinforced clostridial medium (D.R.C.M).

#### **Chemical tests**

Biochemical oxygen demand (BOD) test was carried out with the Hach Manometric biochemical oxygen demand apparatus (Hach Chemical Company, U.S.A.), using the methods described in the Hach manual (Manometric BOD Apparatus Manual, 1979). The quantity of water sample in each bottle was 157 ml to enable a direct scale reading. The apparatus was incubated at 25 °C.

Tests for dissolved oxygen, nitrate, phosphate, silica and chloride were carried out as previously described (Hach Chemical Methods Manual, 1973) using Hach direct reading engineer's laboratory

Parameter	Percentage reduction (%)
†TBC	52.7
Coliform density	85.4
†BOD	83.9
Nitrate	39.1
Phosphate	37.5
Chloride	41.9

<sup>+</sup> TBC - Total bacteria count and BOD - Biochemical oxygen demand. Percentage reduction = 100 ( $F_1 - F_2$ ) /  $F_1$ . where  $F_1$  = Influent and  $F_2$  = Effluent.

apparatus (Model-EL/2). All chemicals used were supplied by Hach Chemical Company (U.S.A.). Dissolved oxygen tests were performed immediately at the sites of collection.

## **RESULTS AND DISCUSSION**

The temperature and pH readings obtained throughout the period of this investigation ranged from 25.5 - 27 °C and pH 6.7 - 7.5 respectively. There was no discernible relationship between total bacterial density and the temperature and pH of the sewage. This perhaps may be explained by the fact that most bacteria are able to tolerate slight temperature and pH changes (Nester et al., 1978). However, Smith et al. (1989) reported that significant microbial activity in water is found at temperatures of 15 °C or higher. Since the water temperature of the pond is always above 25 °C, the occurrence and density of coliform bacteria is always higher.

The results show that the total bacterial load in the pond effluent was less than in the pond influent, the mean percentage reduction being approximately 53% (Table 1). Similar observations were obtained for the coliform density, the percentage reduction being approximately 85%. Both the total bacterial count and the coliform count of the stream increased after being joined by the pond effluent (Figure 1).

The coliform density in the pond effluent-stream mixture was approximately  $5.1 \times 10^5$  per 100 ml of water. This is approximately fifty times  $10^4$  at which point most natural waters are considered as having evidence of fresh sewage pollution and degradation of a natural waterway (the Opa River) which a little further down, was being utilized for various domestic purposes by the villagers along the river bank. Coliform bacteria are used as a criterion to assess the public health safety of discharges while faecal coliforms are used for modeling bacterial removal (Jagals and Lues, 1996).

Tests carried out showed that faecal coliform and *C. perfringens* were present in all the sampling sites, thus indicating presence of both recent and remote faecal

pollution. Positive results were similarly obtained for faecal enterococci, confirming the faecal nature of the pollution. Presence of these organisms is also indicative of a possible health hazard to the consumers of water from the stream, especially after being joined by the pond.

The biochemical oxygen demand (BOD) decreased across the pond to the effluent (Figure 2). On the other hand the dissolved oxygen content of the effluent was higher than that of the influent (Figure 3). Thus the total bacterial count and the coliform density were directly related to the biochemical oxygen demand, but inversely related to the dissolved oxygen. The percentage BOD reduction is an essential criterion for determining the efficiency of a sewage oxidation pond, viz. the greater the percentage reduction, the greater is the pond efficiency (American Public Health Association, 1971). Among factors contributing to significant removal of bacteria in the oxidation pond are lower BOD and sunlight intensity (James, 1987; Saqqar and Prescod, 1992; Ainon and Chuan, 2002). The dissolved oxygen content of the stream decreased on being joined by the pond outflow, indicating pollution of the stream by sewage effluents. During oxidative decomposition of the organic matter in sewage, a demand is exerted upon the dissolved oxygen present therein, resulting in a decrease in the dissolved oxygen content. Thus tests for dissolved oxygen are of utmost importance in controlling pollution of natural waters, especially streams into which sewage effluents are being discharged (Imhoff and Fair, 1956; Smith, 1990).

The nitrate content decreased from the influent through the pond to the effluent (Table 1, Figure 4). Thus the total bacterial count and the coliform count were directly related to the nitrate content. Two interrelated and or interdependent principles are feasible in explaining the nitrate balance of a sewage oxidation pond. Nitrate is an essential source of nitrogen to both bacterial and algal flora of the pond. This will cause a decrease in the nitrate brought to the pond by the influent. On the other hand,



Sample/Distance (m)

**Figure 1.** Relationship between total bacteria count and coliform density in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.



**Figure 2.** Relationship between total bacteria count and biochemical oxygen demand (BOD) in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.



**Figure 3.** Relationship between total bacteria count and dissolved oxygen content in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.



# Sample / Distance (m)

**Figure 4.** Relationship between total bacteria count and nitrate content in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.



**Figure 5.** Relationship between total bacteria count and phosphate content in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.

the end products of organic matter degradation will include nitrate. High microbial decomposition of the organic matter content of sewage will therefore result in high nitrate content. However if the rate of nitrate consumption is higher than the rate of nitrate replenishment. a decrease in overall nitrate content of the sewage will result. This could account for the present result. High nitrate levels in wastewater could contribute to eutrophication effects, particularly in freshwater (OECD, 1982). The phosphate content of the pond influent was higher than that of the pond effluent (Figure 5). The results also showed that the phosphate content decreased as the total bacteria decreased. Bacteria and algae exert a considerable pressure on available phosphate as they are capable of utilizing phosphates for their physiological activities (American Public Health Association, 1971). This will result in low phosphate concentration in the pond effluent.

The silica content increased from the pond influent to the effluent (Figure 7). Silica is utilized by only a relatively few number of microorganisms, such as the diatoms which utilize silica in their skeletal structure. Silica removed from the water may be returned by resolution of the dead organisms. Thus accumulation of silica resulting from dead organisms will contribute to higher silica content in pond effluent than in pond influent. Ogunfowokan et al. (2008) had reported that effluent discharge from the sewage pond into receiving stream led to increase in concentrations of heavy metals such as cadmium, lead, arsenic and cobalt downstream thus impacting the receiving stream negatively and posing health risk to users downstream.

Throughout the sampling period, the chloride content decreased as we passed from the influent to the effluent (Figure 6). Chloride, in the form of chloride ion is one of the major inorganic anions which increased in sewage over the raw water because sodium chloride is a common article of diet and passes unchanged through the digestive system. It may also be increased by industrial processes. Chloride can serve as nutrient for microorganisms, and hence high microbial load may result in its depletion.

The results also show that the total bacterial count, coliform density, biochemical oxygen demand, phosphate, silica and chloride content of the receiving stream were increased by its being joined by the outflow from the sewage oxidation pond. Effluent discharge from sewage and industries are major component of water pollution, contributing to oxygen demand and nutrient loading of the water bodies, promoting toxic algal blooms and leading to a destabilized aquatic ecosystem (Morrison et al., 2001). Both total bacterial count and coliform density increased



Figure 6. Relationship between total bacteria count and chloride content in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.



Figure 7. Relationship between total bacteria count and silica content in a sewage oxidation pond. Each sample point is the mean of 4 replicate analysis.

by 66.3 and 94.6% respectively in the effluent-stream mixture. Thus the performance of the sewage oxidation pond was poor. During this investigation, it was noticed that a lot of sludge accumulated in the pond, and this will contribute immensely to the poor performance of the oxidation pond. Constant removal of the sludge is therefore to be recommended. Building of additional sewage oxidation ponds is another possibility. An alternative will be to add a primary sewage treatment to the existing system. This will involve construction of a 'digester' to care for the solid portion of the sewage, and thus allow the liquid portion (99.9%) to be treated more efficiently by the existing sewage oxidation pond. Cases have also been made for the use of particular aquatic weeds in sewage oxidation ponds, at little or no extra cost, which enhance the efficiency of the pond with improvement in organic matter mineralization and removal of heavy metals (Makinde et al., 2007; Abbasi and Abbasi, 2010).

# ACKNOWLEDGEMENTS

The authors wish to appreciate the assistance of staff of the Obafemi Awolowo University sewage treatment plant as well as the laboratory staff of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria during the course of this study.

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