# Biochemical Oxygen Demand and Carbonaceous Oxygen Demand of the Covenant University Sewage Oxidation Pond

**Ajayi A.A.[1], Peter-Albert C.F.[1], Ajojesu T.P.[1], Bishop S.A.[3], Olasehinde G.I.[1], Siyanbola T.O.[2]**

[1]Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria
[2]Department of Chemistry, College of Science and Technology, Covenant University, Ota, Nigeria
[3] Department of Mathematics, College of Science and Technology, Covenant University, Ota, Nigeria

Corresponding Author: Ajayi, Adesola Adetutu
E mail: adesola.ajayi@covenantuniversity.edu.ng

***Abstract***: Biochemical Oxygen Demand (BOD) is a measure of the dissolved oxygen consumed by microorganisms during the oxidation of reduced substances in waters and wastewaters. It is often used ambiguously in relation to Carbonaceous Oxygen Demand (CBOD) which is the oxygen consumed during the oxidation of carbonaceous compounds to carbon dioxide ($CO_2$) and other oxidized end product. BOD is actually the sum of CBOD and NBOD where NBOD is the Nitrogenous Oxygen Demand which is the oxygen consumed during the oxidation of nitrogenous compounds (mainly $NH_3$) to nitrates with nitrites being an unstable intermediate. The major difference between CBOD and NBOD is that there are two classes of bacteria believed to be responsible for the oxidation of reduced nitrogen. The $BOD_5$ value of Sewage samples collected from Covenant University oxidation pond was therefore measured and the samples examined for the presence of *Escherichia coli*. The sewage samples collected from four points (starting point (A), two middle points (B, C), and end point (D) were inoculated on an Eosin Methylene Blue agar plates and the presence of *E. coli* was confirmed by the appearance of greenish metallic sheen colonies on the agar plates and biochemical Tests. The BOD of the effluent at the different points (A, B, C, D) respectively showed a reduction in microbial load. The ultimate CBOD was also estimated based on the $BOD_5$ value which is based upon the exponential (first-order) nature of oxygen demand. This research describes the formulations of CBOD breakdown using simplified oxidation kinetics.

***Keywords***: Biochemical Oxygen Demand; Carbonaceous Oxygen Demand; *Escherichia coli*; Wastewater

## Introduction

Biochemical oxygen demand (BOD) is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period (Manyuchi and Ketiwa , 2013 ). The BOD value is most commonly expressed in milligrams of oxygen consumed per litre of sample over five days of incubation at 20 °C and it is often used as a robust surrogate of the degree of organic pollution of water (Virendra *et al.,* 2013). BOD can be used to gauge the effectiveness of wastewater treatment plants (Penn *et al*., 2013). Chemical Oxygen Demand (COD) is a measurement of the oxygen depletion capacity of a water sample contaminated with organic waste matter. It is similar in function to Biochemical Oxygen Demand (BOD) because they both measure the amount of organic compounds in water and they are the most commonly used parameters for the characterization of wastewaters (Abdalla and Hamman, 2014). COD also used to estimate BOD because a strong correlation exists between them, however COD is a much faster and more accurate test but it is less specific, since it measures everything that can be chemically oxidized, rather than just levels of biologically active organic matter (Sawyer *et al*., 2003). The conventional standard method for the determination of BOD measures the microorganisms' oxygen consumption or respiration over a period of 5 days and it is reported as $BOD_5$ (Liu *et al*., 2014). The BOD measurement is a good indicator of the concentration of organic pollutants in water but it is extremely slow hence not suitable for process control (Chen *et al*., 2002) but it is essential to obtain a correlation between $BOD_5$ and COD for various wastewater treatment plants to help in the design and operation of treatment plants (Abdalla and Hamman, 2014). However, BOD is often used ambiguously in relation to Carbonaceous Oxygen Demand (CBOD) which is the oxygen consumed during the oxidation of carbonaceous compounds to carbon dioxide ($CO_2$) and other oxidized end product (Penn *et al*., 2009). BOD is actually the sum of CBOD and NBOD where NBOD is the Nitrogenous Oxygen Demand which is the oxygen consumed during the oxidation of nitrogenous compounds (mainly $NH_3$) to nitrates with nitrites being an unstable intermediate (Yudianto and Yuebo, 2008). *Escherichia coli* is an index organism used for the determination of faecal contamination it can be used to measure the effectiveness of the disposal mechanisms or treatment plants in ensuring that the effluents are environmental friendly (Naidoo and Olaniran, 2014). This research work was therefore carried out to isolate *Escherichia coli* from the Covenant University Oxidation pond, evaluate the BOD of the oxidation pond and determine the CBOD based on the $BOD_5$ values.

## Materials and Methods

### Collection of Samples

Four sewage water samples were obtained from four different point of the Covenant University Oxidation pond. The samples were collected with the aid of sterile sampling bottles and a long rope tied around the neck of each bottle was allowed to gradually sink into the sewage to fill the bottles. The bottles were covered aseptically and transported to the Microbiology Laboratory of the Department of Biological Sciences, Covenant University, Ota. The samples were analyzed immediately.

**Cultivation of *Escherichia coli***

Ten milliliter (10ml) of water sample was dispensed into three test tubes containing ten milliliters of double strength McConkey broth (10ml), one milliliter of the water sample was dispensed into single strength McConkey broth (10ml) in each of three test tubes and 0.1ml of the water sample into another set of three test tubes containing single strength McConkey broth (10ml). The inoculated broths were incubated at 37$^o$C for 24 - 48h and they were monitored for acid and gas production. The pour plate method was used for the presence of *E.coli.* One milliliter of each sample was aseptically transferred into a sterile petridish to which about fifteen milliliter of cooled molten agar was poured. The organisms were subcultured on EMB to obtain pure cultures and they were thereafter streaked on nutrient agar slant and incubated at 37$^o$C for 24h and stored as stock cultures.

**Measurement of Dissolved Oxygen of the Covenant University Oxidation Pond**

The dissolved oxygen of the samples collected at the four points was measured using the MW600 Dissolved Oxygen Meter. The device was calibrated according to manufacturer's specification. The probe was verified to be polarized and probe meter calibrated. The tip of the probe was immersed in the samples (A, B, C, D) respectively. For accurate Dissolved Oxygen (DO) measurements a minimal water movement of 0.3m/sec was required and each sample was dispensed into a sterile beaker and placed upon a stirrer. To check if the water speed was sufficient, a waiting period was observed for the reading to stabilize and move the DO probe.

**Measurement of Biochemical Oxygen Demand of the Covenant University Oxidation Pond**

The Biochemical Oxygen Demand of the samples was carried out according to the methods described in UGA extension (2013) whereby a DO meter was used to measure the initial dissolved oxygen concentration in the sample bottle collected from point D of the oxidation pond and the bottle was placed in a dark incubator at 20$^o$C for five days. After five days, the DO meter was used to measure a final dissolved oxygen concentration. The Final DO reading is then subtracted from the initial DO reading and the result is the BOD concentration.

$$[BOD_5] = [DO]_{Final} - [DO]_{Initial}$$

**Mathematical Determination of CBOD**

This was determined according to the method described by Penn *et al.*

(2009) whereby the equation for the determination of CBOD is:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O \quad (1)$$

$$2H_3^{+} + O_2^{-} = 2H_2O + 2H^{+} \quad (2)$$

$$[BOD_5] = [DO]_{Final} – [DO]_{Initial} \quad (3)$$

$$d [DO]/dt = d [CBOD]/dt = -K[CBOD] \quad (4)$$

The BOD exerted (Oxygen Demand) increases with time, therefore,

$$[CBOD] = [CBOD]_o \times e^{-kt} \quad (5)$$

Where K = First-order reaction rate constant

T = Time in days

$[CBOD]_o$ = initial CBOD concentration

Ultimate CBOD using the approximation of the $BOD_5$ which is based on using the exponential (first-order) nature of oxygen demand is therefore,

$$\text{Ultimate-CBOD} = BOD_5 \times (1-e^{kt})^{-1} \quad (6)$$

$$\text{Ultimate} – [CBOD] = [BOD_5] \times (1–e^{kt})^{-1}$$

Where ($BOD_5$) = the Biochemical Oxygen Demand exerted over the five day period

**Results**

***Escherichia coli* strains obtained from the Covenant University Sewage pond**

All the samples from the sewage oxidation pond investigated revealed the presence of *E.coli* as shown by the Most Probable Number (MPN) test whereby all the samples showed gas production (Table. 1). The appearance of greenish metallic sheen colonies on Eosin Methylene Blue agar further confirms the presence of *E.coli* (Table. 2). The biochemical characteristics of the *E. coli* isolates (Table. 3 ) revealed that the *E.coli* were Indole positive, Methyl red positive, Catalase positive and Voges Proskauer negative, Starch hydrolysis negative, Urease negative and Citrate negative. They appeared as Gram-negative rods under the microscope.

**Determination of Dissolved Oxygen at points of collection**

The Dissolved oxygen measurement for samples taken at four random points along the oxidation pond decreased from a value of 10.1 mg/l to 7.9 mg/l from point A to point D respectively (Fig. 1).

**Determination of Biochemical Oxygen Demand**

The BOD values obtained is as follows:

$[BOD_5]$ = [DO] Final – [DO] Initial

$[BOD_5]$ = 39.5mg/l – 7.9mg/l

$[BOD_5]$ = 31.6 mg/l

**Mathematical Determination of Ultimate Carbonaceous Biochemical Oxygen Demand**

This was determined using the following:

DO final =39.5 mg/l

DO Initial =7.9 mg/l

Time in Days = Five Days; 5 x 24=120h

K = ranging from 0.3 to 0.7

Ultimate [CBOD] = [BOD5] x (1–e-kt)-1

Ultimate CBOD = 31.6mg/1 for K= 0.3 and K= 0.7

**Table 1: The Most Probable Number of Organisms from the Covenant University Oxidation Pond**

| Sample Zones | Combinations | MPN Index per g/ml |
|---|---|---|
| A | 3-3-1 | 4.6 |
| B | 3-2-2 | 2.1 |
| C | 3-3-3 | >11 |
| D | 3-2-2 | 2.1 |
| | | |

**Table 2: Growth of *Escherichia coli* on Eosin Methylene Blue Agar**

| Samples | Growth on EMB | Presence of *E.coli* |
|---|---|---|
| A | + | + |
| B | + | + |
| C | + | + |
| D | + | + |

**Keys**

+ indicates a positive test
- Indicates a negative test

**Table 3: Biochemical Characterization of *Escherichia coli* strains isolated from Covenant University Oxidation Pond**

| Sample | Oxidase Test | Catalase Test | Urease test | Citrate Test | Voges Proskauer | Methyl Red | Indole Test | Starch Hydrolysis test | Gram Staining Reaction |
|---|---|---|---|---|---|---|---|---|---|
| **A** | - | + | - | - | - | + | + | - | Negative Rods |
| **B** | - | + | - | - | - | + | + | - | Negative Rods |
| **C** | - | + | - | - | - | + | + | - | Negative Rods |
| **D** | - | + | - | - | - | + | + | - | Negative Rods |

**Key**

**+ indicates a positive result**
**- indicates a negative result**

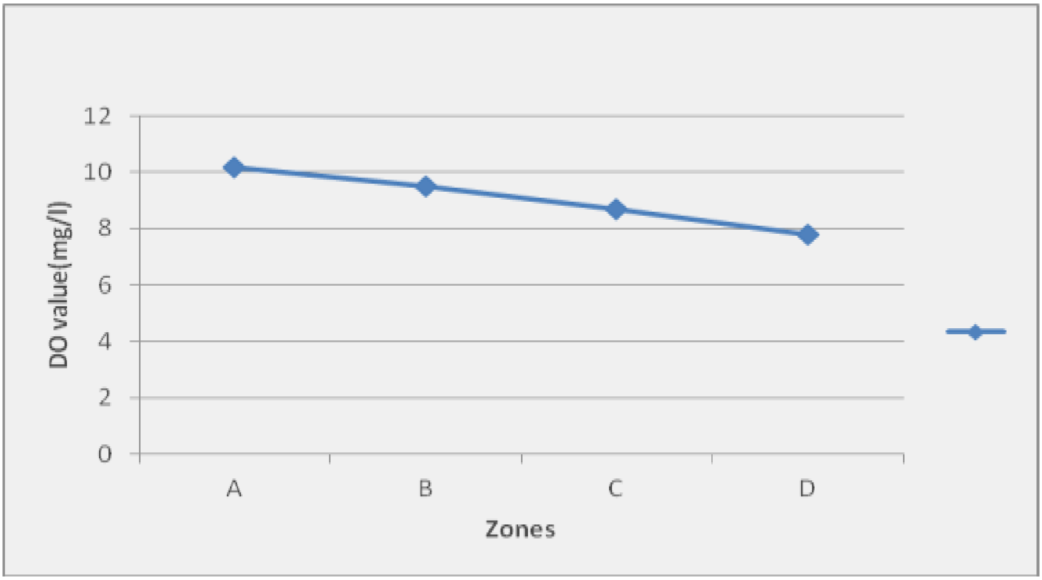


**Fig 1: Dissolved Oxygen at the various points (A-D) of the Covenant University Oxidation Pond**

## Discussion

The results of this investigation revealed that the Biochemical Oygen Demand was employed as a parameter to define the strenght and examine the efficiency of the Covenant University Oxidation Pond. The goal of wastewater treatment is to protect and maintain healthy rivers and oceans which is the aim of evaluating the $BOD_5$ and COD of waste water (Abdalla and Hamman, 2014). If pollutants in wastewater are not removed, they flow directly into our waterways and this can threaten public health, fisheries, wildlife habitat, recreation opportunities and ultimately, our quality of life (Metro Vancouver, 2013). Two of the important components of wastewater addressed through treatment are: Total Suspended Solids (TSS) and the Biochemical Oxygen Demand. The amount of total suspended solids and biochemical oxygen demand removed from wastewater is used to gauge the effectiveness of wastewater treatment plants (Penn *et al.*, 2009).

The Dissolved oxygen values of the water samples obtained from the Covenant University oxidation pond decreased in value from the first point of collection from 10.1mg/l to 7.9mg/l respectively for four random collection points. This shows that the water is in a healthy condition and is fit for aquatic life. It also reveals that the Covenant University treatment plant is effective. Nester *et al*. (2001) reported that when there is excessive BOD, there will be deficiency of DO and water will be in anaerobic condition resulting in mortality of living aquatic organisms; release of ammonia, methane, $CO_2$ in the absence of oxygen, anaerobic bacteria becomes active. CBOD is a method defined test which is measured by the depletion of dissolved oxygen by biological organisms in a body of $H_2O$ in which the contribution from nitrogenous bacteria has been suppressed (Penn *et al*., 2009). It is used as an indicator of the pollutant removal from wastewater.

The results of this investigation revealed the presence of *Escherichia coli* in the four sewage samples obtained. The strains of *E.coli* isolated were found to be urease positive with the exception of the strains isolated from the last collection point (D). Also, the strains were found to be indole positive, Methyl red positive, Catalase positive fermenting Glucose, Lactose, Maltose and Sucrose, with exception of the strain from the third collection point(C). *E.coli* has been used as an indicator for water pollution since it is entirely foreign to water (Akande *et al*., 2011; Health Canada, 2012). *E.coli* is a facultative anaerobe, mixed acid fermenter, able to convert formic acid to hydrogen and carbondioxide, lactose fermenter and unable to utilize citrate as the sole carbon source (Holt *et al*., 1994). The presence of *E.coli* in water samples such as sewage implies faecal contamination and strongly suggests the possible presence of enteric pathogenic bacteria, enteric viruses and protozoans (Feng *et al*., 2002). Apart from being an indicator of faecal pollution of water, *E.coli* has been implicated in diseases, although most strains are harmless (Nataro *et al*., 1998). The Ultimate CBOD has the same value as $BOD_5$ for $K = 0.3$ and $K = 0.7$ when calculated mathematically with the time for five days recorded in hours but an undefined result was obtained for the time calculated in seconds. This is probably due to the exponential function. The importance of the oxygen demand of wastewater for a healthy living condition cannot be overemphasized for two major related purposes which are: To provide an indirect measure of the total amount of organic matter in the wastewater and to provide a basis for assessing the effects of the natural water receiving it. CBOD is sometimes advantageous when compared with BOD because it measures just the oxygen demand exerted by organic (carbonaceous) compounds, excluding the oxygen demand exerted by the nitrogenous compounds. The CBOD accomplishes this by inhibiting the nitrifying organisms from using oxygen by the addition of a nitrification inhibitor to the samples (Acton, 2012).

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