Biodegradability Study of Potassium Hydrogen Phthalate and Benzene Using BOD5 Seed as Inoculum

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Abstract

results. In addition to this, the use of a mixed microbial inoculum from an activated sludge treatment plant, leads to concerns with regards to inoculum sampling, difficulty in maintaining viability and controlling quantitative and qualitative characteristics, and the possible presence of pathogenic microorganisms [4,5].

The definition of a 'standard' inoculum could be the best solution to this potential problem. The 'standard' inoculum could be formed by mixing specific microbial strains conserved in microbial collections with fine knowledge of its relevant biochemical properties. Standardized microbial communities as inoculum give way to more homogenous and comparable results that eliminates bias with the results. An example of a standardized inoculum is the BOD5 Seed Inoculum which is known as the standard inoculum use in BOD5 test.

In this present work, BOD5 Seed Inoculum had been used as a standardized seed inoculum for biodegradation of Benzene and Potassium Hydrogen Phthalate. The seed was composed of specialized microbial cultures which contain species from the genera Pseudomonas, Nocardia, Streptomyces, Bacillus and Micromonospora. BOD5 seeds inoculums are non-pathogenic, convenient, fast and cost-effective thus such seeds have the potential for widespread use in water pollution laboratories [6]. The BOD5 Seed Inoculum had also been used to treat aqueous organic waste sample to test the

solution and the seeding of the samples. The pre-treatment of the samples was done by adjusting the pH to neutral and lowering the temperature up to 20°C to preserve the content of the sample. The dilution water was prepared by adding the nutrients needed by the microbes for biodegradation. Magnesium sulfate solution, Calcium carbonate solution and Ferric sulfate solution were added for the nutrients of the microbe. All the components were mixed and preserved up to 20 ± 3°C before analysis. The seed solution was then prepared by pouring entire content of the Polyseed® capsule in a half liter of dilution water, aerated and stirred for an hour then settled for 5 to 15 minutes. A Bran, which acts as the carrier for the microorganisms, will neither dissolve or nor inhibit microbial activity, this must be settled out of the solution necessarily. When the solution is settled, decant the supernatant carefully then transfer decanted solution to a clean beaker and was gently stirred in the remainder of the test. The prepared samples were transferred in an amber bottle and added with 300 mL dilution water along with 4 mL of the seed solution which is prescribed to the method of the BOD. Aside from the biodegradability of the samples, a negative, blank, and sample control were used. The Negative control was used to determine the possible abiotic degradation wherein the test water and seed was used as the negative control. The blank was used to check the possible effect between the seed and dilution water only. The sample control is the unseeded samples which is composed of two: the aerated and sealed. The first COD monitoring started after three (3) to fifteen (15) hours of the completion of the set-up. The Chemical Oxygen demand was used to monitor the biodegradation of the samples and controls.

Set-up: Four (4) Amber bottles with a capacity of four (4) liters were filled with the one liter of sample. A silicone tube with a small diameter is dipped in the samples and connected with a splitter connector for aeration of samples. The silicone tubes are connected along the other sample's splitter and to the air pump, which is elevated from the samples, for the distribution of air. The set up consists for degradations of the negative, blank, sample controls and the samples.

Final analysis

After the biodegradation of the samples, samples then undergone final analysis. Similar in the initial analyses, the pH, dissolved oxygen content, and chemical oxygen demand was determined.

Results and Discussion

The biodegradation process was caused mainly by the microorganisms in the BOD5 Seed Inoculum, wherein they consume the organic pollutants as a part of their metabolism thus leading to its growth and to the biodegradation of the samples.

Initial and final dissolved oxygen

Sample	Dissolved Oxygen(DO) (mg/L)	
	Initial	Final
Benzene	3.8	6.1
KHP	2.4	8.6
Waste	1.1	1.7

Table 1: Dissolved Oxygen.

This section reveals the result obtain from the biodegradation of the samples using BOD5 seed inoculum. The biodegradation of the samples was assessed using COD concentration which was measured at time intervals throughout the biodegradation.

The Dissolved Oxygen (DO) was determined using the Winkler-Azide Modification Method. The results were obtained before and after the biodegradation process to compare and prove the degradation method proposed. Table 1 shows the initial and final Dissolved Oxygen of benzene and KHP and waste. As shown in Table 1, DO concentration of benzene increased from 3.8 mg/L to 6.1 mg/L while DO of KHP increased from 2.4 mg/L to 8.6 mg/L. Same instance was observed for the waste sample in which its DO concertation increased from 1.1 mg/L to 1.7 mg/L.

The results obtained showed that dissolved oxygen content of the samples. The increased DO indicate that the samples were degraded since increased DO means that organic substances present in the sample have decreased which allows more oxygen to be dissolved

However, after that period, COD concentration of both benzene and KHP then decreased slightly as biodegradation proceeds. Furthermore, Figure 2 also shows that the COD concentration of both samples slightly increased at time interval 3 to 75 hours. In addition to the degradation caused by the seed, the exponential decreased of COD concentration observed after 3 hours of biodegradation may also be due to the addition of 300 mL dilution water which contains the nutrients needed by the microorganisms to survive. On the other hand, the sudden increase of COD at time interval 3 to 75 hours could be explained by the factor caused by filtration in obtaining samples. Filtration eliminates interferences from the seed itself. The significance of the filtration of the samples was then justified by the result obtained after 123 hours which shows a 92.99% decrease in the COD concentration of Benzene 1 and 14.82% in KHP 1.

Biodegradation of aqueous organic waste

The biodegradability of aqueous organic waste sample using BOD seed inoculum in 250 hours (10 days) was studied. As shown in Figure 2, COD concentration of aqueous organic waste sample continuously decreases as biodegradation proceeds. It is shown that COD of the waste sample was reduced by 68.71% from the initial COD of 48360 mg O_2/L to final COD of 15131 mg O_2/L after 243 hours or 10 days of biodegradation. At first COD monitoring (3 hours after seeding), 22.95% COD reduction was observed. While at second COD monitoring, 17.54% of COD was reduced after 24 hours from first COD monitoring. On the other hand, at third COD monitoring (after 96 hours of biodegradation), 41.37% of COD was reduced from COD concentration at second monitoring which may suggest that about 10% COD was reduced every 24 hours of biodegradation from third COD monitoring to second COD monitoring. Moreover, 15.99% of COD was reduced from COD concentration at third monitoring after another 120 hours of biodegradation which may also suggest that about 3% COD was reduced every 24 hours of biodegradation from fourth COD monitoring to third COD monitoring. This implies that the rate of COD concentration reduction decreases the longer the biodegradation of the aqueous organic waste sample.

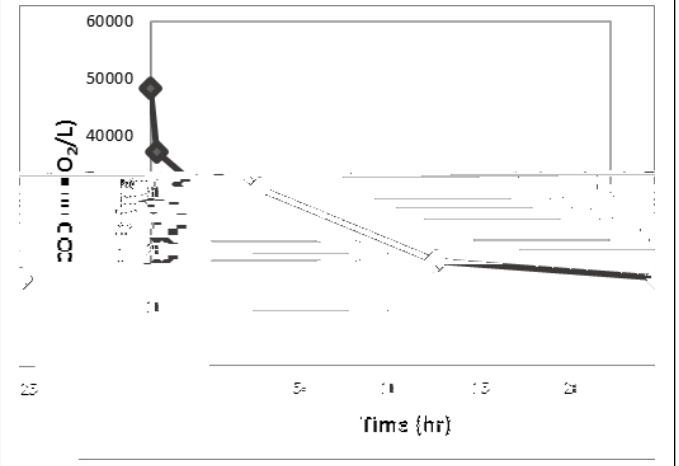

Figure 2: The graph obtained from plotting from plotting COD concentrations of aqueous organic waste sample against biodegradation time.

The initial concentration of 48360 ppm of the organic waste depletes rapidly at the first

of the seeded sample ranges from 6-8 which is the required pH for the continuous survival of the seed.

Controls