

# CARBON EMISSION PATHWAYS OF BIODEGRADABLE THERMOPLASTICS SPECIES MINERALIZED IN NATURAL AND SIMULATED AQUEOUS CONDITIONS IMPLICATIONS FOR ENVIRONMENTAL BIOSAFETY

## Abstract

This study assessed the biosafety of the biodegradation process of bio-based thermoplastic moieties in two aqueous (surface and simulated marine water) environments and its implication on environmental quality. The physicochemical parameters of the two aqueous media used were determined using standard methods. The American Society for Testing and Materials' standard was used to assess amount of CO<sub>2</sub> evolved. Cellulose, bioplastic and polyethylene were inserted in two aquatic environments and arranged thrice in a randomized experimental arrangement of 2x4x3. Ultimate biodegradations of the test films were monitored using Scanning Electron Microscopy. The amount of CO<sub>2</sub> evolved was assayed using the titration method. Data obtained were subjected to descriptive and inferential statistical analyses using Statistical Packages for Social Sciences (SPSS) version 25.0. After biodegradation, there was little or no deviation (less than 2%) from the initial values of the physicochemical parameters with the values within recommended values of the WHO standards. Moreover, CO<sub>2</sub> captured from the two aqueous conditions were lower than the amount of CO<sub>2</sub> evolved in aqueous solution with cellulose which is a natural polymer in this order: 88.725×10<sup>2</sup> mg from the soaked cellulose samples in marine > 85.215×10<sup>2</sup> mg of CO<sub>2</sub> evolved from cellulose entrenched in surface water > 82.758×10<sup>2</sup> mg of CO<sub>2</sub> evolved from bioplastic soaked in marine water > 82.758×10<sup>2</sup> mg of CO<sub>2</sub> evolved from bioplastic soaked in surface water > 65.046×10<sup>2</sup> mg of CO<sub>2</sub> evolved from polyethylene soaked in marine water > 60.152×10<sup>2</sup> mg of CO<sub>2</sub> evolved from polyethylene soaked in surface water. This study concluded that bioplastic can biodegrade totally in aquatic environments without the release of toxic residues that can alter the environmental quality/ Thus, the biodegradation of the bioplastics are environmentally safe.

**Keywords:** Carbon Emission, Bioplastic Packaging Nylon, Freshwater, Marine water, Bioremediation, Plastic pollution

## 1.0 Introduction

There has been a growing demand to use biodegradable bioplastics of natural origin as a suitable replacement for non-biodegradable traditional plastics because the proportion of non-biodegradable single-use plastics with short-term benefits that are produced globally is up to 900 billion [1]. This amount constitutes a significant portion of the plastic waste structure in the environment in which the terrestrial and aquatic environments are affected as many single-use plastic wastes form the bulk of the litter found in them [2]. The use of natural biogenic polymers such as starch, and fiber in bioplastics provides a solution for waste disposal and reduction of carbon and energy footprints. Besides, natural polymers that are used to prepare bioplastics are renewable, biodegradable, cost effective, low density, sustainable, and environmentally-friendly [5]. These attributes confer the biodegradability potential of bioplastics. However, the biosafety of the bioplastic biodegradability process is germane to encouraging public acceptability and adoption of the bioplastic species in society across the globe. According to [4] the evolution of carbon dioxide gases during biodegradation confirms the ultimate biodegradation of bioplastic entities in any environment without toxic residues. Under specific conditions, water, carbon dioxide, and methane are the natural elements reported after the biodegradation of bioplastics as well as increased cell biomass [5]. Therefore, biodegradable bioplastics can be composted in industrial composting facilities or home composting systems leading to the production of nutrient-rich compost. Thus, waste could be easily diverted from landfills through composting; thereby reducing environmental pollution [6]. Moreover, in assessing the biosafety of the biodegradation process, the amount of carbon dioxide released during bioplastic biodegradation is usually lower than the carbon footprint of conventional plastics derived from petrochemicals and some natural plastics [7]. Consequently, mitigating the impact of greenhouse gas emissions as well as contributing to the overall goal of reducing carbon emission pathways. The ecological benefits of this closed-loop cycle of organic matter ensures wastes minimization, reduced reliance on non-renewable resources while fostering sustainable practices that support the regeneration of natural systems [8]. In addition, microbial diversities responsible for the breakdown of the bioplastic's species increase in cell biomass after assimilating part of the carbon-based nutrient released during the mineralization of the biodegradable bioplastics [9]. In another vein, physico-chemical properties of the medium such as aquatic environment are safe for use as microplastics and nanoplastics are not found in the environment as carbon-based residues [10].

In this study, the carbon emission footprints of the biodegradable thermoplastic blends were evaluated. Using the International Standard methods, three species of plastics which are biodegradable plastic blends of thermoplastic base, cellulose (positive control) and polyethylene (negative control) were subjected to two aqueous (surface water and simulated marine water) conditions with a view to assess the biosafety of the real biodegradable bioplastics in aqueous environment.

## **MATERIALS AND METHODS**

### **Preparations of Natural and Simulated Aqueous Conditions**

The natural and simulated aqueous conditions were the two aqueous environments used in this study. The natural aqueous conditions were obtained from flowing stream in an unperturbed environment while the simulated aqueous conditions were prepared by adding each compound of 24.53 g/l of NaCl, 25.20 g/l of  $MgCl_2 \cdot 6H_2O$ , 4.09 g/l of  $Na_2SO_4$ , 1.16 g/l of  $CaCl_2$ , 0.695 g/l of KCl, 0.201 g/l of  $NaHCO_3$ , 0.101 g/l of KBr, 0.027 g/l of  $H_3BO_3$ , 0.025 g/l of  $SrCl_2 \cdot 6H_2O$ , 0.003 g/l of NaF dissolved in one liter of distilled water to simulate marine water conditions. The mean monthly temperature ranged between 25°C and 30°C while the mean monthly relative humidity was below 65.

### **Sources of The Test Plastics**

Three test materials used for the study are nylon 6 packaging bag, biodegradable thermoplastic blends and cellulose. The biodegradable thermoplastic blend was PBAT–PBS (30/70) (commercially named Bionolle 1020) obtained from the Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa. The Nylon 6 was bought from a local store in Akure, Ondo State-Nigeria while cellulose was obtained from the Environmental Management and Toxicology (EMT) Laboratory, Department of Biological Sciences, Elizade University, Ilara-Mokin, Ondo State-Nigeria.

### **Determinations of the Physicochemical Properties of Water**

The temperature readings of the natural and simulated aqueous conditions were taken with the aid of a digital thermometer on-site. The pH and total dissolved solids (TDS) and electrical conductivity (EC) of the water samples were measured with HANNA HI 9810 pH-TDS meter.

The meter was standardized with a buffer solution (i.e., buffer 7 and 9). Total Suspended Solids (TSS) in natural and simulated aqueous conditions were measured using the filtering technique according to the established protocols of APHA (2005) [11]. The Dissolved Oxygen (DO) method was used using titration methods while turbidity of the natural and simulated aqueous conditions were determined using Nephelometric methods as described in [12]. The heavy metal contents of the natural and simulated aqueous conditions were determined using digestion and atomic spectrophotometric methods.

### **Experimental Design**

Following the procedures of American Society of Testing and Materials [13], five hundred mls of natural aqueous solution (surface water) and simulated aqueous conditions (marine water) were added respectively to each 1000 mls of twenty-four respirometric glass jar of height 12 cm and width 4 cm. The test materials of bioplastic, nylon-6 films were cut into sizes of 2 cm by 2 cm. Five hundred grammes each of the bioplastic, nylon-6 films and the powdered cellulose as well as the blank were released into the surface and marine water samples. The experimental design was arranged in three replicates in a randomized design of 4x2x3. Thereafter, 40 ml of 1 N KOH (Potassium hydroxide) were poured into a sterilized 50 ml glass beaker and placed in all the respirometric glass jars to capture the evolved CO<sub>2</sub>. The respirometric jars were incubated at 58°C for four months. Readings were taken every other day.

### **Analytical Characterization**

#### **Scanning Electron Microscopy (SEM)**

The morphology of the polymers was characterized using a Scanning Electron Microscope (SEM). The samples sent out for the imaging were cleaned using distilled water, air dried and put into paper envelopes before the analysis. The standard procedures for sample preparation and analysis were followed to ensure the maximum results.

#### **Calculations for Biodegradation test**

To determine the concentration of CO<sub>2</sub> evolved by titration method, calculations were made using the formular below as described by [14]:

$$\text{CO}_{2(g)} = (\text{N KOH} \times \text{ml KOH} - \text{ml HCl} \times 1\text{N HCl}) \times 44 / 2.$$

CO<sub>2(g)</sub> is the amount of Carbon (iv) oxide evolved, N KOH is the normality of KOH used during titration, ml HCl is the amount of HCl used during titration to get the endpoint of titration process.

1N HCl is the normality of the HCl used.

## **Results and Discussion**

### **Physicochemical Properties of Natural Aqueous Conditions before and After Biodegradation**

As reported, Table 1 displayed the physicochemical properties of natural aqueous condition before and after the biodegradation process. The pH reported shows that the natural aqueous sample was slightly alkaline (7.7) in nature before and 7.8 after the test in water with cellulose sample; other samples, such as natural aqueous condition embedded with bioplastic, was 7.7 before and 7.9 after the test, whereas water with nylon 6 was 7.7 before and 7.6 after the test, showing a decrease in pH by 0.1 after the test. The pH of the control sample (blank) was 7.7 before the experiment and same 7.7 after the experiment. This may be because no test polymer was added. Furthermore, the values of the other parameters such as electrical conductivity, temperature, dissolved oxygen, turbidity, total dissolved solids, and total suspended solids, obtained before and after the test had slight differences across all water samples with values within the recommended values of the World Health Organization (WHO). This revealed that the aqueous media was obtained from unperturbed surface water because human activities were minimal around the sampling area. The concentrations of metals found in water were within the permissible level recommended by WHO. However, the concentrations of lead found in the

initial concentrations of the aqueous solution were higher than the recommended allowance for aqueous medium. However the concentrations of iron in the water after each medium with the polymers varies slightly at almost 2% reduction in the concentrations. This may be attributed to the fact that iron is a strong reducing agent because iron releases electrons to the available oxygen in the aqueous media containing the polymers (.

**Table 1:** Physicochemical properties of The Natural Aqueous Condition Before and after

Analysis	Cellulose		Bioplastics		Nylon-6		Blank		WHO/ USEPA limits
	Before	After	Before	After	Before	After	Before	After	
Electrical conductivity ( $\mu\text{S}/\text{cm}$ )	306.00	307.00	306.00	308.00	306.00	306.00	306.00	305.00	NA
Temperature ( $^{\circ}\text{C}$ )	20.30	20.40	20.30	20.50	20.30	20.20	20.30	20.30	NA
pH	7.7	7.8	7.7	7.9	7.7	7.6	7.7	7.7	6.5–8.5
Dissolved Oxygen (DO)	2.67	2.68	2.67	2.69	2.67	2.65	2.67	2.66	NA
Turbidity (m)	7.80	7.90	7.80	8.00	7.80	7.80	7.80	7.70	NA
Total Dissolved Solid (mg/L)	153.00	154.00	153.00	155.00	153.00	152.00	153.00	152.00	1000
Total Suspended Solid (mg/L)	1	1	1	0.8	1	1	1	0.9	NA
Cadmium (Cd) (ppm)	0.008	0.007	0.008	0.007	0.008	0.008	0.008	0.008	0.01
Lead (Pb) (ppm)	0.032	0.028	0.032	0.031	0.032	0.031	0.032	0.032	0.00001

Zinc (ppm)	(Zn)	0.272	0.262	0.272	0.270	0.272	0.270	0.272	0.271	3.0
Iron (ppm)	(Fe)	0.2185	0.2181	0.2185	0.2178	0.2185	0.2180	0.2185	0.2182	0.3

### Physicochemical Qualities of Marine Water Samples Before and After Biodegradation

The physicochemical properties of simulated aqueous condition (marine water) before and after the biodegradation of the test polymers are shown in Table 1. The value (8.2) of the pH before biodegradation was basic in nature. The pH increased (8.3) after the test in water embedded with cellulose sample. A similar increase in pH values from 8.2 to 8.3 was reported in the natural aqueous condition with bioplastic sample. However, water with nylon 6 showed a decrease in the pH (8.2 before and 8.1 after the test) by 0.1 after the test whereas, in water as a control sample (blank), no changes in the pH (8.2) even after the test. Nevertheless, the increase in the pH by 0.1 after the test in water samples with cellulose and bioplastic ascertains the validity criteria of the test. The values of the other variables, such as electrical conductivity, temperature, dissolved oxygen, turbidity, total dissolved solids, and total suspended solids, reported before and after the test also had slight differences between them across the different water samples; although with values within the World Health Organization recommended permissible limits. Similarly, the metal concentrations for cadmium, Lead, Zinc and Iron reduced after soaking the test polymers in this order respectively: cadmium ( ), Lead ( ), Zinc ( ), and Iron ( ).

**Table 2:** Physicochemical properties of simulated aqueous condition before and after Biodegradation Processes of the Polymers

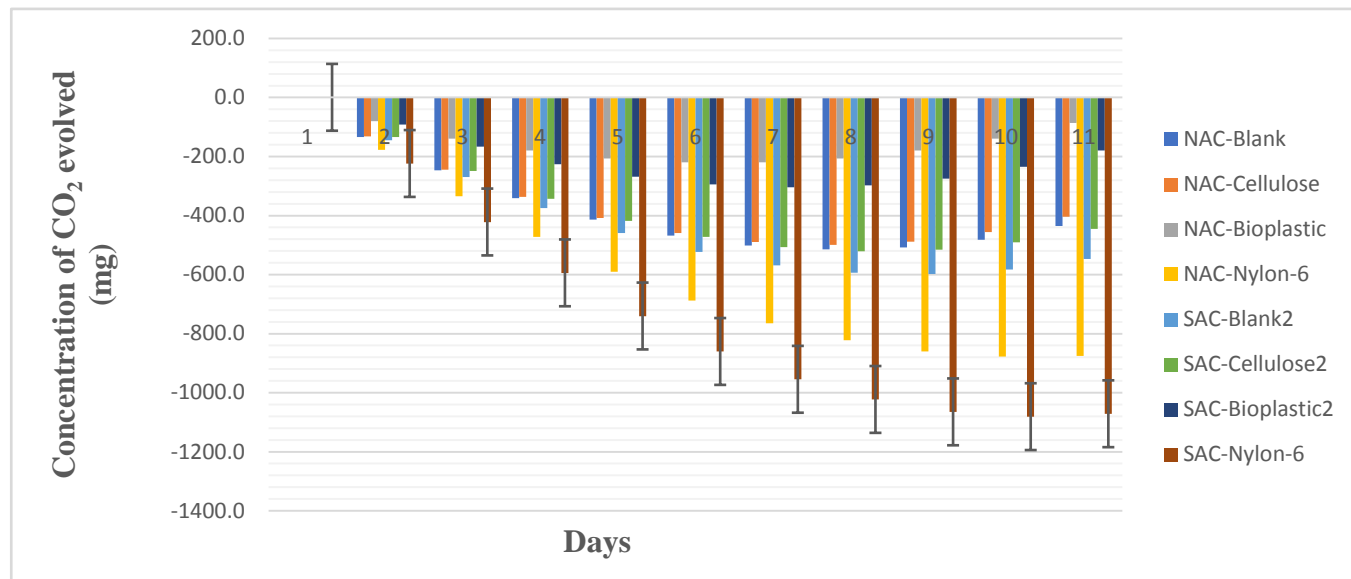
Analysis	Cellulose		Bioplastics		Nylon-6		Blank		WHO/US EPA limits
	Before	After	Before	After	Before	After	Before	After	
Electrical conductivity ( $\mu\text{S}/\text{cm}$ )	385.00	386.00	385.00	387.00	385.00	384.00	385.00	385.00	NA
Temperature ( $^{\circ}\text{C}$ )	26.20	26.30	26.20	26.40	26.20	26.10	26.20	26.10	NA
pH	8.2	8.3	8.2	8.3	8.2	8.1	8.2	8.2	6.5–8.5
Dissolved Oxygen (DO)	2.31	2.32	2.31	2.33	2.31	2.31	2.31	2.32	NA
Turbidity (m)	6.30	6.90	6.30	6.80	6.30	6.30	6.30	6.20	NA
Total Dissolved Solid (mg/L)	155.00	157.00	155.00	159.00	155.00	155.00	155.00	154.00	1000
Total Suspended Solid (mg/L)	1.1	1.1	1.1	1.2	1.1	1.1	1.1	1.0	NA

Cadmium (Cd) (ppm)	0.0009	0.0006	0.0009	0.0008	0.0009	0.0009	0.0009	0.0009	0.01
Lead (Pb) (ppm)	0.0225	0.0223	0.0225	0.0225	0.0225	0.0225	0.0225	0.0955	0.00001
Zinc (Zn) (ppm)	0.0955	0.0950	0.0955	0.0957	0.0955	0.0955	0.0955	0.7700	3.0
Iron (Fe) (ppm)	0.780	0.772	0.780	0.774	0.780	0.779	0.780	0.5685	2.0

### **Carbon (iv) Oxide (CO<sub>2</sub>) Evolution of Bioplastic Packaging Nylon, Cellulose and Synthetic Nylonin Natural and Simulated Aqueous Conditions Across Thirty (30) Days**

Figure 1 revealed the carbon (iv) oxides (CO<sub>2</sub>) evolved in both natural aqueous condition with test materials for the first month. In the surface water, the amount of CO<sub>2</sub> evolved from natural aqueous conditions sunk with test materials ranging from  $-0.799 \times 10^2$  mg to  $-1.775 \times 10^2$  mg. The CO<sub>2</sub> evolved from bioplastic sunk in freshwater was  $-0.799 \times 10^2$  mg while that of cellulose was  $-1.322 \times 10^2$  mg with the synthetic nylon 6 having CO<sub>2</sub> evolution at  $-1.775 \times 10^2$  mg and control were  $-1.337 \times 10^2$  mg. At the initial stage (Day 3) of experiment no ( $-1.322 \times 10^2$  mg,  $-0.799 \times 10^2$  mg, and  $-1.775 \times 10^2$  mg) CO<sub>2</sub> evolution was reported in natural aqueous condition with cellulose, bioplastics and synthetic nylon 6 respectively. Similarly, throughout the days in the first month up till the end of the month (Day 30), there were no amount of CO<sub>2</sub> captured. However, mean values of CO<sub>2</sub> recorded at the end of the month for natural aqueous condition containing the test materials were presented as followed; cellulose ( $-4.040 \times 10^2$  mg), bioplastic ( $-0.858 \times 10^2$  mg), synthetic nylon 6 ( $-8.751 \times 10^2$  mg) and control ( $-4.349 \times 10^2$  mg). This result was similar to the report of the study carried out by [17]. The lack of CO<sub>2</sub> evolved may be due to the fact that the microbial communities responsible for the degradation processes are in their lag phase.

According to [18] at this phase the microbes are acclimatizing to the environmental conditions of the medium as well as attempting to colonize the polymers respectively as potential carbon sources.



**Figure 1: Carbon (iv) Oxide (CO<sub>2</sub>) Evolution in Natural and Simulated Aqueous Conditions Across Thirty (30) Days**

**Key:** NAC-BL = Natural Aqueous Condition-Blank, NAC-CE = Natural Aqueous Condition-Cellulose, NAC-BP = Natural Aqueous Condition-Bioplastics, NAC-NY = Natural Aqueous Condition-Nylon 6.

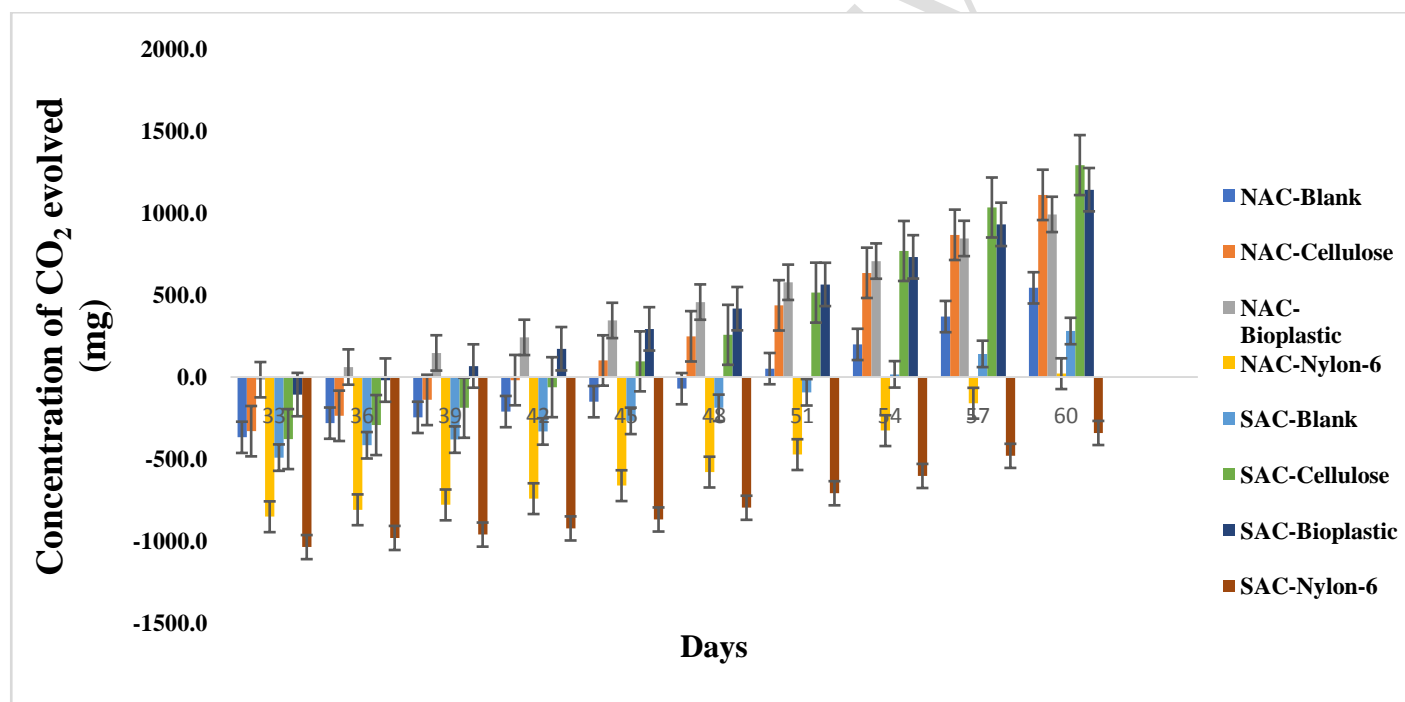
SAC-BL = Simulated Aqueous Condition-Blank, SAC-CE = Simulated Aqueous Condition-Cellulose, SAC-BP = Simulated Aqueous Condition-Bioplastics, SAC-NY = Simulated Aqueous Condition-Nylon 6.

Similarly, in simulated aqueous samples, CO<sub>2</sub> evolution was not captured across all samples at the beginning of the experiment (Day 3). The CO<sub>2</sub> concentration were in this trend,  $-1.447 \times 10^2$  mg (control),  $-1.344 \times 10^2$  mg (water with cellulose),  $-0.917 \times 10^2$  mg (simulated aqueous samples with bioplastic), and  $-2.241 \times 10^2$  mg (simulated aqueous condition with synthetic nylon 6). All through the month up to the end of the month (Day 30), CO<sub>2</sub> evolution was not reported in all simulated aqueous condition inserted with cellulose, bioplastic, synthetic nylon 6

and control in this trend respectively;  $-4.447 \times 10^2$  mg,  $-1.796 \times 10^2$  mg,  $-10.715 \times 10^2$  mg, and  $-5.473 \times 10^2$  mg. This may be due to the fact that the microbes responsible for the biodegradation processes are yet at the lag phase of their growth attempting to acclimatize to the conditions as well as trying to form biofilms around the polymeric samples [19].

### Carbon (iv) Oxide (CO<sub>2</sub>) Evolution of Bioplastic Packaging Nylon, Cellulose and Synthetic Nylonin Natural and Simulated Aqueous Conditions Across Sixty (60) Days

The concentrations of carbon (iv) oxide (CO<sub>2</sub>) evolved in both freshwater and marine water with test materials were revealed in Figure 2. As shown, CO<sub>2</sub> evolved was not captured in the fresh water sample at the beginning of the month (Day 33) across all samples. However, there was a



**Figure 2: Carbon (iv) Oxide (CO<sub>2</sub>) Evolution in Natural and Simulated Aqueous Conditions Across Sixty (60) Days**

**Key:** NAC-BL = Natural Aqueous Condition-Blank, NAC -CE = Natural Aqueous Condition-Cellulose, NAC -BP = Natural Aqueous Condition-Bioplastics, NAC -NY = Natural Aqueous Condition-Nylon 6.

SAC-BL = Simulated Aqueous Condition-Blank, SAC-CE = Simulated Aqueous Condition-Cellulose, SAC-BP = Simulated Aqueous Condition-Bioplastics, SAC -NY = Simulated Aqueous Condition-Nylon 6.

gradual improvement in the amount of CO<sub>2</sub> evolved from control with mean value of  $0.504 \times 10^2$  mg at Day 51. Similarly, for the other samples, the amount of CO<sub>2</sub> evolved were  $1.002 \times 10^2$  mg for water embedded with cellulose at Day 45,  $0.602 \times 10^2$  mg for freshwater embedded with bioplastic at Day 36, and  $0.197 \times 10^2$  mg for water embedded with synthetic nylon 6 at Day 60, which was the last day of the second month. Moreover, at the last day of the month, the final amount of CO<sub>2</sub> evolved from the freshwater embedded with the test materials improved in this trend:  $11.098 \times 10^2$  mg (freshwater with cellulose),  $9.907 \times 10^2$  mg (freshwater with bioplastic) and  $5.429 \times 10^2$  mg (freshwater with synthetic nylon 6) respectively. At this stage, the polymers contain significant biofilms on it which increase the rates of biodegradation and the amount of CO<sub>2</sub> evolved [20].

Conversely to the evolution of CO<sub>2</sub> recorded at the second month in surface water, there was no CO<sub>2</sub> captured at the beginning of the second month (Day 33) in marine water samples; across all the samples with values ranged from  $-1.077 \times 10^2$  mg (marine water embedded with bioplastics) to  $-10.377 \times 10^2$  mg (marine water embedded with synthetic nylon 6). According to [21] this may be attributed to the fact that fewer microbes that are referred to as stenohalides are usually found as normal flora of marine environment. These stenohalides are the ones responsible for the biodegradation of the polymers. Although, the CO<sub>2</sub> evolution had an increase on Day 54 in the control sample (blank) ( $0.156 \times 10^2$  mg), also in water with cellulose ( $0.947 \times 10^2$  mg) at Day 45 and in water with bioplastic ( $0.664 \times 10^2$  mg) at Day 39. However, in water embedded with a nylon-6 sample, there was no CO<sub>2</sub> evolution captured throughout the month of March. This may be due to the recalcitrant nature of the synthetic nylon 6 polymer [22]. Moreover, at Day 60, water sample as control, water sample embedded with cellulose and bioplastic were  $2.797 \times 10^2$  mg,  $12.914 \times 10^2$  mg,  $11.415 \times 10^2$  mg, respectively.

In comparing the amount of CO<sub>2</sub> evolved in freshwater and marine water, the highest ( $12.914 \times 10^2$  mg) CO<sub>2</sub> evolved was in marine water samples with cellulose and  $11.415 \times 10^2$  mg for marine water with bioplastic sample; followed by that of freshwater samples, which were  $11.098 \times 10^2$  mg for water with cellulose and  $9.907 \times 10^2$  mg for water embedded in bioplastic sample in the second month. According to [23] naturally occurring mineral components in marine water are higher than that of surface water and these available mineral wealth may increase the

biodegradation process of the polymers by microbial communities. Thereby, increasing the amount of CO<sub>2</sub> evolved.

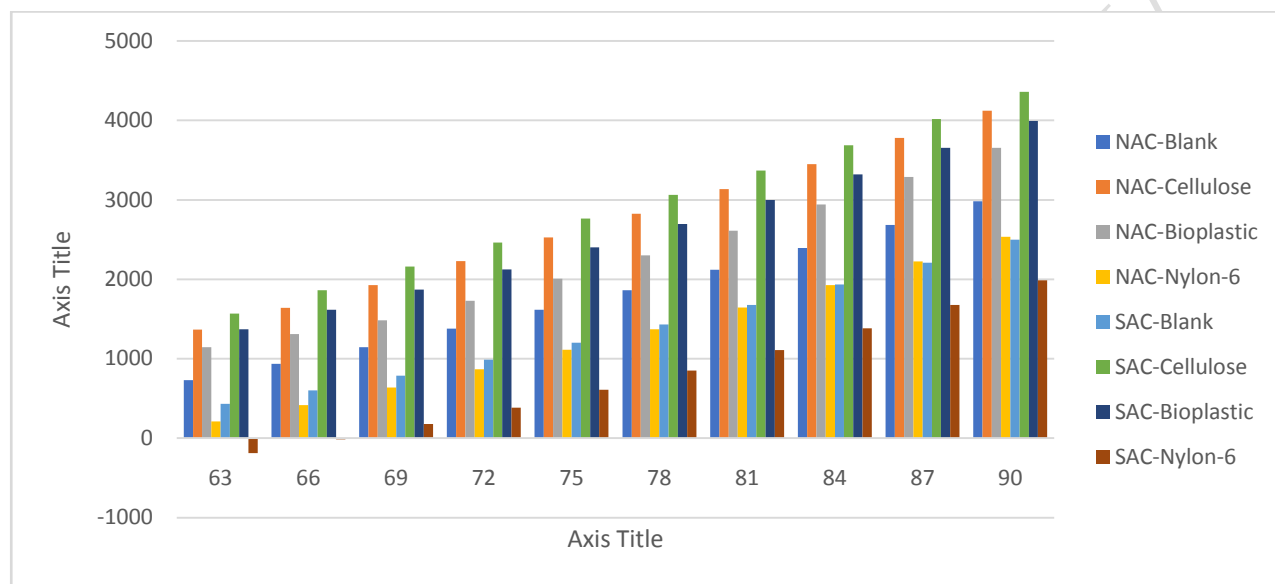
### **Carbon (iv) Oxide (CO<sub>2</sub>) Evolution of Bioplastic Packaging Nylon, Cellulose and Synthetic Nylonin Natural and Simulated Aqueous Conditions Across Ninety (90) Days**

The report of the concentration of carbon (iv) oxide (CO<sub>2</sub>) evolved in fresh and marine water samples with test materials in the third month were shown in Figure 3. The CO<sub>2</sub> evolved in fresh water at the beginning of the month (Day 63) was  $7.321 \times 10^2$  mg for the control water sample,  $13.670 \times 10^2$  mg for the water with cellulose sample  $11.460 \times 10^2$  mg for water embedded with bioplastic and  $2.128 \times 10^2$  mg for water with synthetic nylon 6. There was a constant increase in the CO<sub>2</sub> evolution across the whole month in all the samples up till the end of the month on Day 90 with mean values of  $29.840 \times 10^2$  mg,  $41.210 \times 10^2$  mg,  $36.553 \times 10^2$  mg, and  $25.360 \times 10^2$  mg for the control, water embedded with cellulose, bioplastic, and synthetic nylon 6 samples, respectively.

Moreover, in marine water samples, CO<sub>2</sub> evolution also maintained a constant increase, the same as that of fresh water samples from the beginning of the month at Day 63,  $4.343 \times 10^2$  mg for control,  $15.692 \times 10^2$  mg for water samples with cellulose,  $13.723 \times 10^2$  mg for water with bioplastic, which continuously increased in the CO<sub>2</sub> evolved throughout the month up to Day 90 (end of the month). In the control samples, water with cellulose, and bioplastic, the values were  $24.996 \times 10^2$  mg,  $43.601 \times 10^2$  mg, and  $39.937 \times 10^2$  mg, respectively. The amount of CO<sub>2</sub> released from the experiment throughout the months revealed that amount of CO<sub>2</sub> released from bioplastic in both surface and marine water were lower than amount of CO<sub>2</sub> released from the two aqueous solution

containing cellulose which is a natural bio-based plastics. This revealed that the biodegradation processes of bioplastic biodegradation in the water environment is safe [24] and the method is sustainable [25]. Physical examination of the water revealed that residues of cellulose and

bioplastics are not present in the aqueous solution. This showed that at the end of 120 days, there were no residues observed in the medium. This result corroborated with the report carried out by .....



**Figure 3: Carbon (iv) Oxide (CO<sub>2</sub>) Evolution in Natural and Simulated Aqueous Conditions Across Ninety (90) Days**

**Key:** NAC-BL = Natural Aqueous Condition-Blank, NAC -CE = Natural Aqueous Condition-Cellulose, NAC -BP = Natural Aqueous Condition-Bioplastics, NAC -NY = Natural Aqueous Condition-Nylon 6.

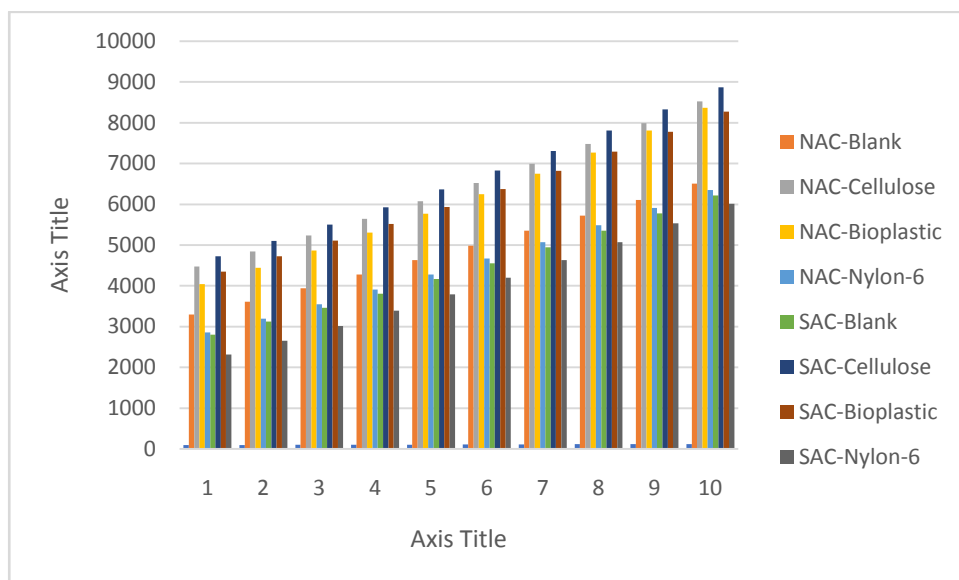
SAC-BL = Simulated Aqueous Condition-Blank, SAC-CE = Simulated Aqueous Condition-Cellulose, SAC-BP = Simulated Aqueous Condition-Bioplastics, SAC -NY = Simulated Aqueous Condition-Nylon 6.

nylon 6 sample had a value of  $-1.855 \times 10^2$  mg at Day 63, up until Day 69, there was an improvement ( $1.784 \times 10^2$  mg) of CO<sub>2</sub> evolved, similarly, up to the end of the month (Day 90) the amount of CO<sub>2</sub> evolved were  $19.871 \times 10^2$  mg.

Moreover, cellulose and bioplastic in marine water had the highest ( $43.601 \times 10^2$  mg and  $39.937 \times 10^2$  mg respectively) amount of  $\text{CO}_2$  evolution than that of fresh water, with the mean values of  $41.210 \times 10^2$  mg for water sunk with cellulose and  $36.553 \times 10^2$  mg for water sunk with bioplastic films. Similarly, this result corroborated with the result obtained from [26] Böer]in which the presence of minerals salts in marine environment. Although in fresh water, the control and water embedded with synthetic nylon 6 have the highest ( $29.84 \times 10^2$  mg and  $25.36 \times 10^2$  mg, respectively) concentrations of  $\text{CO}_2$  than that of marine water, which had  $\text{CO}_2$  concentration of  $24.996 \times 10^2$  mg for the control and  $19.871 \times 10^2$  mg for the water sunk with synthetic nylon 6 sample.

#### **Carbon (iv) Oxide ( $\text{CO}_2$ ) Evolution of Bioplastic Packaging Nylon, Cellulose and Synthetic Nylonin Natural and Simulated Aqueous Conditions Across One-hundred and Twenty (120) Days**

Figure 4 showed the carbon (iv) oxide evolution in the fresh and marine water samples with test materials in the fourth month of the experiment. In fresh water samples, the  $\text{CO}_2$  evolution was in this order,  $32.929 \times 10^2$  mg for control,  $44.725 \times 10^2$  mg for water sunk with cellulose,  $40.394 \times 10^2$  mg for water embedded with bioplastic and  $28.591 \times 10^2$  mg in water embedded with synthetic nylon 6 at the beginning of the month (Day 93). There was a constant increase in the concentration of  $\text{CO}_2$  evolved across all the samples up to Day 120. This results were similar to the reports presented by [27]on plastic biodegradation processes. The amount of  $\text{CO}_2$  evolved reported were in this trend;  $65.046 \times 10^2$  mg,  $85.215 \times 10^2$  mg,  $83.660 \times 10^2$  mg, and  $63.514 \times 10^2$  mg for control, water embedded with cellulose, bioplastic, and synthetic nylon 6, respectively.



**Figure 4: Carbon (iv) Oxide (CO<sub>2</sub>) Evolution in Natural and Simulated Aqueous Conditions Across One-hundred and Twenty (120) Days**

**Key:** NAC-BL = Natural Aqueous Condition-Blank, NAC -CE = Natural Aqueous Condition-Cellulose, NAC -BP = Natural Aqueous Condition-Bioplastics, NAC -NY = Natural Aqueous Condition-Nylon 6.

SAC-BL = Simulated Aqueous Condition-Blank, SAC-CE = Simulated Aqueous Condition-Cellulose, SAC-BP = Simulated Aqueous Condition-Bioplastics, SAC -NY = Simulated Aqueous Condition-Nylon 6.

Similarly, in marine water samples, the CO<sub>2</sub> evolved at the beginning of the fourth month (Day 93<sup>rd</sup>) was in this order; 28.040×10<sup>2</sup> mg for control, 47.214×10<sup>2</sup> mg for water sunk with cellulose, 43.50×10<sup>2</sup> mg for water with bioplastic, and 23.135×10<sup>2</sup> mg for water embedded with synthetic nylon 6. However, the amount of CO<sub>2</sub> evolved increased throughout the months across all samples in this trend; 62.185×10<sup>2</sup> mg for control > 82.725×10<sup>2</sup> mg for water embedded with cellulose > 82.758×10<sup>2</sup> mg for water with bioplastic > 60.152×10<sup>2</sup> mg for water embedded with synthetic nylon 6 by the end of the fourth month (Day 120).

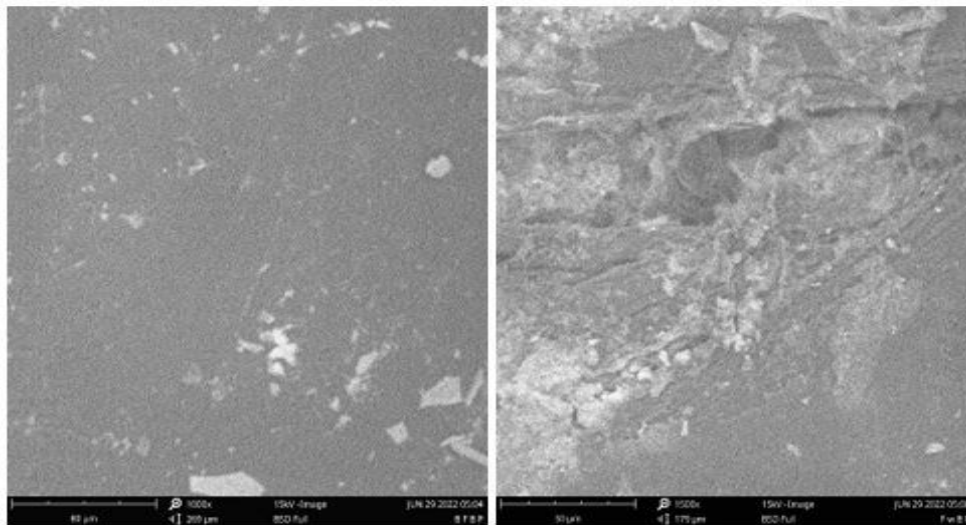
In equating the concentration of CO<sub>2</sub> evolved in fresh and marine water samples, cellulose samples had the highest (88.725×10<sup>2</sup> mg) concentration of CO<sub>2</sub> evolved, followed by bioplastic (82.758×10<sup>2</sup> mg) in marine water samples while that of fresh water samples, cellulose (85.215×10<sup>2</sup> mg) and bioplastic (83.660×10<sup>2</sup> mg) were reported [28]. In contrast, the concentration of CO<sub>2</sub> evolved in fresh water samples had the highest (63.514×10<sup>2</sup> mg for control

>  $65.046 \times 10^2$  for synthetic nylon 6) than that of marine water samples, the concentration of  $\text{CO}_2$  evolved were  $60.152 \times 10^2$  mg for synthetic nylon 6 and  $62.185 \times 10^2$  mg for control. The reduction in the amount of  $\text{CO}_2$  evolved could be attributed to the recalcitrant nature of the nylon 6. This is because polymer characteristics such as tacticity, crystallinity, molecular weight, type of functional groups and substituents such as plasticizers or additives presents in the chemical structure of the polymer germane factors influencing the biodegradation of polymers [29].

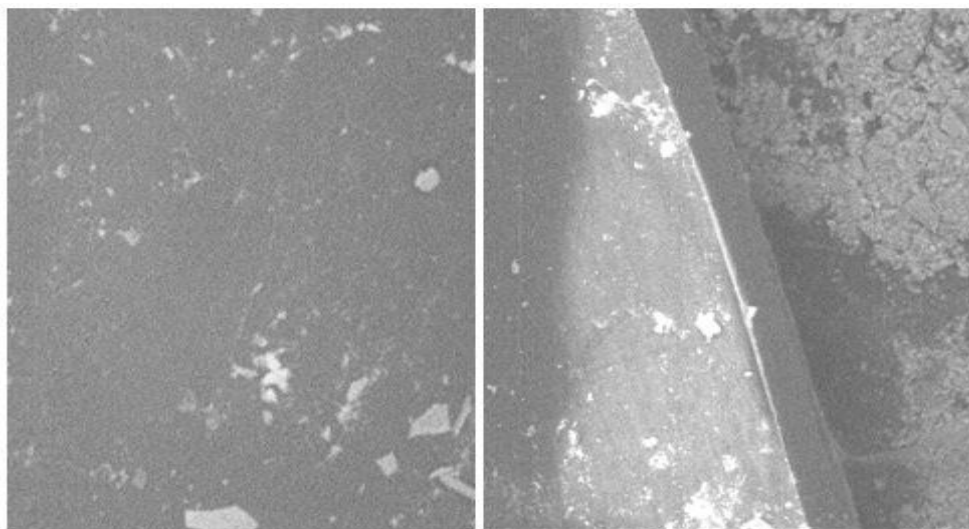
### Scanning Electron Microscope (SEM) of the Test Materials

SEM imaging showing the changes in surface morphology due to microbial activity on the plastic films (bioplastics and nylon 6) used in the degradation test is shown below. The SEM imaging was done on the samples before and after the degradation test (four months).

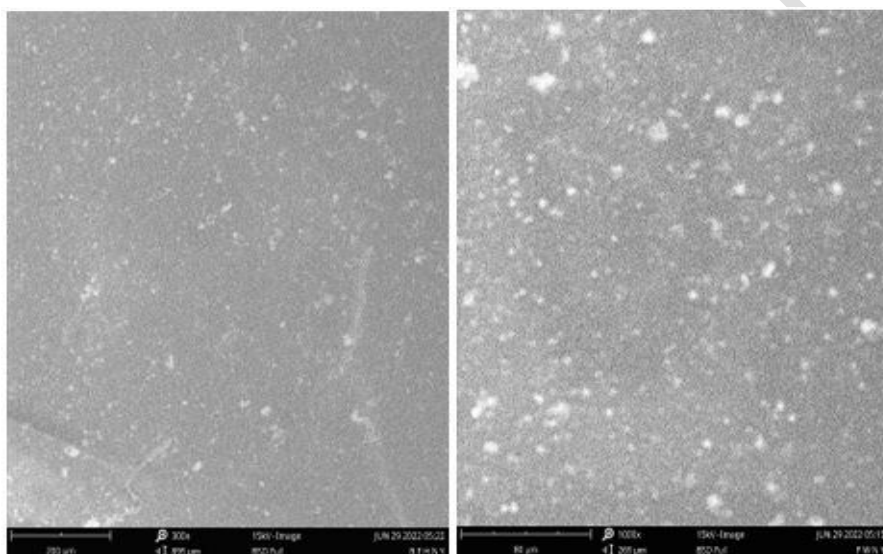
The following plates (Plates 1 and 2) showed the difference in the morphologies of bioplastic material (PBS 1020) samples before microbial attack and after microbial activity after soaking into natural and simulated marine aqueous solutions while Plates 3 and 4 showed that of nylon 6 in the different sample environments [30] Muniyasamy and dada]. The plates showed the level of degradation of bioplastics with the production of biofilms after the degradation test in the different sampled environments [31]. Nylon 6 shows little or no biofilms before or after the degradation test due to its slow rate of degradation across the four months.



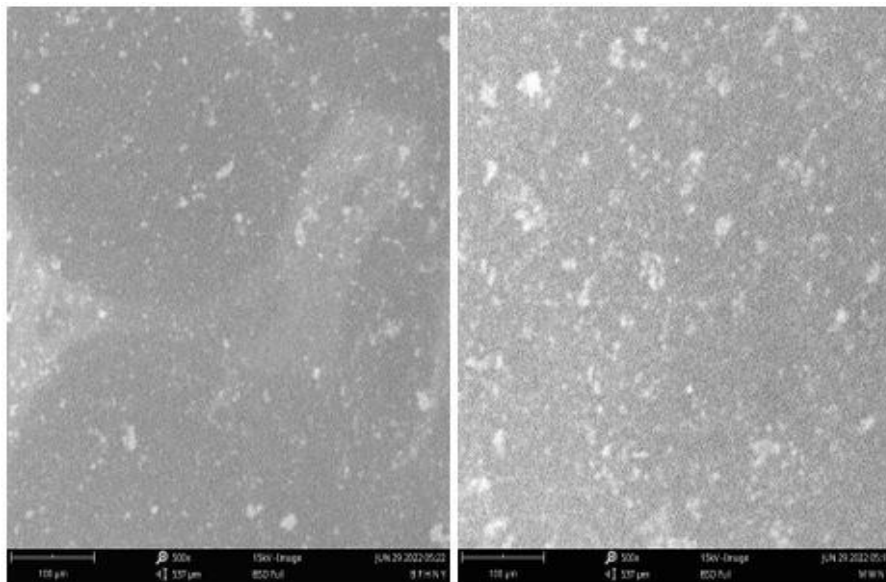
**Plate 1: SEM Image of Bioplastic (PBS 1020) Before Test and 4 Months After in Fresh Water**



**Plate 2: SEM Image of Bioplastic (PBS 1020) Before Test and 4 Months After in Marine Water**



**Plate 3: SEM Image of Synthetic Nylon 6 Before Test and 4 Months After in Fresh Water**



**Plate 4:SEM Image of Synthetic Nylon 6 Before Test and 4 Months After in Marine Water**

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