Natural Biodegradation Rates of Single-Use Blended Bioplastic Packaging Nylon Entrenched In Freshwater and Marine Water Environments of the Tropics

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Article Info	ABSTRACT
Article type: Research Article **Article history:** Received: 09 Mar 2023 Revised: 29 May 2023 Accepted: 07 Jun 2023 **Keywords:** *Biodegradation* *Bioplastic* *Nylon* *Freshwater* *Marine water*	The biodegradation rates of single-use blended bioplastic packaging nylon, nylon 6, and cellulose polymer were assessed in aquatic environments in an attempt to identify real biodegradable bioplastics (RBB). The natural biodegradation rates of the test samples in freshwater and marine water were assessed by respirometric method following the procedure of the American Standard Testing and Materials. The experimental design was arranged thrice in a completely randomized design of 2x4x3. The physicochemical parameters were obtained using the standard methods while the rates of biodegradation were obtained by titration method. Data obtained were analyzed using descriptive statistical method. At the end of 120 days, there were steady increase in the rates of biodegradation of cellulose and bioplastic samples across the fourth month in both freshwater and marine water. However, the rate of biodegradation in marine water were higher than in freshwater following the trend cellulose in marine (342 %) > cellulose in freshwater (259%) > bioplastics packaging nylon in marine (193%) > bioplastics packaging nylon in freshwater (175%). For nylon 6, the rate (-14) of retardation in the biodegradation process in Nylon 6 soaked in marine water is greater than that of Nylon 6 soaked in freshwater (-13). Consequently, nylon 6 was recalcitrant to biodegradation both in freshwater and marine water. The study concluded that the blended bioplastic packaging nylon is a real biodegradable bioplastic and could be suggested as a feasible and environmentally-friendly option to replace traditional plastics in the society.

INTRODUCTION

Pollution of the aquatic ecosystem by single-use plastic waste has been noted as one of the most significant challenges of the 21st century. Across the globe, five hundred marine locations have been identified as dead zones with a size of approximately 245,000 km² each due to polymer pollution effects (European-Bioplastics, 2019). To solve the problem of single-use plastic waste in the aquatic ecosystem, scientists have suggested a nature-based solution that involves the replacement of synthetic plastics with biodegradable plastics termed ''bioplastics''. Apart from the bioplastic; other treatment options pollute the ecosystems as well as leave millions of tonnes of plastic waste to accumulate in the ecosystems (Dada, 2019; Abdelmoez et al., 2021; Nomadolo et al., 2022). Thus,

the need for a sustainable environmentally-friendly option to replace synthetic non-degradable plastics such as bioplastics.

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However, the major concern with bioplastic usage is the false claim made by some manufacturers who do not meet the required criteria and standards of biodegradability. (Ariole and George-West, 2020). Such fake bioplastics have been confirmed to release microplastics and thereafter end up polluting the environment with effects similar to or worse than those of conventional plastics (Saalah et al., 2020; Mroczkowska et al., 2021).

This clarification of the real biodegradable bioplastics (RBB) is germane in order to avoid being greenwashed by the false claims of some manufacturers.

Globally, the American Society for Testing and Materials (ASTM) method is useful to confirm the claims made by most bioplastic industries about the biodegradability potentials of their products. This method is based on certain criteria and requirements to assess the validity of any biodegradability test. Hence, non-conformity to these criteria shall be considered an invalid test. The criteria revealed that the reference material may be at least 90% biodegradable within six months with evidence of carbon dioxide evolution. This is necessary as carbon dioxide is an end product of biodegradation (Tourova et al., 2020; Atanasova et al., 2021). In addition, to assess the rate of bioplastic biodegradation, changes in the physical and chemical properties of the bioplastic materials can be monitored using analytical and microscopy methods (Kjeldsen, et al., 2019; Oliveira et al., 2021). In this way, they contribute to environmental enrichment, remove plastic accumulations in the environment, and reduction in the cost of waste management (Haider et. al., 2019; Abed et al., 2020; Abe et al., 2021).

The idea of replacing synthetic plastic with bioplastic is widely acceptable and had been confirmed to degrade in temperate and sub-tropical region. Conversely, there is need to study the biodegradability potentials of bioplastics in tropical environment. Presently, the degradability testing of bioplastics has been successfully carried out in most developed countries with temperate and sub-tropical environment using American Standard for Testing and Materials (ASTM) methods (Muniyasamy and Dada, 2021; Eronen-Rasimus et. al. 2022). Here, in tropical environment, there is a dearth of knowledge on researches involving biodegradation of bioplastics in the tropical aquatic environment using the ASTM methods as a certified testing method for materials such as bioplastics. In this study, biodegradable packaging nylon bag is subjected to the natural biodegradable activities

of Indigenous microflora of the freshwater and marine water environment using the methods of the American Standards Testing and Materials (ASTM, 2017). This is with a view to assessing the rate of biodegradation of the bioplastic packaging film and its suitability in tropical aquatic environment.

MATERIALS AND METHODS

The freshwater samples were obtained from Isokun River, Ogo-Oluwa Street, Ilara-Mokin, Ondo State-Nigeria at latitude 5.1023° E and longitude 7.3491° N (Figure 1) where anthropogenic activities were minimal. The wet season covers March–October with an average rainfall of 1900 mm, while the dry season covers November–February. The mean monthly temperature ranges between 25°C and 30°C while the mean monthly relative humidity is below 65. The freshwater was filtered through a 200 μm mesh, in order to remove the zooplanktonic organisms and stored at 4°C in a refrigerator. To prepare the simulated marine water; one litre of fresh water samples were obtained and the pH adjusted to 8.2 using 0.1 N solution of hydrochloric acid.

The appropriate quantity of each component of salts in g/l as indicated in Table-1 were dissolved in one litre of distilled water to mimic marine water condition following standard methods as described by Lake Products Company (2021). Three sterile test materials used for the study are nylon 6 packaging bag, Bioplastic packaging bag and cellulose. The bioplastic was PBAT–PBS (30/70) (commercially named Bionolle 1020) obtained from Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa. The Nylon 6 were sourced from the local stores on campus
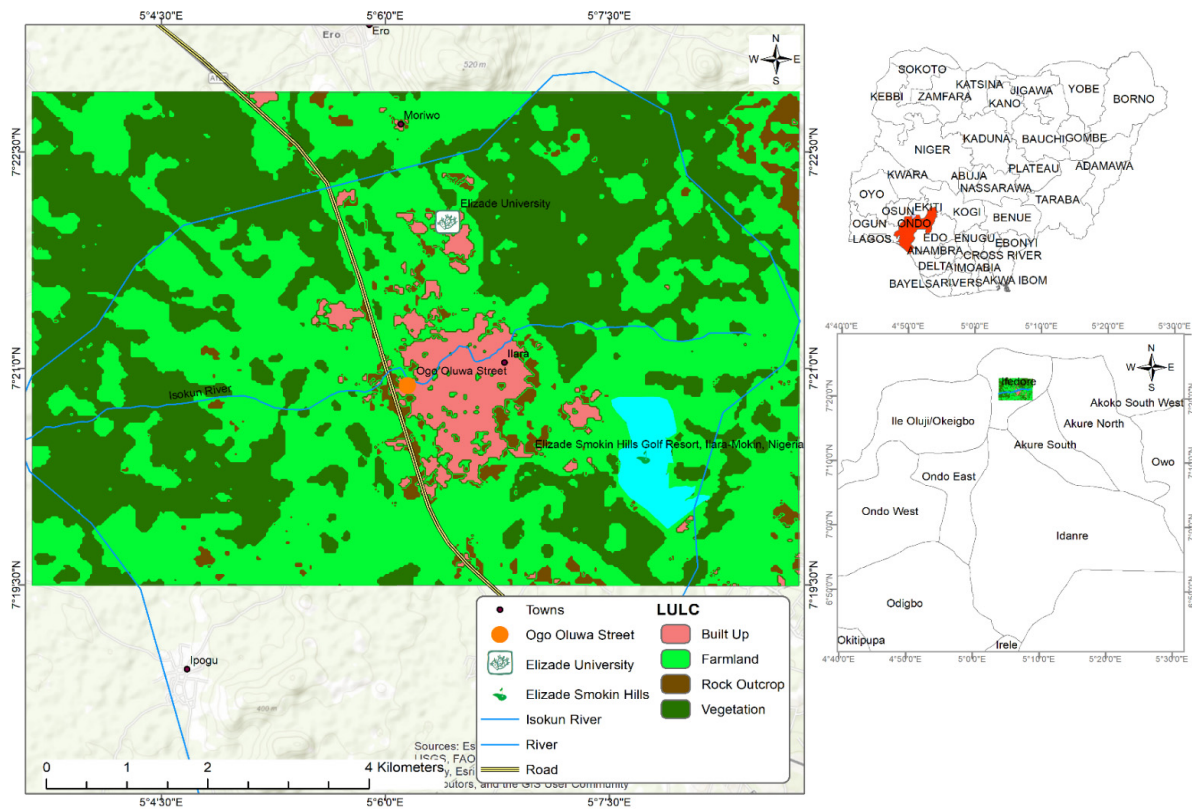
Fig. 1. Map showing Sources of The Freshwater and Marine water (Isokun River)

Table 1. Composition of Simulated Marine Water

Percentage of Salts in Simulated Marine water		Conc. of Salts Used for Simulated Marine Water
NaCl	58.490%	24.53 g/l
$MgCl_2 \cdot 6H_2O$	26.460%	25.20 g/l
Na_2SO4	9.750%	4.09 g/l
$CaCl_2$	2.765%	1.16 g/l
KCl	1.645%	0.695 g/l
$NaHCO_3$	0.477%	0.201 g/l
KBr	0.238%	0.101 g/l
H_3BO_3	0.071%	0.027 g/l
$SrCl_2 \cdot 6H_2O$	0.095%	0.025 g/l
NaF	0.007%	0.003 g/l
Density of seawater equals	1.025 at 15°C	988.968 g/l

The physicochemical parameters of the freshwater and marine water samples were determined using standard methods. The Experimental Design of the biodegradation of the test samples was conducted under controlled conditions in accordance with the International Standard of the American Society for Testing and Materials (ASTM). For the fresh water, the procedure highlighted in ASTM D 1141-98 (ASTM, 2017) for surface water were followed; while conditions and criteria indicated in ASTM D6691 were followed to set up biodegradation test of plastics entrenched in simulated marine water. Twenty-four of 1 litre respirometric glass jars of height 15 cm and width 7 cm were washed and sterilized by drying at 170 Celsius for 15 minutes. Twelve respirometric

glass jars were prepared for the fresh water and marine water respectively.

Five hundred mls of water samples of fresh water and marine water were added respectively to each of the 1000 mls of respirometric glass jar. Five hundred miligrammes each of test film materials (bioplastic and nylon-6 films) were cut into sizes of 3 cm by 4 cm and immersed in fresh and marine water respectively. Besides, 0.5 g of cellulose powder were poured in fresh and marine water respectively as positive control. Blank was set as control and prepared both for freshwater and marine water respectively. Thereafter, for trapping the CO_2 evolved from the three test samples and the blank as biodegradation sets in, 40 ml of 1 N KOH (Potassium hydroxide) were poured into a sterilized 50 ml glass beaker and were positioned in the respirometric glass jars to capture the evolved CO_2. The experimental design was in replicate and arranged in a randomized design of 2x3x4. Each test flask was tightly closed and incubated at 35ºC for four months. Readings on evolved CO2 were taken at every other days by titration method (Demirkan et al. 2020). To determine the percentage (%) or rate of degree of degradation and the concentration of CO_2 evolved, the method described below was used following the procedures highlighted by Muniyasamy and John (2017).

Degree of degradation (D_t) = ($CO_{2(t)}$) - ($CO_{2(b)}$) / $ThCO_2$ x 100

From the accumulated amounts of biologically produced carbon dioxide, measured in the test vessels (CO_2) (t) and in the blank control (CO_2) (b), the % degree of biodegradation (Dt)

$$\textbf{Moles } CO_2 = \frac{\textbf{HCl (N) x ml HCl}}{\textbf{2}}$$

(CO_2) (t) or (CO_2) (b) can be determined using the formular below:

$$\textbf{CO}_2 \textbf{ (g) = (N KOH x mls KOH- ml HCl x 0.5 NHCl) x 44}$$
$$\textbf{2}$$

Normality (N) of KOH: Volume of HCL used at titration / 10 x NKOH
mls KOH: 40 mls of KOH placed in the inset jar
mls HCl: Volume of HCl used for titration
NHCl: The normality of HCL used at titration

The theoretical amount of carbon dioxide ($ThCO_2$ in g per vessel) which can be produced by a total oxidation of the added test or reference material was calculated by using the formula below:
**$ThCO_2 = M_t$ x C_t x 44/12**
where M_t, is the total weight of the test samples which was 500 milligrams or 0.5 g (after conversion to S.I. unit), C_t is the % carbon (58 %) of the test samples which is a constant , 44 is the molar mass of carbon dioxide and 12 is the atomic mass of carbon.

RESULTS AND DISCUSSION

The physicochemical parameters for the aquatic environments were presented in Table 2. The pH of the freshwater and simulated marine water were 7.70 and 8.20 respectively. The value of the dissolved oxygen reported in the freshwater samples was above 2.60 while that of the marine water was around 2.31. In addition, a 306.00 µS/cm value was estimated as the

Table 2. Physicochemical Properties of Fresh Water and Marine Water

Analysis	Fresh water	Marine water
Electrical conductivity (µS/cm)	306.00	385.00
Temperature (°C)	20.30	26.20
pH	7.70	8.20
Dissolved Oxygen (DO)	2.67	2.31
Turbidity (m)	7.80	6.30
Total Dissolved Solid (mg/L)	153.00	155.00
Total Suspended Solid (mg/L)	1.00	1.10
Cadmium (Cd) (ppm)	0.008	0.023
Lead (Pb) (ppm)	0.032	0.096
Zinc (Zn) (ppm)	0.272	0.780
Iron (Fe) (ppm)	0.219	0.559
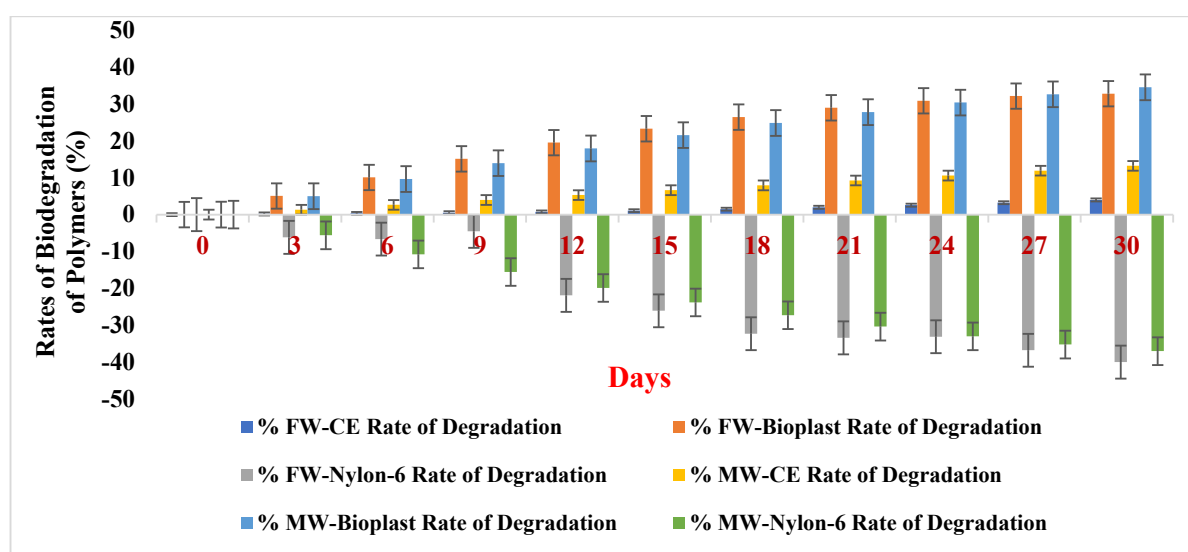
Fig. 2. Rates of Biodegradation of Bioplastics, Cellulose and Nylon 6 in Freshwater and Marine Water across Thirty (30) Days

Key: FW-CE = Fresh Water Cellulose, FW-BP = Fresh Water Bioplastics, FW-NY = Fresh Water Nylon 6. MW-CE = Marine Water Cellulose, MW-BP = Marine Water Bioplastics, MW-NY = Marine Water Nylon 6.

electrical conductivity of the freshwater sample. However, mean value of up to 385.00 µS/cm was reported for the marine water. The average temperature for the freshwater was 20.30°C while that of marine water was 26.20°C. Moreover, the turbidity values for the freshwater and marine water were 7.80 m and 6.30 m respectively. For total dissolved solids, the highest (155.00 ml/L) value was reported in marine water while 153.00 ml/L was reported in the freshwater sampled. However, the mean values reported for total suspended solids were 1.00 ml/L and 1.10 ml/L for both freshwater and marine water respectively.

For the metal analyses conducted on the aquatic environments, the concentrations of cadmium (Cd), lead (Pb), zinc (Zn), and iron (Fe) for freshwater were 0.008 ppm, 0.032 ppm, 0.272 ppm, and 0.219 ppm, respectively while in marine water, the concentrations were in this order: zinc (Zn) (0.780 ppm) > iron (Fe) (0.559 ppm) > lead (Pb) (0.096 ppm) > cadmium (Cd) (0.023 ppm). These values are within the acceptable ranges that could be used to assess the biodegradation rates of polymer samples in aquatic environments (Ahmed et al. 2018; Harrison et al., 2018; Guerrera et al. 2021).

As shown below, Figure 2 displayed the rates of biodegradation of the three test polymers

both in freshwater and marine water samples in the first month of the experiment. The rates of biodegradation of the test samples on the 3rd day in fresh water samples were about 0.2 % for cellulose, 5% for bioplastic and 1% for synthetic nylon -6. However, at the same period, in the marine environment, cellulose biodegraded by about 1% while bioplastic biodegraded by 5% with synthetic nylon 6 revealing evidence (-6%) of retardation in rate of biodegradation. Initially, the low rates of biodegradation may be due to the fact that the plastic-degraders were trying to form biofilms around the test materials as reported by Dada (2020). Besides, retardation in the rate of biodegradation of the nylon 6, may be due to the recalcitrant nature of nylon material (Ganesh et al., 2020). Therefore, for the nylon 6, retardation in the rates of biodegradation processes by -40% was reported in the first month. Correspondingly, cellulose and bioplastics maintained gradual biodegradation rates (4% and over 33%) till the end of the month in fresh waters respectively while in marine water, the rate of biodegradation of cellulose and bioplastic increased steadily throughout the first month in this order: Bioplastic (35%) > Cellulose (13%).

The rates of biodegradation of the test materials entrenched in both fresh and marine water samples respectively in the second month were shown in Figure 3. For the second month, in the fresh water, the rate of biodegradation of cellulose was 5% while 33% was reported for bioplastic. However, the synthetic nylon 6 had retarded (-40) rates of biodegradation. Similarly, at the end of the month, there was an increase in the rate of biodegradation of cellulose by over 73% while the rate of biodegradation of bioplastics increased by over 42% respectively. For nylon 6, retardation in biodegradation rates by -39 were reported till the end of the month (60th day). The rate of retardation reported on the 60th day may be due to the reduction in microbial loads of the plastic degrader as they were exposed to their wastes during biodegradation processes. These results corroborated with the outcome of Nomadolo et al. (2022) which revealed that blended biopolymer biodegraded in this same trend under controlled aerobic laboratory conditions. Similarly, on the 60th day (Figure 2), cellulose degraded at a 70% rate while bioplastic degraded at over 42%. This is because cellulose is a purely natural biopolymer while bioplastic packaging nylon is made up of blended polymeric substances (Nwinyi and
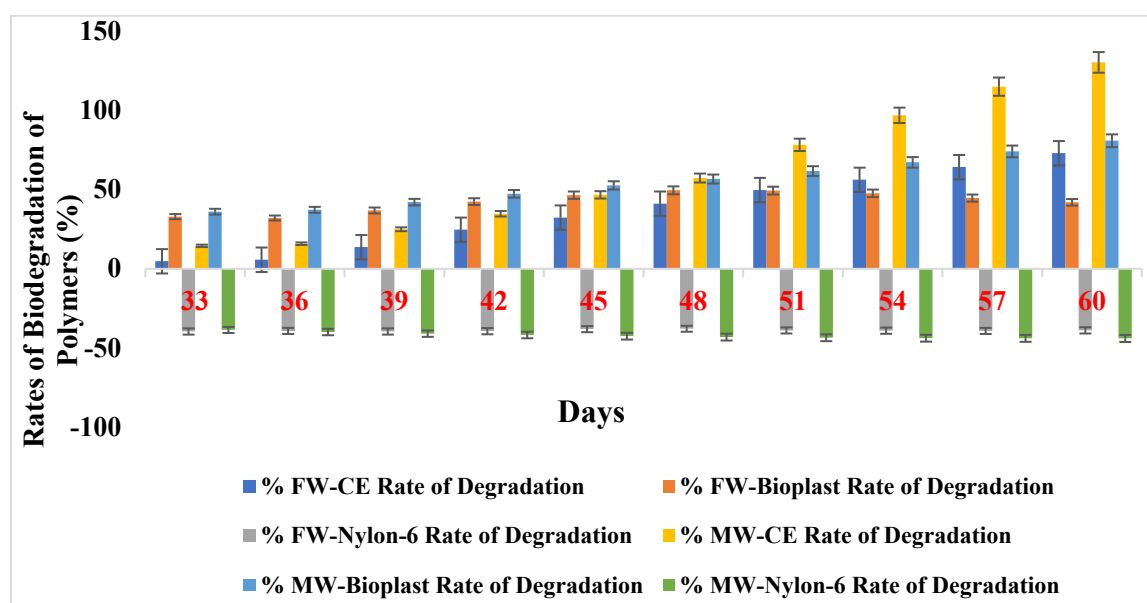

Fig. 3. Rates of Biodegradation of Bioplastics, Cellulose and Nylon 6 in Freshwater and Marine Water across Sixty (60) Days

Key: FW-CE = Fresh Water Cellulose, FW-BP = Fresh Water Bioplastics, FW-NY = Fresh Water Nylon 6. MW-CE = Marine Water Cellulose, MW-BP = Marine Water Bioplastics, MW-NY = Marine Water Nylon 6.
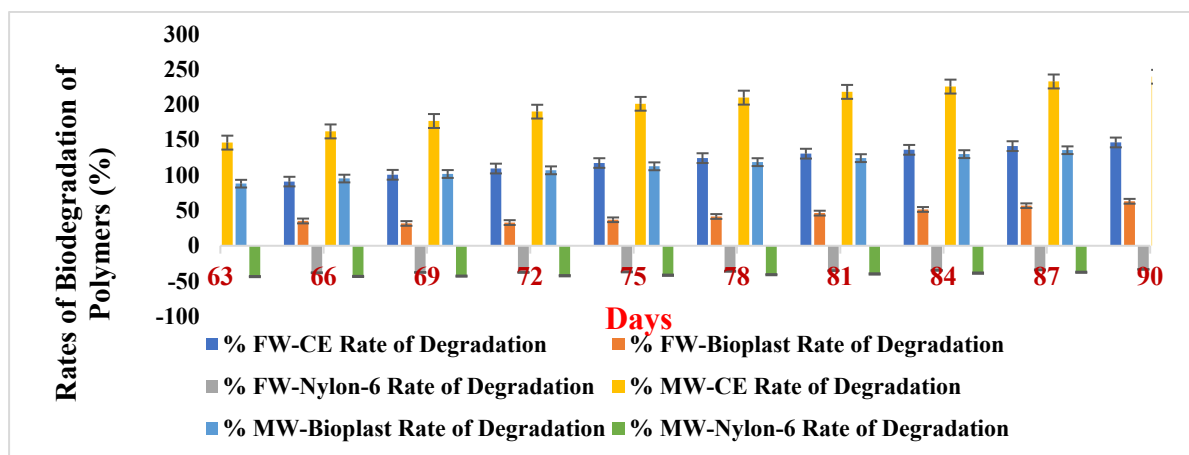
Fig. 4. Rates of Biodegradation of Bioplastics, Cellulose and Nylon 6 in Freshwater and Marine Water across Ninety (90) Days
Key: FW-CE = Fresh Water Cellulose, FW-BP = Fresh Water Bioplastics, FW-NY = Fresh Water Nylon 6. MW-CE = Marine Water Cellulose, MW-BP = Marine Water Bioplastics, MW-NY = Marine Water Nylon 6.

Owolabi, 2019; Zhong et al., 2020; Abe et al. 2021). In marine water conditions, cellulose was biodegraded by over 15% rate.

However, the rate of bioplastic biodegradation improved by up to 3-folds than the value reported for cellulose with synthetic nylon 6 retarded in biodegradation process at the beginning of the second month (33rd day) in fresh water. Moreover, cellulose had over 130% level of biodegradation and over 81% rates of biodegradation reported in bioplastic with synthetic nylon 6 been resistant and retarded in biodegradation process by - 44% retardation rates.

Thereafter, at 48th day of aerobic biodegradation, cellulose had 57% level of biodegradation. Moreover, at 51st, 54th, 57th and 60th days, the rates of biodegradation of cellulose were in this order: 78%, 96%, 115% and 130%. At the same period, the rates of biodegradation of bioplastics also increased across the days in this order 56%, 61%, 67%, 74% and 80%; although with biodegradation rates lower than that of cellulose. At this stage, the gradual increase may be associated to the fact that the bioplastic is almost at the peak of its ultimate biodegradation and mineralization stage (Fesseha and Abebe, 2019; Dada, 2020; Ribba et al., 2022). Similarly, in marine water samples, cellulose biodegraded faster (over 130%) than bioplastics (80%) throughout the test periods. This may be as a result of the presence of some salts and nutrients in marine water that may accentuate the potentials of indigenous marine microbes to degrade the polymers as reported by Muniyasamy and Dada (2021).

In the third month, Figure 4 reported the rates of biodegradation of test materials in both fresh and marine water samples. In freshwater samples, at the beginning of the month (63rd day), cellulose and bioplastic had biodegradation rates of over 82% and 39% with synthetic nylon 6 having no sign of biodegradation all through the month with rates of retardation as -39% and -34% at 63rd and 90th day respectively. Both cellulose and bioplastic degradation rates (147% and 63%) increased constantly till the end of the third month (90th day). In marine water samples, there was a gradual increase in the rate of biodegradation starting from the 63rd day with over 146% degradation reported for cellulose and over 88% level of degradation reported for bioplastics. The rates of biodegradation increased continuously throughout the month, reaching over 240% degradation for cellulose and 140% degradation of bioplastic on the 90th day. Contrarily, the rate of retardation in the biodegradation process of synthetic nylon 6 samples was -44. Likewise, the rates at which cellulose entrenched in freshwater biodegraded
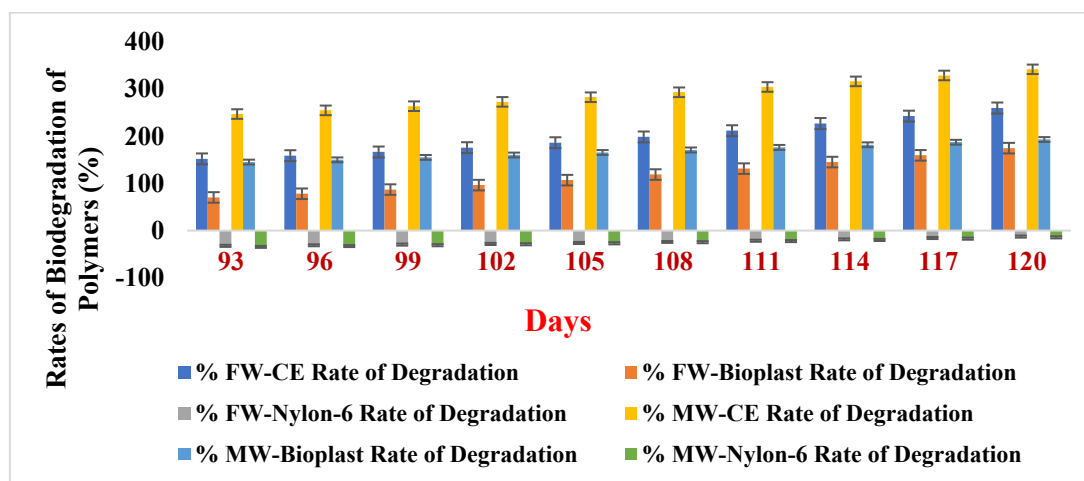
Fig. 5. Rates of Biodegradation of Bioplastics, Cellulose and Nylon 6 in Freshwater and Marine Water across One Hundred and Twenty (120) Days
Key: FW-CE = Fresh Water Cellulose, FW-BP = Fresh Water Bioplastics, FW-NY = Fresh Water Nylon 6. MW-CE = Marine Water Cellulose, MW-BP = Marine Water Bioplastics, MW-NY = Marine Water Nylon 6.

improved by 147% than that of bioplastic (63%) soaked in the same medium.

The rates of biodegradation of test materials in both fresh and marine water samples were shown in Figure 5 for the fourth month. At the early part of the month, cellulose and bioplastic had biodegradation rate of over 152% and over 70% rates with synthetic nylon 6 sample having no level of biodegradation reported in freshwater samples. There was a constant increase in the rates of biodegradation of cellulose and bioplastic across the fourth month at 120th day. Similarly, degradation of synthetic nylon 6 were reported not to occur with -13% sign of retardation. According to Ciriminna and Pagliaro (2020), synthetic nylon 6 are non-degradable in nature.

However, initially, in marine water for the fourth month, cellulose biodegraded by 247% while bioplastic degraded by 145%. By the end of the month, cellulose and bioplastic biodegraded at the rate of 342% and 193% respectively with synthetic nylon 6 being resistant (-14%) to biodegradation. This report is in line with the findings of Calabro et al (2020) when evaluating the anaerobic biodegradability of three biobased materials used for the production of disposable plastics. This is because at this level, the biodegradation of bioplastics is at the peak and a significant amount of bioplastics were biodegraded. Similarly, when comparing biodegradation rates, cellulose and bioplastics sunk in marine water biodegraded faster than that of freshwater.

CONCLUSION

The replacement of non-biodegradable synthetic plastics with Bioplastics has been regarded as a safe and acceptable nature-based solution to the environmental challenge of plastic pollution. Real biodegradable bioplastics (RBB) are known to biodegrade ultimately in the aquatic environment without the release of toxic moieties. Therefore, the environmental impacts of bioplastics is manageable compared to the damage caused by conventional plastics and can be suggested as feasible alternative to the petroplastics. This breakthrough in the RBB development will positively influence the abatement of plastic pollution in the environment.

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CONFLICT OF INTEREST

The authors declared that there is no any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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