

EARLY RELEASE OF METHANE AND QUICKER DEGRADATION OF BIOPLASTICS USING ACTIVATED SLUDGE

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ABSTRACT

Environmental hazards caused by fossil-based plastics are spread through every ecosystem of the biosphere. As an alternative, biodegradable plastics are environmentally friendly and sustainable. Composting biodegradable plastics is a big challenge. Microorganisms in anaerobic conditions convert biodegradable plastics into methane, carbon dioxide, water, hydrogen sulphide, ammonia, and hydrogen. In the present study, locally procured sugarcane bagasse plates, cornstarch-based shopping bags and bamboo starch-based spoons of size < 1 cm² (cut pieces) were anaerobically digested using activated sludge under mesophilic conditions to evaluate the production of biogas (liquid displacement) and methane (liquid replacement). The structural changes of biodegradable plastics were analysed using Fourier transform infrared spectroscopy - attenuated total reflectance (FTIR-ATR) and scanning electron microscopy (SEM). Peak shifts and increase in the intensities of several functional groups were observed in FTIR-ATR for all three biodegradable plastics. Changes in colour, texture and surface porosity were observed. The methane curve showed that the sugarcane bagasse plates were easily degraded. Biogas and methane were immediately produced. The cornstarch-based shopping bag is degraded after a “lag phase”, indicating hydrolysis as the rate-limiting step in anaerobic digestion. The bamboo starch-based spoon showed inhibition in the initial phase for up to 14 days which reduced the production of methane and then the methane release occurred.

Keywords: *biodegradable plastics, anaerobic biodegradation, methane, biogas, compost*

INTRODUCTION

Our ecosystem faces critical problems related to plastic disposal and in most developing countries plastic is burned in open spaces. It mainly includes plastic wastes generated from

household activities, agricultural work, electronics and open dumps [1, 2]. The burning of plastics seriously affects the environment and the health of living organisms. Harmful pollutants such as polychlorinated dibenzo-p-dioxins,

polychlorinated dibenzofurans, polycyclic aromatic hydrocarbons, etc. are released during burning [3, 4]. The impact of the use of plastic from cradle-to-grave life cycle assessments (LCAs) and by single-use consumer base, the rate at which plastic is produced and disposed of exceeds most other man-made materials, leading to significant environmental hazards [5]. In recent decades, biodegradable plastics have been introduced as an alternative to fossil-based plastics. These plastics are environmentally friendly and sustainable. The sources of bioplastic are starch, cellulose, chitosan, and proteins extracted from biomass [6]. Currently, the produced bioplastic accounts for approximately 10 - 15 % of plastics produced worldwide. Therefore, the huge turnover in the global bioplastics market is a welcome development in reducing environmental pollution. Renewable biomass obtained from food industry such as starch, collagen, cellulose and polylactic acid can become cheap sources for the production of food-based bioplastics [7, 8]. The growing use of bioplastics in applications such as food packaging, pharmaceuticals, and consumer goods requires further encouragement and acceptance by industry, governments and consumers [9]. Biodegradable plastics are those designed to degrade in the presence of microbial flora such as fungi, algae and bacteria under aerobic and anaerobic conditions [10]. They are usually produced from natural by-products and follow controlled conditions of temperature and humidity at the industrial level.

Different types of degradation of biopolymers depending on sunlight, temperature, oxygen, and microorganisms are well explained in the previous studies [11 - 13]. Biodegradable plastics are biopolymers of long-chain molecules that require abiotic chain cutting such as temperature, water, and sunlight to occur before biodegradation, which results in the formation of oligomers, dimers, or monomers. The resulting short chains enter the cell walls of various microorganisms [14]. Microorganisms use them as substrates for their metabolic pathways, resulting in degradation with the help of microbial

enzymes. The biodegradation of these biodegradable plastics occurs in two ways: aerobically and anaerobically. In an environment rich in oxygen (more than 6 %), aerobic biodegradation occurs. In aerobic biodegradation, microorganisms use the polymer as a carbon and energy source and release carbon dioxide and water as the main by-products, along with the remaining part, which is called compost. Anaerobic biodegradation occurs in an oxygen-free environment and under mesophilic or thermophilic conditions. In anaerobic biodegradation, microorganisms use the polymer as a carbon and energy source and convert it into methane, carbon dioxide, water, hydrogen sulphide, ammonia, and hydrogen, which is the result of different sequences of metabolic pathways of different microorganisms [9, 15].

Mesophilic anaerobic biodegradation takes place in a moderate temperature range of 35 - 37 °C. Thermophilic anaerobic biodegradation takes place in a temperature range of 52 - 55 °C. Therefore, most anaerobic biodegradation occurs under mesophilic conditions, as they are more stable and economical in terms of cost and energy [16 - 18]. The novelty of this study is the use the approach of anaerobic digestion of commercially available biodegradable plastics for efficient waste management and building the economy through energy production. Thus, the approach will help in reducing the unnecessary accumulation of biodegradable plastics as occurs with conventional plastic materials. Therefore, this study was undertaken to evaluate the methane produced during mesophilic anaerobic digestion of different types of biodegradable starch-based plastics available locally.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical grade. Biodegradable plastics such as cornstarch-based shopping bags, bamboo starch-based

spoons and sugarcane bagasse plates were purchased from the local market of Mysuru District, Karnataka. Sludge was collected from M/s. Bannari Amman Sugar Factory, Alaganchi, Nanjungud, Mysuru District, Karnataka. The composition of cornstarch-based shopping bags, bamboo starch-based spoons and sugarcane bagasse plates is as follows: (a) cornstarch-based plastic bags (corn starch - 55 %, PLA (polylactic acid) - 25 %, plasticizers (glycerol, sorbitol) - 10 %, polycaprolactone - 5 %, natural fibres, e.g. talc - 3 %, additives, e.g. antioxidants, UV stabilizers - 2 %), (b) sugarcane bagasse plates (sugarcane bagasse pulp - 75 %, binders (starch, cellulose) - 8 %, water - 12 %, additives (PLA, natural fibres) - 5 %) and (c) bamboo starch-based spoons (bamboo fibres - 65 %, binders (cornstarch, PLA) - 15 %, plasticizers (glycerol, sorbitol) - 10 %, additives (PLA, natural fibres) - 5 %).

Methodology

Sludge treatment

Sludge collected from the M/s. Bannari Amman Sugar Factory was kept overnight at 105 °C and then the next day at 300 °C, also overnight. Heating sludge to 300 °C involves a process similar to thermal hydrolysis or mild pyrolysis, which breaks down complex organic compounds into simpler forms, such as volatile fatty acids and other intermediates that are more easily biodegradable by anaerobic microbes and at 105 °C the process enhances sludge availability for microbial degradation during anaerobic digestion. Furthermore, the breakdown of resistant compounds improves the overall biodegradability of the sludge, facilitating a more efficient conversion to biogas. Heat treatment makes more organic material available to anaerobic microorganisms, thereby increasing substrate availability for methanogens, the microbes responsible for methane production, and with the sludge components that are more accessible and less complex, the rate of anaerobic digestion can be significantly accelerated, leading to faster methane production. The sludge was kept for 6 days in

the dark at room temperature to reduce the biogas production activity produced by the sludge and the remaining organic compounds in the sludge [19].

Pretreatment of biodegradable plastics

Biodegradable plastics were cut into small pieces with an area of < 1cm². Pretreatment was carried out by maintaining the pH at 12 using sodium hydroxide (1N) and the temperature was maintained at 55 °C for 24 hours. High pH helps the breakdown of polymers (depolymerisation), making the material more accessible to microbial activity and enhancing solubility, which helps the release of volatile fatty acids that serve as intermediate in anaerobic digestion. Furthermore, elevated temperature increases the reaction rate. It is very significant in removing resistant materials that contribute to slow biodegradation, an unfavourable event in the biodegradation process. After pretreatment, the temperature was maintained at 37 °C, which is optimal for microbial activity [20]. The inoculum (10 % treated sludge as mentioned in section “Sludge treatment”) for biodegradable plastic was maintained at the ratio of 3:2. The inoculum provides adequate nutrients and initiates the repopulation or re-colonisation of anaerobic microorganisms.

Determination of the biochemical methane potential (BMP)

The determination was carried out according to the method from [19] with minor modifications. The reaction was carried out in an airtight bottle with a volume of one liter containing 800 ml of nutrient media consisting of 20 ml macronutrients (potassium, ammonia, magnesium and calcium) and 2 ml micronutrients (Mn, Fe, Zn, Co, Mo, Al, Cu, B). Digestion was carried out for (i) control (sludge only) and (ii) test (sludge and biodegradable plastic). 1.33 g of each biodegradable plastic and 2 g of treated sludge (used as inoculum) were added to the test reactor bottle, and the pH was maintained at 7.1 using sodium hydroxide (1N). Nitrogen

gas was used for 5 min to remove oxygen from the reactor bottle to maintain anaerobic conditions and the bottles were immediately sealed.

Biogas and methane monitoring

Biogas monitoring was carried out using the liquid displacement method according to [21] with slight modifications. In this method, the external system is filled with liquid, which is called a barrier solution. It consists of a vessel and an inverted reservoir that are filled with a barrier solution. A highly acidic or saline barrier solution was used to avoid CO₂ diffusion. If the barrier solution used is an acid, the volume displaced gives the volume of biogas produced. If the barrier solution is basic, the volume displaced gives the volume of CH₄ produced. For the liquid displacement method, about 1000 ml of 0.5 M hydrochloric acid was used to fill a 50 ml measuring cylinder and added to a 1000 ml beaker. The reactor bottle was kept in the water bath at 50 °C, and the hose was opened from the reactor bottle to the barrier solution. Biogas moves through the liquid and replaces the barrier solution in the graduated measuring cylinder. The volume of gas obtained corresponds to the volume of biogas. After that, the pH was raised to 9 using KOH solution to absorb carbon dioxide and hydrogen sulphide. This absorption decreases the volume of gas in the measuring cylinder. The decreased volume corresponds to the volume of methane. The difference in the two volumes gives the volume of carbon dioxide released during the process. The concentration of hydrogen sulphide was taken as negligible [22].

Analytical techniques

Diamond FTIR-ATR and scanning electron microscopy norma system 7 are used for the evaluation of the anaerobic biodegradation of biodegradable plastics. The cut samples of biodegradable plastic with a surface area of 1 cm² were subjected to sequential washing with ethanol, immersing them in solutions of 30 %, 50 %, 70 %, and finally 90 % ethanol for 10

min. After thorough drying, a thin layer of gold particles was applied to the samples. The prepared samples were then mounted onto SEM stubs for analysis. Using Agilent FTIR-ATR (Agilent FT-IR ATR Cary 630) functional groups were identified between wave numbers ranging from 4000 - 400 cm⁻¹. For FTIR-ATR, treated samples were cut and immersed in ethanol and used for analysis.

RESULTS AND DISCUSSION

The changes in appearance of three different biodegradable plastics after 21 days of anaerobic biodegradation are shown in Figures 1(a) to 1(f). A change in colour was observed in a cornstarch-based shopping bag, a change in texture was observed in a sugarcane bagasse plate, and a change in surface porosity was observed in the bamboo starch-based spoon.

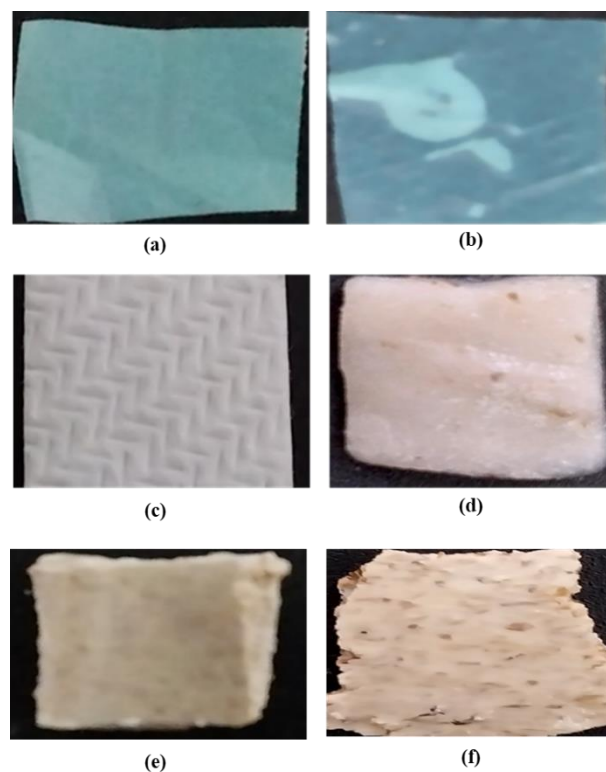


Figure 1. Visual comparison of cornstarch-based shopping bags (a, b), sugarcane bagasse plates (c, d) and bamboo starch-based spoons (e, f) before and after 21 days of anaerobic biodegradation

SEM analysis

The SEM analysis enables examining the morphological changes in the material on a micro-scale. The morphological structures

before and after anaerobic biodegradation of cornstarch-based shopping bags, sugarcane bagasse plates and bamboo starch-based spoons are shown in Figures 2 - 4.

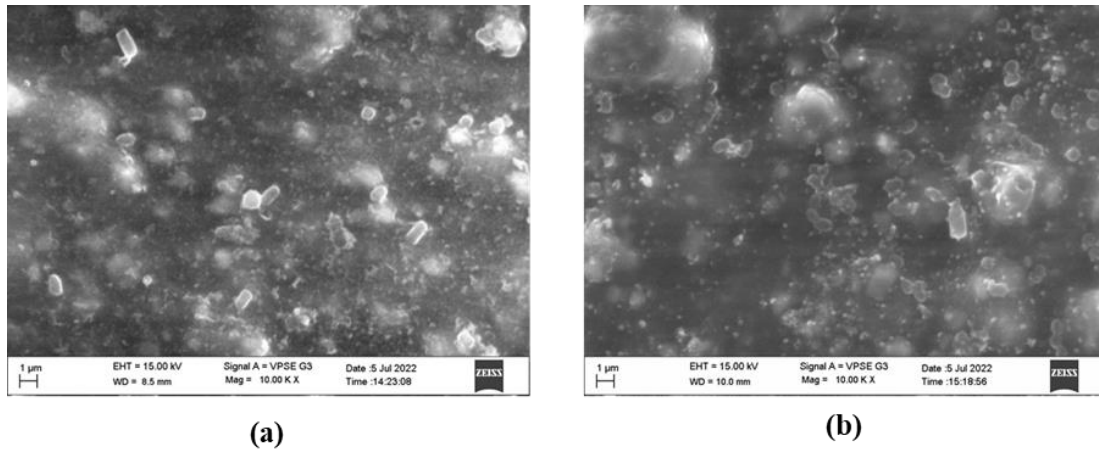


Figure 2. Cornstarch-based shopping bags before (a) and after 21 days (b) of anaerobic biodegradation

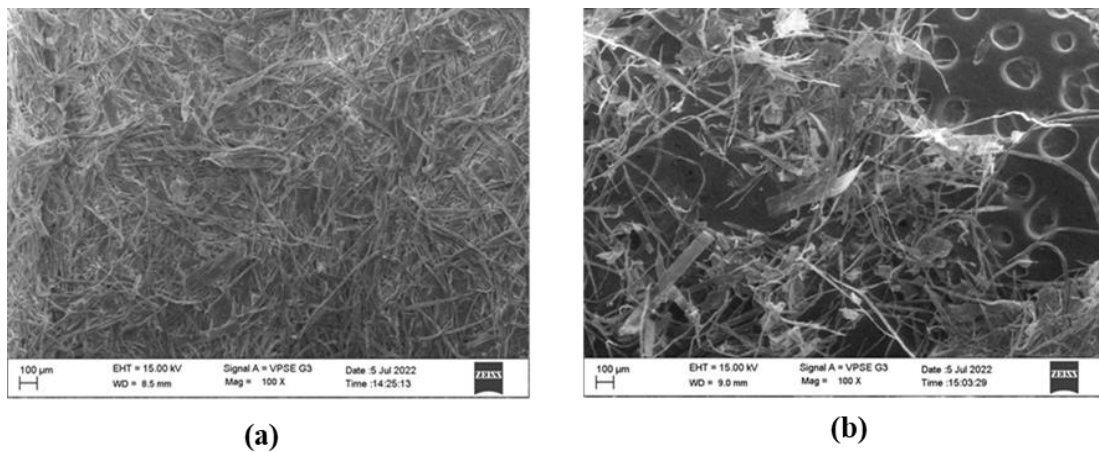


Figure 3. Sugarcane bagasse plates before (a) and after 21 days (b) of anaerobic biodegradation

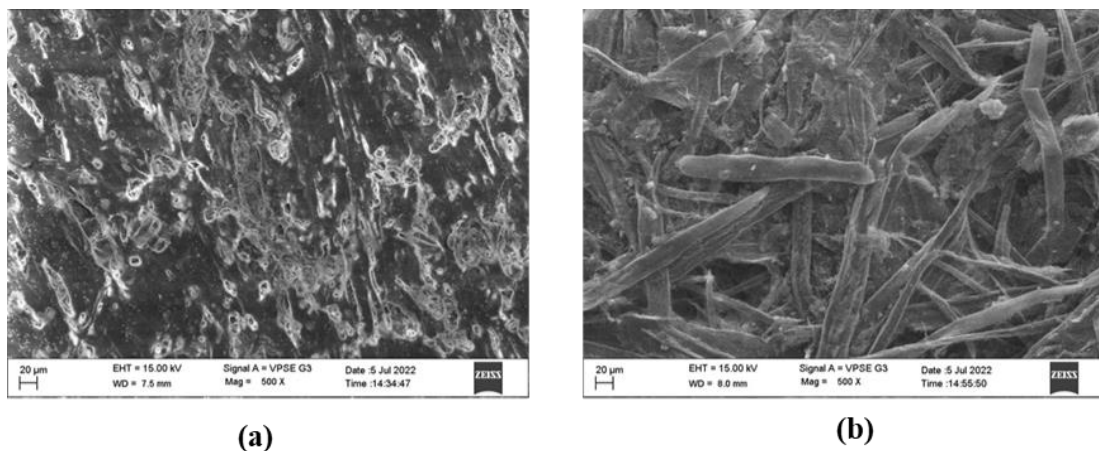


Figure 4. Bamboo starch-based spoons before (a) and after 21 days (b) of anaerobic biodegradation

The increased area of pores in Figure 2(b) indicates that the cornstarch-based shopping bag has been degraded. Figure 3(b) shows a decrease in the amount of fibre-like structure and appearance of pores, indicating that the sugarcane bagasse plate has been degraded. Figure 4(b) shows that surface structure changes and fibre-like structures appear, indicating that the bamboo starch-based spoon has been degraded. In previous studies [23 - 27], the reduction of mechanical properties and changes in the morphological surface confirm the occurrence of degradation. Similar surface changes were observed in the cornstarch-based bags, sugarcane bagasse plates, and bamboo starch-based spoons and showed a decrease in the mechanical properties of bioplastics, which probably confirms that biodegradation has occurred.

FTIR-ATR analysis

FTIR-ATR spectra of the cornstarch-based shopping bags before and after anaerobic biodegradation were recorded at room temperature in the range of 4000 - 400 cm^{-1} . In previous studies, the dissociation of the peak at 1450 cm^{-1} into two peaks at 1454 cm^{-1} and 1447 cm^{-1} corresponds to the CH_3 functional group, and the increase in wave number 1745 cm^{-1} corresponds to the carbonyl group [28] rising peaks for hydroxyl groups [29], changes in CO stretching vibrations for polyhydroxybutyrate and CH_4 deformation vibrations [30, 31]. In this study, after 21 days of biodegradation, a new peak at 3350 cm^{-1} , indicating the OH stretch, was observed. Furthermore, the peak shifts of methyl C-H symmetric stretch from 2955 cm^{-1} to 2892 cm^{-1} and 2926 cm^{-1} , carboxylate from 1321 cm^{-1} to 1319 cm^{-1} , aromatic ether, aryl-O stretch from 1267 cm^{-1} to 1269 cm^{-1} , secondary alcohol C-

O stretch from 1101 cm^{-1} to 1086 cm^{-1} , C-H 1,3 di-substitution from 872 cm^{-1} to 875 cm^{-1} , and methylene-(CH_2) from 728 cm^{-1} to 726 cm^{-1} were also observed, as shown in Figure 5(a) and 5(b). These visible peak shifts confirm the biodegradation of cornstarch-based biodegradable plastic. Thus, the cornstarch-based bags treated with anaerobic microorganisms experienced major structural changes, which directly indicate the biodegradation by microorganisms [32, 33]. The FTIR-ATR spectral analysis of cornstarch-based bioplastics revealed that microbial metabolism results in the breakdown of the bioplastic films, which is reflected in peak shifts and an increase in the intensities of several chemical functional groups.

After 21 days of anaerobic biodegradation of sugarcane bagasse plates, peak shifts of methyl C-H asymmetric stretch from 2974.4 cm^{-1} to 2976.3 cm^{-1} , methyl C-H symmetric stretch from 2894.3 cm^{-1} to 2898.0 cm^{-1} , C=C stretch from 1647.5 cm^{-1} to 1638.2 cm^{-1} , OH bend from 1371.7 cm^{-1} to 1373.5 cm^{-1} , C-C vibrations from 1200.2 cm^{-1} to 1202.1 cm^{-1} and tertiary alcohol C-O stretch from 1161.1 cm^{-1} to 1159.2 cm^{-1} were observed, as shown in Figure 6(a) and 6(b). The FTIR-ATR spectral analysis of sugarcane bagasse plates revealed that microbial metabolism results in the breakdown of the bioplastic films, which is reflected in the peak shifts.

After 21 days of anaerobic biodegradation of bamboo starch-based spoons, the peak shift of the O-H bend from 3391 cm^{-1} to 3377 cm^{-1} , and the alkyne C-H bend from 669 cm^{-1} to 676 cm^{-1} was observed, as shown in Figure 7(a) and 7(b). This visible peak shift confirms the anaerobic biodegradation of bamboo starch-based spoons.

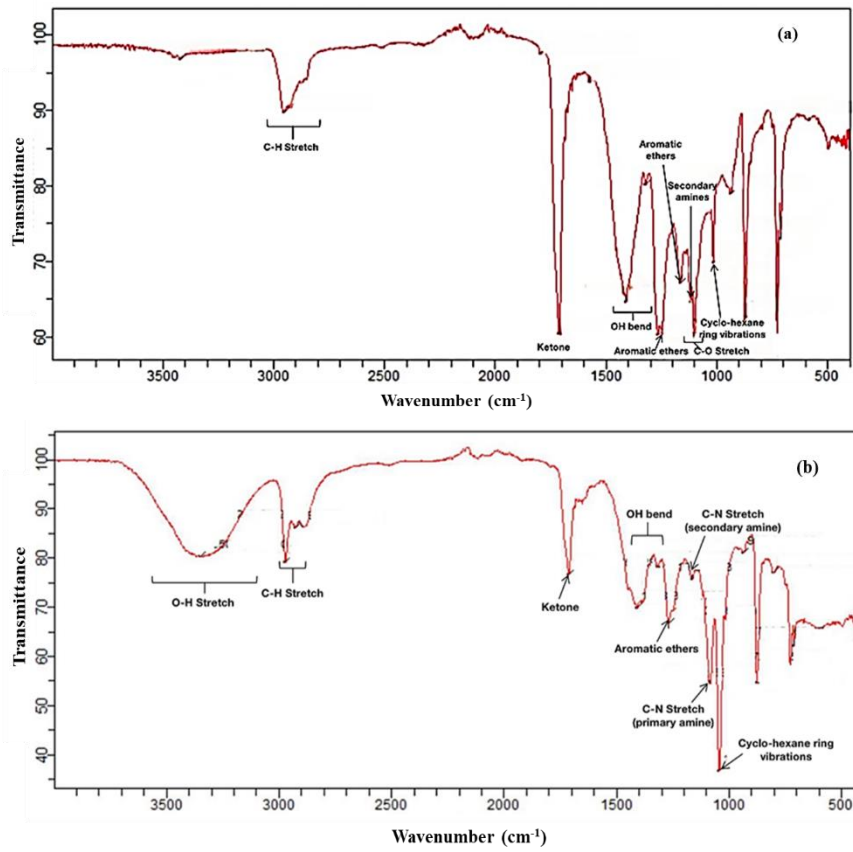


Figure 5. FTIR-ATR spectra of the cornstarch-based shopping bag before (a) and after 21 days (b) of anaerobic biodegradation

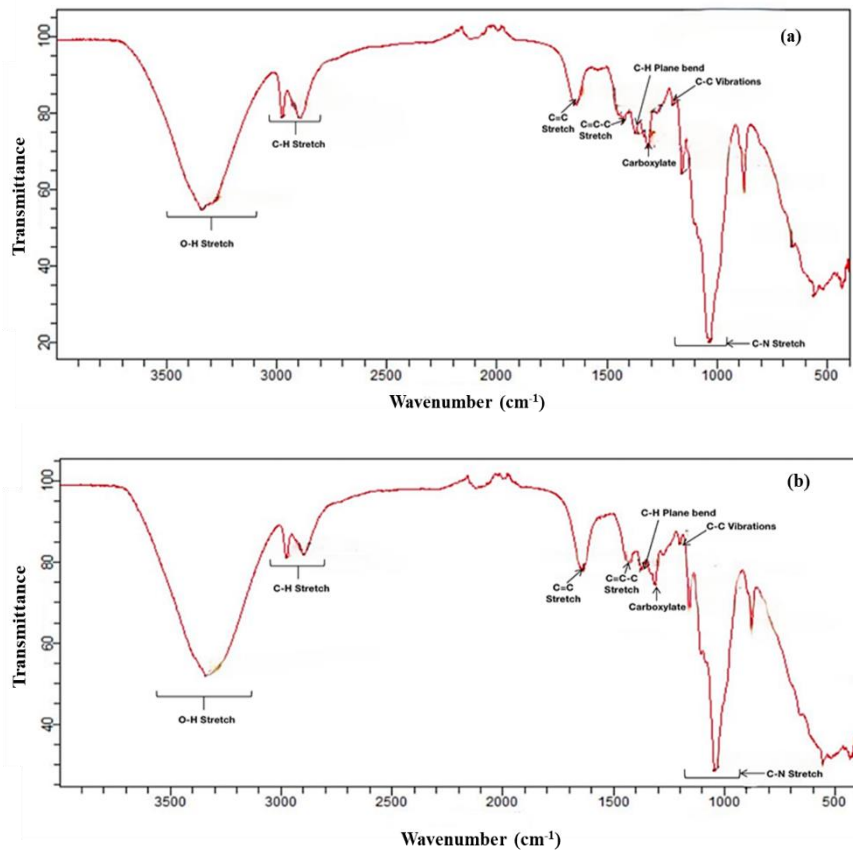


Figure 6. FTIR-ATR spectra of the sugarcane bagasse plates before (a) and after 21 days (b) of anaerobic biodegradation

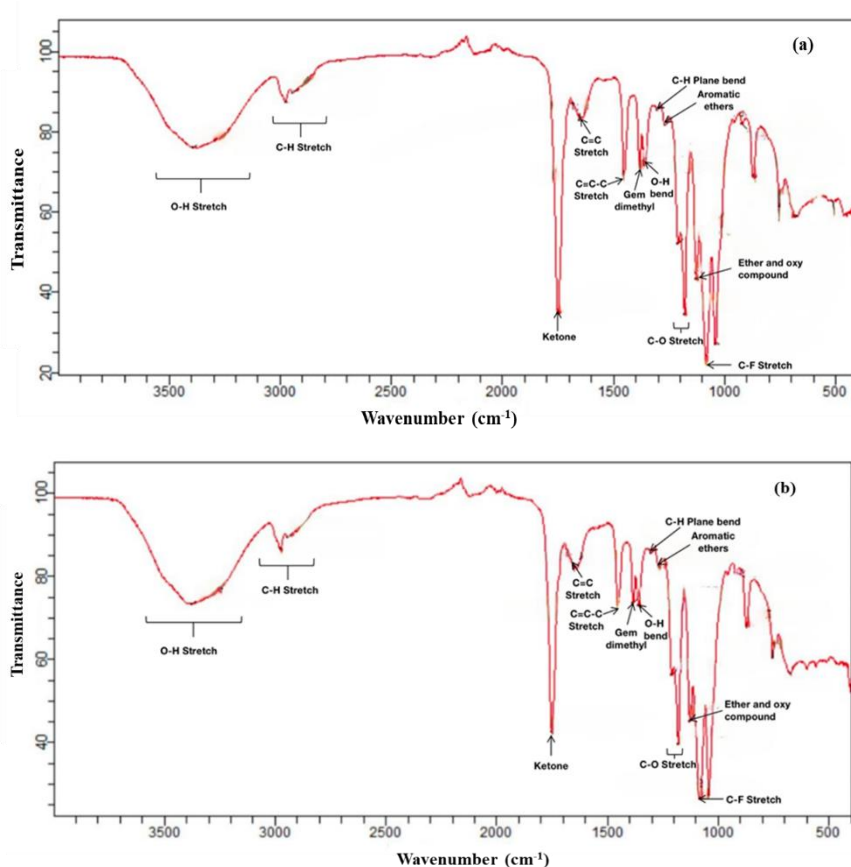


Figure 7. FTIR-ATR spectra of the bamboo starch-based spoons before (a) and after 21 days (b) of anaerobic biodegradation

Table 1 summarises the change in FTIR-ATR band position after 21 days of anaerobic biodegradation compared to the spectra before biodegradation.

These spectral shifts are critical indicators of the anaerobic biodegradation process. In anaerobic conditions, microorganisms break down complex organic polymers into simpler compounds such as volatile fatty acids, alcohols, and gases through a sequence of biochemical processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Methanogenic archaea use these simple compounds, particularly acetate and hydrogen, to produce methane (CH₄) and carbon dioxide (CO₂), contributing to the production of biogas. The observed FTIR-ATR band shifts correlate with these stages, showing the structural changes that occur in bioplastics during anaerobic biodegradation and confirming the subsequent generation of methane.

Table 1. Comparison of the band changes in the cornstarch-based shopping bags, sugarcane bagasse plate and bamboo starch-based spoon before and after 21 days of anaerobic biodegradation

Wavenumber (cm ⁻¹)		Functional groups
Before	After	
Cornstarch-based shopping bags		
-	3350	O-H stretch
2955	2892 and 2926	C-H stretch
1321	1319	carboxyl
1267	1269	aryl O-stretch
1101	1086	C-O stretch
872	875	C-H 1,3 disubstitution
728	726	methylene (CH ₂) _n
Sugarcane bagasse plate		
2974.4	2976.3	C-H asymmetric stretch
2894.3	2898	C-H stretch
1647.5	1638.2	C=C stretch
1371.7	1373.5	O-H bend
1200.2	1202.1	C-C vibrations
1161.1	1159.2	C-O stretch
Bamboo starch-based spoon		
3391	3337	O-H bend
669	676	C-H bend

In summary, the FTIR-ATR spectral analysis provides a comprehensive understanding of the structural changes in bioplastics under anaerobic conditions. These changes confirm the anaerobic process of biodegradation, aligning with the stages leading to methane production. This dual benefit of managing biodegradable plastics and generating renewable energy through anaerobic digestion highlights the potential of bioplastics in sustainable waste management and energy production [34 - 36].

Biogas and methane production

During the 21-day anaerobic degradation of cornstarch-based shopping bags, significant production of biogas and methane was observed, indicating the bioplastic degradation over time (Table 2). In the first week, 2.2 mL of biogas and 1.5 mL of methane were produced per gram of bioplastic, which indicates the initial microbial adaptation and initiation of hydrolysis. By the second week, biogas production increased to 36.1 mL/g, and the methane production reached 23.6 mL/g, indicating efficient microbial breakdown of the bioplastic. This trend continued in the third week, with biogas and methane production peaking at 41.3 mL/g and 29.3 mL/g, respectively. The cumulative production of 79.6 mL/g of biogas and 54.4 mL/g of methane over 21 days highlights sustained microbial activity and efficient substrate utilization.

For sugarcane bagasse plates, the rapid production of biogas and methane was evident

from the very beginning (Table 2). In the first week, 37.5 mL/g of biogas and 26.3 mL/g of methane were produced, indicating readily available fermentable sugars. Although production decreased slightly in the second week (34.6 mL/g biogas and 24.0 mL/g methane), it remained significant, reflecting ongoing microbial degradation of complex carbohydrates. Over 21 days, the total yields reached 103.7 mL/g of biogas and 71.4 mL/g of methane, highlighting the efficient anaerobic digestion of sugarcane bagasse.

In contrast, bamboo starch-based spoons showed initial inhibitory effects on gas production. In the first week, there was gas consumption (- 2.6 mL/g biogas, - 0.75 mL/g methane), which indicates challenges in microbial adaptation due to inhibitory compounds. This trend continued in the second week, with further decreasing (- 3.8 mL/g biogas, - 1.5 mL/g methane), before a significant increase in the third week (33.1 mL/g biogas, 21.8 mL/g methane), indicating microbial adaptation or neutralization of the compound. Overall, over 21 days, bamboo starch-based spoons produced 26.7 mL/g of biogas and 19.55 mL/g of methane, highlighting the complexity of microbial interactions and substrate characteristics in anaerobic degradation processes (Table 2).

These findings illustrate the varying biodegradation dynamics among different bioplastics under anaerobic conditions, highlighting the importance of substrate-specific considerations in optimizing biogas and methane production from biodegradable materials.

Table 2. Comparison of the biogas and methane yield for the cornstarch-based shopping bag, sugarcane bagasse plates and bamboo starch-based spoons

Days	Biogas yield (in mL/g dry weight)			Methane yield (in mL/g dry weight)		
	Cornstarch-based shopping bags	Sugarcane bagasse plate	Bamboo starch-based spoon	Cornstarch-based shopping bags	Sugarcane bagasse plate	Bamboo starch-based spoon
0	0	0	0	0	0	0
7	2.2	37.5	-2.6	1.5	26.3	-0.75
14	36.1	34.6	-3.8	23.6	24.0	-1.5
21	41.3	31.6	33.1	29.3	21.1	21.8
Total	79.6	103.7	26.7	54.4	71.4	19.55

CONCLUSION

This study showed that anaerobic biodegradation can effectively break down bioplastics, resulting in the production of valuable by-products such as biogas and methane. Among the tested bioplastics, sugarcane bagasse plates proved to be the most efficient. This rapid production indicates that sugarcane bagasse plates degrade quickly under anaerobic conditions, with microbial activity starting almost immediately and remaining high throughout the process. The methane yield curve additionally illustrates the distinct biodegradation dynamics of the tested bioplastics. The sugarcane bagasse plates showed immediate and rapid methane production, indicating early and continuous microbial activity. SEM and FTIR-ATR analyses provided additional insights into the structural changes occurring in bioplastics during anaerobic biodegradation. These findings emphasize the importance of considering substrate-specific characteristics when optimizing biogas and methane production from biodegradable materials and further promote the use of anaerobic digestion approaches for commercially available biodegradable plastics for efficient waste management.

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