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# Application of natural biodelignification systems in forest soil for enhanced anaerobic digestion potential of wood waste

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## ABSTRACT

Wood wastes are a valuable potential resource to produce clean and renewable biogas through anaerobic digestion. However, the high lignin content limits its potential as such a biomass resource by inhibiting its biodegradability. Efforts to enhance digestibility of such wastes have mainly focused on chemical and physical methods, although the employment of such tools can be costly and environmentally unfriendly. Forest soil ecosystems have evolved to manage fallen timber and deadwood with efficient processes brought about by a range of organisms including fungi, bacteria, and insects. We propose that such systems are feasible for pre-treatment of lignocellulosic waste and improvement of biogas production during anaerobic digestion. In this paper, we describe the collection and analysis of wood obtained from a semi-natural woodland floor. These samples were characterized and classified into one of five decay classes through a range of chemical and physical analyses including total solids, volatile solids, total organic carbon, carbon to nitrogen ratio, and lignocellulose content. Finally, biomethane potential tests were carried out to determine the effect of reduction of wood particle size, as a proxy for one of the effects of timber biodegradation, on the potential for enhanced biogas production. The outcomes are instructive in exploring the potential for nature-based pre-treatment of lignocellulosic wastes to render them more amenable to anaerobic digestion, which has the potential to make available significant new waste feedstocks (e.g., forestry waste) for biogas and energy generation.

*Keywords: wood waste, anaerobic digestion, biomethane, biodelignification, forest soil*

## 1 INTRODUCTION

As a sustainable and renewable green material, wood plays a vital role for the human beings. The timber trade is a globally expanding business, with significant volumes of roundwood consumed each year. However, as the beginning of the timber supply chain, logging will generate large amounts of wood waste. In general, almost 50% of a tree can be processed into the final product, whereas the rest is retained as wood waste (Turley et al., 2006). Wood waste consists mainly of the residues from forestry operations (branches, treetop, stumps and sawdust) and wood processing (bark, sawdust, trimmings and core) (Souza et al., 2018). The overall amount of global wood waste is estimated to be 232.94 million $m^3$ in 2020 (Gao et al., 2022). However, only a limited amount of wood waste has so far been available for recycling and reuse, and an economical method has not yet been developed to make full use of wood waste. Given the high calorific value of wood waste, the predominant disposal scenario is to generate thermal energy from combustion (Molenda et al., 2021). With the high consumption of fossil fuels (non-renewable resources) and the increasing environmental awareness, direct combustion of wood waste to produce energy or electricity is not considered to be an efficient and environmentally friendly alternative anymore (González-García and Bacenetti, 2019; Vicente et al., 2020). Particularly in small boilers or combustion chambers without emission control systems, the emissions produced could cause a lot of energy waste and serious environmental pollution (Jaworek et al., 2021; Sharma and Dasappa, 2017). Wood waste can be a potential resource for the generation of different materials by reforming or creating new products. Biopolymers derived from wood waste are considered a sustainable resource for the development of advanced materials in high-tech fields such as energy storage, flexible electronics, biomedicine, and water purification (Liu et al., 2021). What's more, biochar made from wood waste through a process of pyrolysis has great application potential (Ferrari et al., 2022; Kim et al., 2020). However, these reproductions require higher energy consumption and more complex processes, as well as a lack of widespread application.

Anaerobic digestion (AD) is an attractive choice that simultaneously helps to mitigate pollution caused by improper organic waste treatment and provides a source of green, sustainable, and renewable energy. Many wastes such as sewage sludge (Liu et al., 2016), food waste (Ren et al., 2018) and animal manure (Sohail et al., 2022; Usman Khan and Kiaer Ahring, 2021) are ideal raw materials for AD, however the high lignin content and crystalline cellulose structure of lignocellulosic wastes, especially wood waste, makes it difficult to produce biogas continuously and efficiently, which limits their large-scale application in AD (Gao et al., 2022). Therefore, the pre-treatment of wood waste to improve the biogas potential from AD process is necessary (Muaaz-Us-Salam et al., 2020; Rashid et al., 2017). Pre-treatment techniques typically include biological, chemical, and physical methods. Among them, physical methods involve a large energy input and a high equipment for extreme environmental conditions (Atelge et al., 2020); chemical methods use plenty of chemicals, which are expensive and may pollute the environment if not treated properly (Yu et al., 2019). Biological methods may have some advantages compared to physical and chemical methods, but isolating microorganisms or enzymes that can break down the lignocellulose structure into more accessible materials is time-consuming and ultimately ineffective in biogas upgrading (Alexandropoulou et al., 2017; Ali et al., 2017). For such consideration, alternative technique for the pre-treatment of wood waste must be paid attention and developed to increase biogas production in a more green and sustainable way.

Forests play an important role in the global carbon cycle, with plants like trees and bushes absorbing energy through photosynthesis and storing it as carbon in wood, which is then partly released into the atmosphere with the decomposition of deadwood in the forest soil (Seibold et al., 2021). Without being harvested, deadwood, comprising fallen dead trees and the remains of branches after logging, cannot escape the fate of being degraded into humus as part of the forest soil (Tatti et al., 2018). Forest soil ecosystems have evolved to manage fallen timber and deadwood with efficient processes brought about by a range of organisms including fungi, bacteria, and insects. Moreover, forestry operations generate large amounts of wood wastes which are left in the forest soil. We propose that the forest soil ecosystems are feasible for pre-treatment of the above wood wastes to improve their biogas production during AD as a result of the increase in accessibility and biodegradability of the lignocellulosic structure. The degradation of deadwood into humus can be divided into five decay classes by its morphology (Tatti et al., 2018), which helps us to investigate the degradation process of deadwood in the forest soil ecosystems on its anaerobic digestion potential. Therefore, the objectives of this study were to determine: (1) the disparity in chemical and physical composition between different decay classes wood samples collected from forest, and (2) the effect of wood particle size on biogas yield from AD as a proxy for natural biodeterioration.

## 2 MATERIALS AND METHODS

### 2.1 The disparity in physicochemical composition of different decay classes wood samples

#### 2.1.1 Study site and field sampling

The wood samples were collected from an unmanaged seminatural forest in Cardiff (51°31'4"N, 3°14'46"W). The wood samples were hardwoods, silver birch (*Betula pendula*), according to the external appearance and the tree species of the surrounding site. We collected 6 dead fallen logs which can be classified as decay class 1 (DC1) and decay class 5 (DC5) based on the visual and mechanical indicators proposed in previous studies (Tatti et al., 2018), and each DC contain three replicates. Specifically, (1) DC1: wood still hard, knife blade penetrates a few millimetres, bark intact; (5) DC5: the wood is very soft and easy to break apart by hand, often covered in moss (Table 1). The fresh samples were cut into 2 mm blocks by a Fritsch 55743 rotary knife mill and finally stored in a refrigerator at 4 ℃ until further processing.

***Table 1. The features of collected wood samples for determining their decay classes (Tatti et al., 2018).***

| Features | DC1 | DC5 |
|---|---|---|
| Bark | Bark normally intact | Absent |
| Texture | Intact | Soft and powdery (very few portions of the log remain coherent) |
| Knife test | Knife blade penetrates a few millimeters | Breaks up easily by hand |
| Color of wood | Original color | Baded to light yellow or grey, or red brown to dark brown |

### 2.1.2 Chemical composition analysis

Total solids (TS) and volatile solids (VS) of the wood samples were measured following the Standard Methods 2540 protocol, where TS was measured at 105 ℃; VS was measured at 550 ℃. Flash*Smart*™ elemental analyser (Thermo Scientific) was used to measure total carbon and total nitrogen (Lee et al., 2021).

The contents of lignocellulose including cellulose, hemicellulose and lignin were determined by thermogravimetric analysis (TGA). Firstly, the fraction of extractives in the dried biomass was determined by using a Dionex® Accelerated Solvent Extractor (ASE100) with 95% ethanol as extraction solvent (Sluiter et al., 2008). Secondly, The TGA was performed on a Mettler Toledo analyser (TGA/SDTA 851e). The analyses were carried out using a nitrogen atmosphere with a flow rate of 50 mL/min. The heating rate used was 5 ℃/min, from 30 ℃ to a final temperature of 1000 ℃. The sample weight was c.a. 8 mg. Additionally, the pyrolysis process in wood sample is the successive separation and escape of the four components (water, hemicellulose, cellulose and lignin). The Gaussian peak fitting module of the software Origin was used to separate the reaction rate curves of the four components from the total derivative thermogravimetry (DTG) curve (Rego et al., 2019). The DTG experimental curve was treated with 7 pseudocomponents, of which 1 on water for 30–150 ℃, 2 on hemicellulose for 200–350 ℃, 1 on cellulose for 250–400 ℃ and 3 on lignin for 150–1000 ℃ (Díez et al., 2020). The correlation coefficients $R^2$ of the correlation results are generally greater than 0.99, showing that the fitting results are accurate and credible. Finally, the lignin content of DC1 and DC5 wood samples were measured using the standard method as showed in Muaaz-Us-Salam et al. (2020) which was used to validate the TGA results.

Total organic carbon (TOC) of solid and liquid samples were measured with a Shimadzu TOC-VCPH following the manufacturer's instructions. To test water extractable organic carbon (WEOC) of decaying wood samples, 5 g of dried wood sawdust was weighed into a 50 mL plastic centrifuge tube containing 30 mL of deionized water and extracted on a reciprocating shaker at 200 rpm for 2 h in room temperature, followed by centrifugation at 4000 rpm for 10 min, and then passed through a 0.45 µm glass-fiber filter membrane (Bai et al., 2020). The carbon content of the filtrate was determined using a Shimadzu TOC-VCPH.

### 2.1.3 Crystallinity measurements

Biomass crystallinity was measured by Siemens / Bruker D5000 X-ray Powder Diffraction (XRD) System operated at 40 kV and tube current of 40 mA. Scans were obtained from 2θ = 10–40° with step size of 0.02 at 0.6 s per step. The cellulose crystallinity index (CrI) of the samples can be calculated according to Eq. (1).

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \tag{1}$$

where $I_{002}$ is intensity of diffraction from 002 plane at 2θ = 22° and $I_{am}$ is the intensity of background measured at 2θ = 18° (Kumar et al., 2019).

### 2.1.4 Statistical analysis

The software SPSS 19.0 was used for the analysis of standard deviations and significant differences with a threshold *p*-value of 0.05, 0.01 and 0.001. The software OriginPro 2022 was used to plot the figures.

## 2.2 The impact of particle size reduction on biogas yield

This preliminary experiment was carried out using softwood timber (redwood pine timber) as a model undegraded wood source to explore the impact of particle size reduction on biogas production. We chose 2 cm and 2 mm as the final sizes for anaerobic digestion, the redwood pine timber was chopped into the two sizes using a Fritsch 55743 rotary knife mill with the corresponding screens. The anaerobic digestion experiment used biomethane potential testing equipment (supplied by Anaero Technology UK) consisting of 1 litre reactors submerged in a water bath to maintain the required temperature. Gas flow meters based on the water displacement method combined with an Arduino (for data logging) were used to monitor the biogas production. Biogas was collected in 5 litre Tedlar® bags attached to the outlets of the gas flow meter for each reactor, and all biogas data are reported at STP. The sludge from a biogas plant digesting sewage sludge in Cardiff (TS 5.52% ± 0.002, VS 59.46% ± 0.023) was used as inoculum of AD. The sludge was sampled using 5-litre high-density polyethene jerrycans, and immediately taken to the lab. Prior to the biomethane potential test, the sludge was incubated at 30 ℃ for 3 days and shaken manually twice a day to ensure homogenization. Each bioreactor comprised 700 ml sewage sludge digestate and 4 g of wood cubes (0 g in controls), together with 300 ml of headspace (Muaaz-Us-Salam et al., 2020). All bioreactors were incubated in the water bath apparatus at 30 ℃ for 30 days and continuously stirred at 45 rpm. The digestions were performed in triplicate.

In order to better understand the influence of particle size on the AD of pinewood samples, the modified first-order kinetic model is used to conduct dynamic analysis of biogas production (Wall et al., 2013). The modified model is shown in Eq. (2).

$$y(t) = y_m \times (1 - \mathrm{e}^{(-k*t)}) \tag{2}$$

where *y(t)* is the cumulative biogas yield at time t (mL/g of TS), $y_m$ is the biogas yield at the end of the 30-day test (mL/g of TS), t is the time (days), and k is the first order decay constant.

# 3 RESULTS AND DISCUSSION

## 3.1 Effect of forest soil on wood physicochemical composition

### 3.1.1 The composition of wood samples

The wood samples collected from the forest can be classified as decay class 1 (DC1) and decay class 5 (DC5), and their characteristics are shown in Table 2. The TS in DC1 were considerably higher than in DC5 indicating much greater absorption of moisture in the latter as a result of the considerably more open structure. VS was slightly reduced with increased decay. The total carbon in DC1 and DC5 were nearly the same, but DC5 had higher nitrogen content, resulting in the C/N ratio of decayed wood much closer to the optimal value (C/N of 20–30) for AD (Ajayi-Banji and Rahman, 2022). In the AD process, biogas (mainly methane and carbon dioxide) is produced through the biodegradation of organic carbon during hydrolysis, acidification and methanation (Provenzano et al., 2014). WEOC is a key contributor in this reaction, as microbial metabolism takes place in the water-soluble phase (Shakeri Yekta et al., 2012; Xing et al., 2012). Therefore, the significant higher TOC (Figure 1a) and WEOC (Figure 1b) in DC5 indicate a better potential for methane production from AD.

***Table 2.*** *Characteristics of wood samples collected from the forest.*

| Parameters | DC1 | DC5 |
|---|---|---|
| Total solids (%)$^a$ | 59.47 ± 15.12 | 18.39 ± 6.80 |
| Volatile solids (%)$^b$ | 99.31 ± 0.60 | 96.61 ± 0.73 |
| C (%)$^b$ | 47.16 ± 0.29 | 50.55 ± 0.77 |
| N (%)$^b$ | 0.33 ± 0.04 | 1.19 ± 0.10 |
| C/N ratio | 146.73 ± 17.62 | 42.92 ± 3.93 |

$^a$ Based on wet weight; $^b$ Based on dry weight; C/N ratio: carbon to nitrogen ratio.

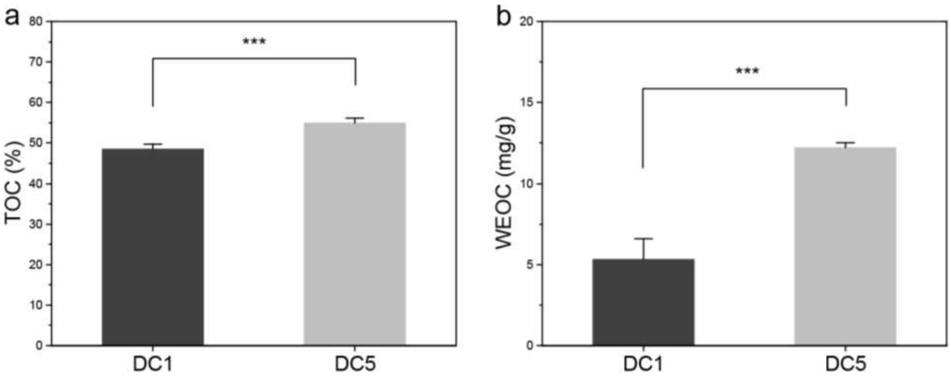

***Figure 1.*** *The total organic carbon (TOC) content in wood samples (a). Water extractable organic carbon (WEOC) in wood samples (b). *** means p < 0.001.*

Figure 2 shows the fitting results to the DTG experimental data using the Gaussian model, and the lignocellulose composition of DC1 and DC5 are provided in Table 3. The lignin content of DC1 and DC5 measured using the standard method were 24.15 ± 1.83% and 46.57 ± 3.59% which were close to the TGA results. The cellulose content decreased from 34.29% in DC1 to 16.24% in DC5. The significant increased lignin content of DC5 may be due to the reduction of other components such as cellulose, indicating that exposure to this natural biodelignification system was not effective in removing lignin and it became proportionally more significant as a component as decay occurred. However, decay has permitted biological access to, and degradation of, cellulose and hemicellulose, suggesting that a certain amount of decay and breakdown may be advantageous as a pre-treatment method for AD. Similar effects have been observed in other pre-treatment studies, such as the application of chemicals (Mohsenzadeh et al., 2012; Salehian et al., 2013), hydrothermal (Karami et al., 2022) and steam explosion (Eom et al., 2019; Mulat et al., 2018) leading to a reduction in the cellulose content and an increase in the lignin content of wood waste.

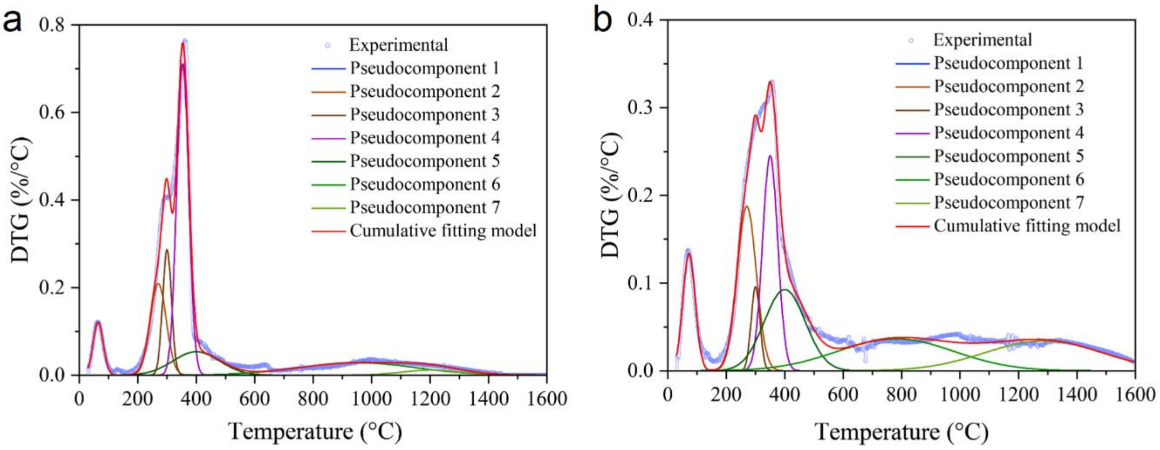

***Figure 2.*** *The model fitted experimental DTG curves. DC1 (a), DC5 (b).*

***Table 3.*** *Lignocellulose composition of wood samples collected from the forest.*

| Parameters | DC1 | DC5 |
|---|---|---|
| Extraction (%) | 7.18 ± 0.59 | 3.67 ± 1.31 |
| Hemicellulose (%) | 23.37 ± 0.61 | 17.94 ± 0.42 |
| Cellulose (%) | 34.29 ± 2.38 | 16.24 ± 1.33 |

| | | |
|---|---|---|
| Lignin (%) | 23.67 ± 2.07 | 49.17 ± 1.90 |
| Ash (%) | 3.34 ± 1.10 | 5.47 ± 0.51 |

### 3.1.2 The crystallinity of wood samples

The crystallinity of lignocellulosic biomass is an important source of its recalcitrance in AD and is closely related to its composition. During the hydrolysis and acidification stage of AD, microorganisms can hydrolyse lignocellulose causing a decrease in crystallinity (Hou et al., 2021). The cellulose CrI was developed to reflect the relative crystallinity of wood cellulose. XRD was performed to determine the changes that occurred in the crystallinity of the wood samples (Figure 3a), and the results showed that the CrI values of DC1 and DC5 samples were 35.43% and 16.47% respectively (Figure 3b). The crystallinity of cellulose for DC5 was reduced by more than a factor of two compared to DC1, which means that the cellulose crystalline region of DC5 was destroyed, resulting in more cellulose being available for microbial hydrolysis. Similar results were observed by He et al. (2022) for corn straw with hydrogen-nanobubble water and by Xu et al. (2018) for corn straw pre-treated with a pure bacteria system. Therefore, the natural biodelignification system could reduce the crystallinity of cellulose and make wood more easily decomposed in AD.

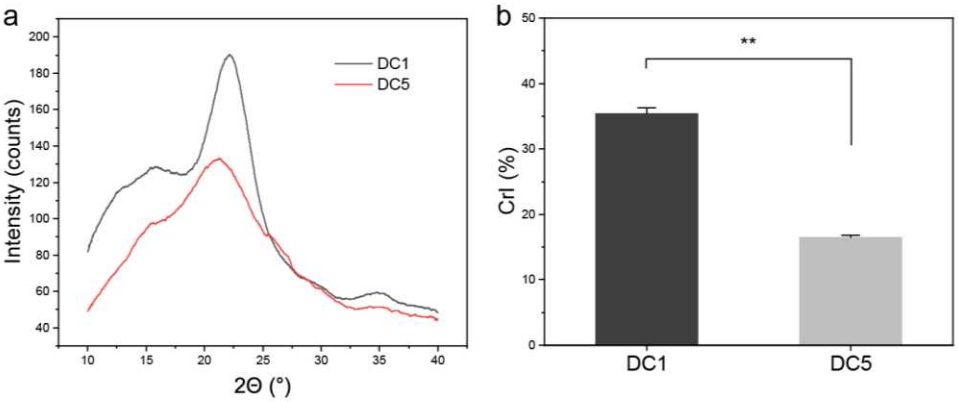

***Figure 3.*** *XRD graph of wood samples (a). Crystallinity Index (CrI) of wood samples (b). ** means p < 0.01.*

## 3.2 Effect of timber particle size on biogas production

The 2 cm and 2 mm wood blocks were anaerobically digested for 30 days, the cumulative biogas production is shown in Figure 4. The 2 mm blocks showed a higher biogas yield with 460.80 ± 13.45 mL/g of TS compared to 222.16 ± 13.64 mL/g of TS in 2 cm blocks. Furthermore, the cumulative biogas production curve shows that the biogas increased rapidly in the first 15 days, reaching 80% of the total output. In the early stage of AD, the feedstock was mainly hydrolysed and acidified, and a significant amount of biogas production was probably contributed by the WEOC of wood samples and the residual organic fraction in the sludge. The rate of gas production was consistently higher with 2 mm blocks, expected to be caused by 2 mm blocks having greater surface area and thus being more susceptible to hydrolysis and acidification compared to 2 cm blocks, producing more feedstock for methanogen utilization. These results were comparable to many studies on enhancing biogas production from other lignocellulosic waste by reducing particle size. Dai et al. (2019) investigated the effect of particle size on biomethane production of rice straw and reported that particle size reduction significantly enhanced the methane production. Liu et al. (2017) revealed that the reduction in particle size from 4 mm to 1 mm of hazel branches and acacia branches resulted in 25.17% and 8.29% higher methane production from AD respectively.

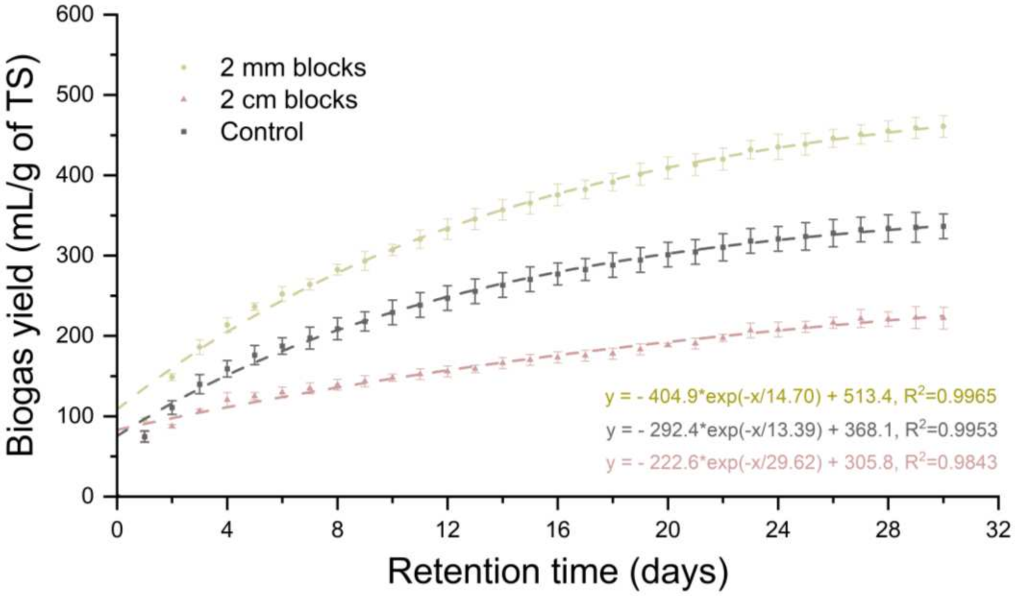

***Figure 4.*** *Cumulative biogas production from 2 cm and 2 mm of pinewood blocks.*

## 4 CONCLUSIONS

Analysis of naturally decayed timber demonstrated that a natural biodelignification system significantly affected the physical and chemical nature of the wood. Decayed timber was physically considerably more friable and able to absorb moisture whilst decay reduced the crystallinity of cellulose and increased TOC and WEOC alongside other significant changes. These changes are expected to lead to enhanced biogas production in anaerobic digestion as a result of a breakdown of the lignocellulosic structure, increasing access to microorganisms involved in AD. As an initial test of this hypothesis, anaerobic digestion of initially undecayed softwood demonstrated that reduction in particle size, as a proxy for physical breakdown, significantly increased biogas production. Further work will explore the impact of these decay effects on biogas production in greater detail and attempt to identify the optimal level of decay in terms of pre-treatment for AD. However, these initial outcomes do indicate that a low cost, low impact nature-based system without any physical processing may contribute to enhanced biogas production.

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