

Effect of Incubation Time of Activated Vermiculite on the Biodegradation of Microcrystalline Cellulose

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Abstract

This study examined the impact of the preparation method and incubation time of activated vermiculite on the biodegradation of microcrystalline cellulose. Vermiculite was pre-incubated for 1-4 days using compost extract or a commercial organic fermentation agent before use as inoculum. Results showed highest percentage biodegradation (64.9%) after 15 days with 1-day pre-incubation using compost extract. With the commercial agent, the highest rate (45.3%) was achieved after 3 days. The study provided insights into optimizing vermiculite preparation for reliable biodegradation tests, indicating the need to distinguish validity criteria for vermiculite and compost inoculum.

Keywords

activated vermiculite, incubation time, microcrystalline cellulose, biodegradation, inoculum.

1. Introduction

In the process of municipal solid waste treatment, mature compost and activated vermiculite are typical aerobic treatment inoculums, primarily used to simulate the biodegradation process of the organic fraction. Mature compost, being a highly heterogeneous and complex material, presents challenges in quantifying the polymer material remaining in the inoculum at the end of the test, as well as in evaluating changes in biomass after degradation. Furthermore, mature compost may exhibit the so-called “priming effect,” where the addition of a large quantity of organic matter triggers polymer-induced degradation, complicating the measurement of biodegradation capacity [1].

In contrast, vermiculite, an inorganic substance, is non-toxic, environmentally benign, and easily regenerable, which can significantly reduce the priming effect and improve the reliability of biodegradation tests [2, 3]. Due to its low organic matter content, it makes it suitable for assessing the biodegradability of materials with low degradation potential but unaffected by the priming effect [1, 4]. However, the culture solution added to the vermiculite contains organic components that are

easily decomposed by microorganisms, leading to a high overall level of biodegradation of the inoculum itself during the first ten days of incubation. As a result, the pre-incubation time of activated vermiculite can significantly affect the background carbon dioxide production and the biodegradation level of the inoculum.

Biodegradation, the process by which microorganisms break down organic materials into simpler compounds, plays a crucial role in environmental sustainability, particularly in waste treatment and recycling. [5] Numerous studies have focused on understanding the efficiency and effectiveness of various inoculums in promoting biodegradation under different environmental conditions. These inoculums typically consist of microorganisms capable of degrading complex organic compounds, and their performance can vary significantly depending on the substrate, environmental conditions, and microbial community composition. One of the most widely used inoculums in biodegradation studies is activated sludge, which contains a diverse range of microorganisms capable of breaking down a variety of organic materials [6–8]. Research

has shown that inoculum derived from municipal wastewater treatment plants can effectively degrade plastics such as polycaprolactone and starch-based materials [8]. However, the performance of these inoculums is highly dependent on factors such as acclimation to specific substrates, environmental conditions, and the microbial community present in the sludge [9]. In some cases, commercial inoculums have been shown to outperform activated sludge for specific substrates, such as dairy wastewater with high fat content [6].

In addition to activated sludge and commercial inoculum, biochars and other organic amendments have been investigated as potential inoculum in biodegradation processes. Biochar has been demonstrated to influence bacterial community dynamics and enhance the biodegradation of antibiotics in contaminated soils [10, 11]. Furthermore, mixed fungal cultures have been evaluated for their ability to degrade palm oil mill effluent, with inoculum concentration shown to significantly affect biodegradation rates [12]. These findings underscore the importance of selecting an appropriate inoculum based on the specific biodegradation task.

The influence of environmental parameters, such as temperature and oxygen availability, on biodegradation rates has also been a focus of recent studies. For instance, increasing the incubation temperature from 25°C to 37°C showed varying effects on the biodegradation of different polymers, with some polymers exhibiting increased degradation rates, while others did not [9]. Additionally, research on microbial community dynamics under varying biodegradation conditions has provided further insights into how the sequestration of compounds in soil can impact microbial performance [13, 14]. The biodegradation of hazardous compounds, such as the military explosive RDX, has also been studied under controlled redox conditions to optimize mineralization rates [15], highlighting the significance of environmental control in biodegradation processes, particularly for compounds requiring specialized microbial communities for effective degradation.

Given the complex nature of biodegradation, factors such as inoculum composition, environmental conditions, and microbial interactions must be carefully considered when designing biodegradation experiments. Studies have shown that different inoculums can yield significantly different biodegradation results depending on these factors. For example, acclimated microbial communities in activated sludge have been shown to provide superior biodegradation performance, particularly for materials like cellulose and certain plastics [8, 9]. Meanwhile, other studies have highlighted the potential of using alternative inoculum, such as commercial organic fermentation agents, for faster and more reproducible results [16].

While much of the research has focused on the biodegradation of well-established materials like plastics and pharmaceuticals, fewer studies have explored the use of less conventional inoculum, such as activated vermiculite, in biodegradation processes. Vermiculite is known for its ability to stabilize errors between repetition [17]. However, the influence of factors such as the preparation method and incubation time

Instrument Name	Model	Manufacturer
Total Organic Carbon Analyzer	enviro TOC	Elementar Trading Co.,Ltd (Shanghai)
Intelligent Muffle Furnace	MFLC-16/12P	Tianjin Test Instrument Co., Ltd.
Handheld Gas Detector	APES-Z2-F	Shenzhen Ampel Technology Co., Ltd.

Table 1. Experimental Instrument Information

on activated vermiculite performance remains underexplored.

This study aimed to investigate the effect of the preparation method and different incubation times of activated vermiculite on the biodegradation performance of microcrystalline cellulose. Using both mature compost extract and commercial organic fermentation inoculum to prepare vermiculite, we evaluated the biodegradation of microcrystalline cellulose over 15 days after pre-incubation for 1 to 4 days. The goal was to identify the optimal pre-incubation time, where the inoculum's own biodegradation level is relatively low, while the sample degradation rate remains high and stable. This study provides valuable insights into optimizing the preparation method and pre-incubation time of activated vermiculite, thus improving the effectiveness and reliability of biodegradation tests.

2. Experimental Section

2.1. Main Materials

Microcrystalline Cellulose (TLC (thin-layer chromatography) grade cellulose), batch number 20220530, molecular formula: $(C_6H_{10}O_5)_n$, molecular weight: $(162.14)_n$, CAS No.: 9004-34-6.

Vermiculite, coarse type, 5-8 mm.

Commercial Organic Fermentation Agent: 1 kg/package, produced by Yancheng Shenwei Microbial Strain Technology Co., Ltd.

2L polypropylene vacuum vessels (with three-way vessel caps)

2.2. Main Instruments

The main instruments used are listed in Table 1.

2.3. Experimental Method

2.3.1. Preparation of Activated Vermiculite and Analysis of Its Physicochemical Properties

Vermiculite was prepared using homemade mature compost extract as the first inoculum. The preparation method was as follows: The homemade mature compost was mainly composed of kitchen waste, such as vegetable leaves, small amounts of fish and poultry offal, sawdust, and a small amount of mature compost, obtained after 4 months of aerobic composting. The culture solution was prepared according to the composition and formula in ISO 14855-1:2012, section 8.6, with a vermiculite to culture solution ratio of 1:3 (mass/volume) and mixed thoroughly. The names, batch numbers and manufacturers of the reagents used to prepare the culture solution are listed in Table 2.

Commercial organic fermentation agent culture solution was used as a substitute for compost extract to prepare vermiculite as the second inoculum. Vermiculite was also prepared using different batches of commercial organic fermentation agents as the third inoculum. The preparation method for the second and third inoculums is as follows: Commercial organic fermentation agents were purchased to replace the mature compost. The procedure followed the manufacturer's instructions, specifically by diluting the organic fermentation agent and brown sugar with water in a ratio of 1:0.2:100

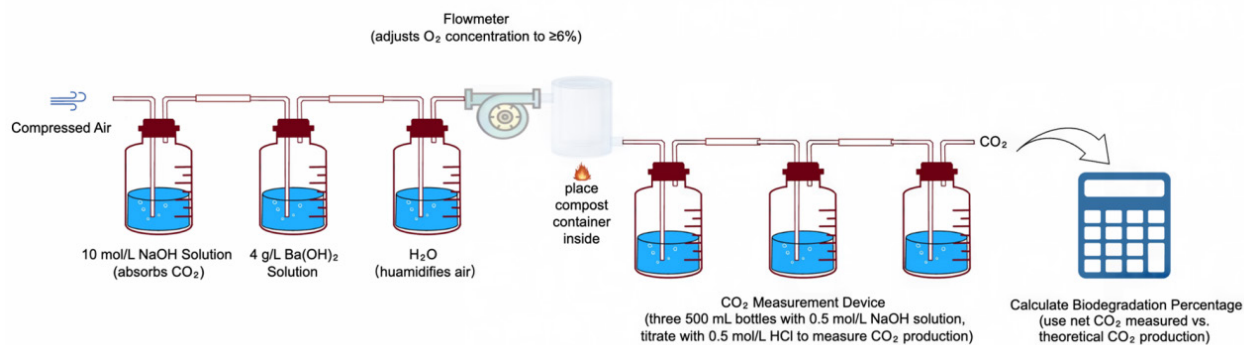


Fig. 1. Aerobic Biodegradation System Flowchart
Source: Author's drawing

Component	Batch Number	Manufacturer
Tryptic Soy Broth	1114012	Beijing Land Bridge Technology Co., Ltd.
CO(NH ₂) ₂	2203302	Xilong Scientific Co., Ltd.
Corn Starch	C13665033	Shanghai Macklin Biochemical Co., Ltd.
Cellulose	20220105	Sinopharm Chemical Reagent Co., Ltd.
KH ₂ PO ₄	20210901	Xilong Scientific Co., Ltd.
MgSO ₄	B2112221	Xilong Scientific Co., Ltd.
CaCl ₂	20220103	Tianjin Damao Chemical Reagent Factory
NaCl	B2202282	Xilong Scientific Co., Ltd.
H ₃ BO ₃	20220311	Tianjin Yongda Chemical Reagent Co., Ltd.
KI	C13184641	Shanghai Macklin Biochemical Co., Ltd.
FeCl ₃	20230103	Fortune (Tianjin) Chemical Reagent Co., Ltd.
MnSO ₄	20220115	Tianjin Yongda Chemical Reagent Co., Ltd.
(NH ₄) ₆ Mo ₇ O ₂₄	2203052	Xilong Scientific Co., Ltd.
FeSO ₄	B2202181	Xilong Scientific Co., Ltd.

Table 2. Experimental Reagent Information

and incubating the mixture at $50^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 12-24 hours, to create an alternative to compost extract. The remaining steps followed the ISO 14855-1:2012, section 8.6, composition and formula for preparing the culture solution, with a vermiculite to culture solution ratio of 1:3 (mass/volume) mixed thoroughly. The names, batch numbers and manufacturers of the reagents used to prepare the culture solution are listed in Table 2.

For each type of vermiculite inoculum, the necessary amounts of vermiculite and inoculum solution were mixed to form a homogeneous mixture, and the mixture was dispensed into the bioreactors (about 1 kg of mixture in each). Each bioreactor with its contents was weighed and incubated at $50^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for one to four days. The total organic carbon content

and volatile solid content (incineration at about 550°C) were measured for each of the three types of vermiculite inoculum at 0, 1, 2, 3, and 4 days of pre-incubation. The trends in their changes were analyzed.

2.3.2. Biodegradation Experiment and Calculation

The biodegradation performance of microcrystalline cellulose was tested using the three types of activated vermiculite inoculum (pre-incubated for 1 to 4 days, respectively), with reference to ISO 14855-1:2012. The experimental system is as follows:

a) First Experiment:

Three vessels with activated vermiculite

prepared from homemade mature compost extract and pre-incubated for 1 day were used as the blank group, with about 134 g of the total dry solid content of the inoculum in each vessel.

Three vessels with the same activated vermiculite and microcrystalline cellulose (approximately 134 g of inoculum and 34 g of microcrystalline cellulose per vessel) were used as the sample group. The same procedure was followed to prepare experimental groups with activated vermiculite pre-incubated for 2, 3, and 4 days.

b) Second Experiment:

Three vessels with activated vermiculite prepared using commercial organic fermentation agent culture solution instead of compost extract and pre-incubated for 1 day were used as the blank group, with about 134 g of the total dry solid content of inoculum in each vessel.

Three vessels with the same activated vermiculite and microcrystalline cellulose (approximately 134 g of inoculum and 34 g of microcrystalline cellulose per vessel) were used as the sample group. The same procedure was followed to prepare experimental groups with activated vermiculite pre-incubated for 2, 3, and 4 days.

c) Third Experiment:

The third experiment was a repeat of the second one, using different batches of commercial organic fermentation agent to prepare the inoculum solution.

Types of Inoculum	Parameter	Incubation Time of Activated Vermiculite				
		0d	1d	2d	3d	4d
1st	Volatile Solids Content, %	16.51	13.57	12.11	10.00	9.52
	Total Organic Carbon Content, %	5.33	4.91	3.90	2.87	1.00
2nd	Volatile Solids Content, %	15.79	10.71	8.80	9.31	8.95
	Total Organic Carbon Content, %	5.26	4.11	3.01	2.61	1.64
3rd	Volatile Solids Content, %	18.70	12.34	10.00	10.47	8.65
	Total Organic Carbon Content, %	5.25	3.89	3.53	2.66	1.96

Table 3. Inoculum Physicochemical Properties

Types of Inoculum	Incubation Time of Activated Vermiculite			
	1d	2d	3d	4d
1st	574.08	455.14	392.64	354.90
2nd	515.61	414.52	402.13	388.54
3rd	673.61	384.11	357.82	349.62
Avg	587.77	417.92	384.20	364.35

Table 4. CO₂ Production in Blank Group for 10 Days, mg/g Volatile Solids (Average)Fig. 2. Experimental System Diagram
Source: Author's photo

The test vessels were placed in the test environment at 58°C ±2°C, and aeration was initiated using water-saturated, carbon-dioxide-free air. The airflow rate was controlled at approximately 50 mL/min by an air flowmeter. The exhaust air from each vessel could be absorbed in a carbon dioxide trap containing three bottles of 0.5 mol/L solution of sodium hydroxide in water. During the experiment, daily checks were performed for system gas tightness, incubation temperature monitoring, air supply flow check, and oxygen concentration measurement in the reaction vessels. On a weekly basis the mixture in the reaction vessels was stirred and homogenized, the appearance observed, and the pH of the mixture measured. For the aerobic

biodegradation system flowchart and experimental system diagram see Figure 1 and Figure 2.

The amount of carbon dioxide in the absorption solution was measured daily using a 0.5 mol/L hydrochloric acid standard titration solution. Each trial lasted for 15 days. Calculation of the theoretical amount of carbon dioxide (ThCO₂) and the percentage biodegradation (*Dt*) of microcrystalline cellulose was performed using the Equations 1 and 2:

$$\text{ThCO}_2 = M_{\text{TOT}} \times C_{\text{TOT}} \times 44/12 \quad (1)$$

$$Dt = ((\text{CO}_2)_T - \text{CO}_2)_B / \text{ThCO}_2 \times 100 \quad (2)$$

where: M_{TOT} - the total dry solids, in grams, in the microcrystalline cellulose introduced into the vessels at the start of the test; C_{TOT} - the proportion of the total organic carbon in the total dry solids in the microcrystalline cellulose, in grams per gram; 44 and 12 - the molecular mass of carbon dioxide and the atomic mass of carbon, respectively; $(\text{CO}_2)_T$ - the cumulative amount of carbon dioxide evolved in each vessel containing microcrystalline cellulose, in grams per vessel; $(\text{CO}_2)_B$ - the mean cumulative

amount of carbon dioxide evolved in the inoculum vessels, in grams per vessel.

3. Results and Analysis

3.1. Physicochemical Properties of Activated Vermiculite

The total organic carbon content and volatile solid content of the three types of vermiculite prepared by different methods, before and after incubation for 1 to 4 days, are shown in Table 3. The cumulative carbon dioxide production and the ratio of volatile solid content in the blank group after 10 days of incubation, using activated vermiculite incubated for 1, 2, 3, and 4 days as inoculum, are shown in Table 4.

Homemade mature compost is structurally complex, with a long composting time, and its sources have significant regional variation, which may lead to poor reproducibility of test results across different laboratories. In contrast, commercial organic fermentation agents can be mass-produced, with uniform management of raw material sources and composting time, making the reproducibility of composting activity better. During the experiment, it was observed that during the early stages of vermiculite activation, unpleasant odors were produced. However, the odor weakened with the extension of the incubation time, which is consistent with the results shown in Table 3 and Table 4.

From Table 3 and Table 4, it can be seen that with the increase in pre-incubation time, the volatile solids content and the total organic carbon content of activated vermiculite show a significant downward trend. The CO₂ production in the blank group for the first 10 days decreases in varying degrees. This indicates that as the pre-incubation time increases, microorganisms utilize the easily degradable nutrients for extensive reproduction, leading to an increase in the microbial content (i.e., activity) of the inoculum, while the background organic matter content decreases. This helps avoid the “priming effect”.

Biodegradation Experiment Repeats	Percentage Biodegradation of Microcrystalline Cellulose on the 15 th Day, %			
	Pre-incubation 1d	Pre-incubation 2d	Pre-incubation 3d	Pre-incubation 4d
1st	64.9	21.5	14.2	15.5
2nd	27.9	41.9	40.4	38.7
3rd	29.1	36.9	50.2	29.6
Avg	40.6	33.4	34.9	27.9

Table 5. Summary of Percentage Biodegradation Results of Microcrystalline Cellulose over 15 Days

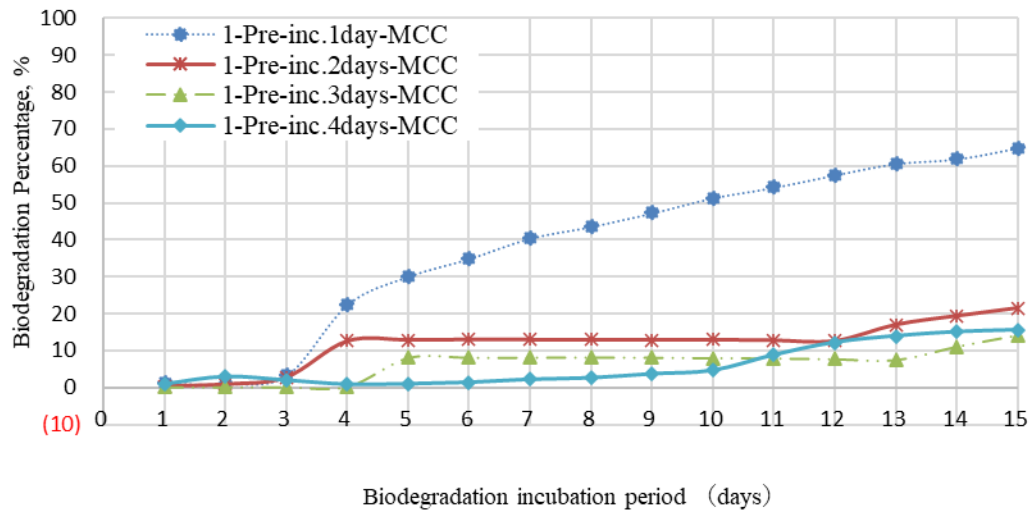


Fig. 3. Percentage Biodegradation-Time Curve of Microcrystalline Cellulose with the First Type of Activated Vermiculite Pre-incubated for 1 Day to 4 Days (1st Experiment)

Source: Author's drawing

When using activated vermiculite pre-incubated for 4 days as the inoculum, the CO₂ production in the blank group for the first 10 days is the lowest, but it still far exceeds the result validity requirements of ISO 14855-1:2012, which states that “the inoculum in the blank has produced more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids (mean values) after 10 days of incubation”. Based on the results of this study, the theoretical analysis of low volatile solid content in activated vermiculite, and the validity requirements of similar standards (GB/T 39951-2021), we believe that the experimental validity criterion “the inoculum in the blank has produced more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids (mean values) after 10 days of incubation” is not applicable to tests using activated vermiculite as a biodegradation inoculum. The validity criteria for biodegradation tests using compost and vermiculite as inoculum should be distinguished in ISO 14855-1:2012.

3.2. Percentage Biodegradation of Microcrystalline Cellulose

The three types of activated vermiculite, pre-incubated for 1 to 4 days, were used as inoculum for a 15-day biodegradation test of microcrystalline cellulose, with three repetitions for each type of vermiculite. The data on the percentage biodegradation of microcrystalline cellulose over 15 days of the experiment are shown in Table 5.

The average percentage biodegradation of microcrystalline cellulose for each pre-incubation time (1 to 4 days) was plotted as a time curve for each of the three experiments. Figures 3-5 show the percentage biodegradation-time curves of microcrystalline cellulose with activated vermiculite pre-incubated for 1 to 4 days. (Note: “MCC” stands for Microcrystalline Cellulose)

From Table 5 and Figures 3-5, it can be observed that the biodegradation results

of microcrystalline cellulose show a high degree of variability depending on the preparation method of activated vermiculite. When activated vermiculite prepared with compost extract was used as the inoculum, the highest percentage biodegradation of microcrystalline cellulose after 15 days was 64.9% when the vermiculite had been pre-incubated for 1 day, showing the highest degradation rate and optimal inoculum activity. As the pre-incubation time of vermiculite increased, a degradation trend of microcrystalline cellulose was observed. When the inoculum was prepared with vermiculite pre-incubated for 2 to 4 days, microcrystalline cellulose reached the biodegradation phase only after approximately 10 days, following a lag phase.

When activated vermiculite prepared using commercial organic fermentation agent culture solution replaced compost extract as the inoculum, the biodegradation of microcrystalline cellulose exhibited lower variability,

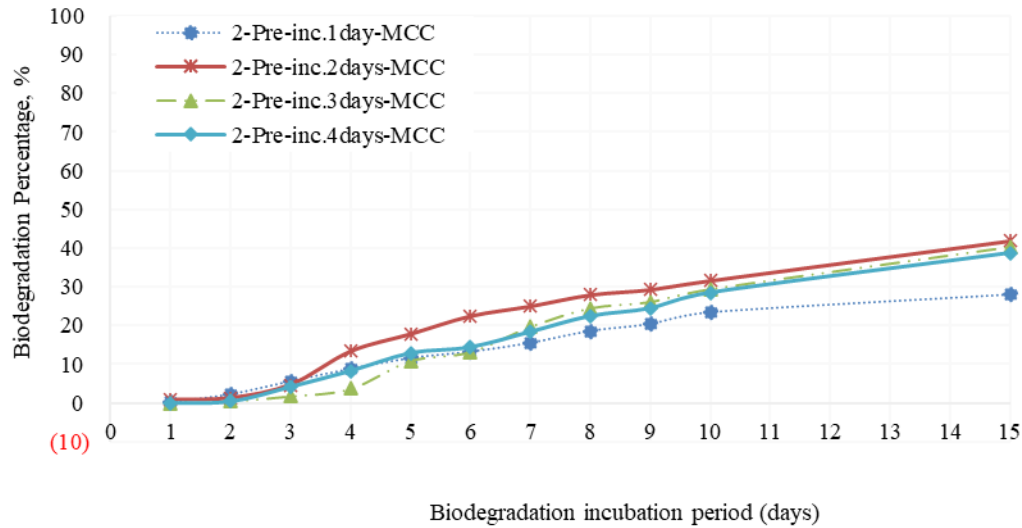


Fig. 4. Percentage Biodegradation-Time Curve of Microcrystalline Cellulose with the Second Type of Activated Vermiculite Pre-incubated for 1 to 4 Days (2nd Experiment)

Source: Author's drawing

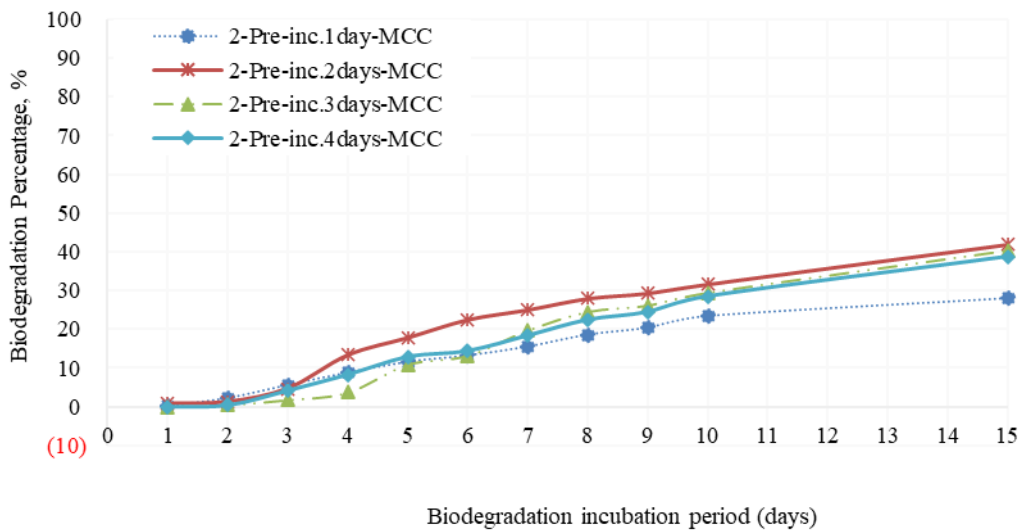


Fig. 5. Percentage Biodegradation-Time Curve of Microcrystalline Cellulose with the Third Type of Activated Vermiculite Pre-incubated for 1 to 4 Days (3rd Experiment)

Source: Author's drawing

with a favorable degradation trend. When vermiculite pre-incubated for 1 to 4 days was used as the inoculum, microcrystalline cellulose exited the lag phase and entered the biodegradation phase after approximately 5 days.

The average results of the second and third experiments showed that when activated vermiculite prepared with commercial organic fermentation agent culture solution was used as the inoculum, the highest percentage biodegradation of microcrystalline cellulose (45.3%) after 15 days occurred when the vermiculite

had been pre-incubated for 3 days, with the highest degradation rate and optimal inoculum activity.

Studies have shown that activated vermiculite as an inoculum may exhibit instability during the biodegradation, followed by a potential degradation plateau, requiring the addition of compost extract to improve the stability and sustainability of the experimental system[18].

This study only tested the biodegradation performance of microcrystalline cellulose

over 15 days, which is relatively short, with a single material. Further research is needed on the biodegradation performance of different materials over 180 days and on the sustainability and stability of activated vermiculite as an aerobic biodegradation inoculum.

4. Conclusion

This study investigated the effect of the preparation method and incubation time of activated vermiculite on the biodegradation performance of

microcrystalline cellulose. The results indicate the following:

1. With an increase in incubation time, the total organic carbon content and volatile solids content of activated vermiculite decreased, and the unpleasant odor generated during the incubation process weakened. When activated vermiculite is used as the inoculum for biodegradation testing, the “priming effect” can be reduced. The low volatile solids content of activated vermiculite and the organic components within it are easily utilized by microorganisms, leading to the production of more than 150 mg CO₂/g volatile solids (mean value) during the first 10 days of biodegradation testing. Therefore, the experimental validity criterion “the inoculum in the blank has produced more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids (mean values) after 10 days of incubation” is not applicable to tests using activated vermiculite as a biodegradation inoculum. ISO 14855-1:2012 should distinguish the validity criteria for biodegradation tests using compost and vermiculite inoculums.

2. The preparation method of activated vermiculite significantly affects the biodegradation results of microcrystalline cellulose. When the culture solution and vermiculite mixture prepared with compost extract was used, the highest percentage biodegradation of microcrystalline cellulose (64.9%) after 15 days was achieved when the vermiculite was pre-incubated for 1 day, showing the highest degradation rate and optimal inoculum activity. As the incubation time of vermiculite increased, the degradation trend of microcrystalline cellulose slowed down.

3. When activated vermiculite prepared with commercial organic fermentation agent culture solution replaced compost extract as the inoculum, the biodegradation of microcrystalline cellulose exhibited lower variability and a favorable degradation trend. When activated vermiculite pre-incubated for 1 to 4 days was used as the inoculum, microcrystalline cellulose exited the lag phase and entered the biodegradation

phase after approximately 5 days. The highest percentage biodegradation (45.3%) after 15 days was achieved when the vermiculite pre-incubated for 3 days was used as the inoculum, with the highest degradation rate and optimal inoculum activity.

This study provides an important reference for optimizing the preparation method and pre-incubation time of activated vermiculite to improve the effectiveness and reliability of biodegradation tests. By reasonably controlling the preparation method and the pre-incubation time of activated vermiculite, the stability of the inoculum and the biodegradation performance of the sample can be enhanced, providing more effective methods and technical support for the biodegradation treatment of municipal solid waste.

5. Main Error Control Explanations

1. Source of Compost, Medium, and Reagents Used: The conditions for the biodegradation of materials must include the presence of a large number of microorganisms. Mature compost is one of the determining factors for the source of microorganisms in biodegradation [19]. Its components, structure, age, and physicochemical properties are difficult to control, and the standard regulations are relatively general. Therefore, using different composts for testing can result in large short-term errors in percentage biodegradations. Laboratories should establish stable methods or procurement channels for preparing mature compost and use compliant mature compost. Different sources and batches of media and reagents may affect microbial growth, thus influencing the results of biodegradation. Therefore, qualified media and reagents should be used and detailed information recorded during the experiment to ensure the stability and reproducibility of the results.

2. Experimental System: The biodegradation system is complex and requires a long test period. Timely attention should be paid to the airtightness

of the system, the suitability of microbial growth conditions (temperature, humidity, pH, nutrients etc.) in the composting vessel, and the stability and accuracy of the equipment used during the test process.

3. CO₂ Measurement Method: CO₂ can be measured directly in the exhaust gas of the composting vessel using a continuous infrared analyser or gas chromatograph, or it can be completely absorbed by an alkaline solution and the dissolved inorganic carbon measured using titration or total organic carbon analyser methods to calculate CO₂ production. This study used an alkaline solution to completely absorb the CO₂ released from the composting vessel, and the amount of CO₂ produced was calculated using a hydrochloric acid standard titration solution to measure the dissolved inorganic carbon. Different analytical methods vary in cost, operational convenience, and precision.

By controlling the above error factors, the accuracy and reliability of biodegradation can be improved, thereby enhancing the operability of biodegradation and providing a more solid technical foundation for the development of biodegradation.

Conflicts of Interest

The authors declare no conflict of interest.

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References

- ISO. (2012). ISO 14855-1:2012 - Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions -- Method by analysis of evolved carbon dioxide -- Part 1: General method.
- Hu, G.-S., & Li, Z.-Y. Application of natural vermiculite in wastewater treatment. *Journal of Beijing Technology and Business University (Natural Science Edition)*. 2006; 24(3), 13-16. <https://doi.org/10.3969/j.issn.1671-1513.2006.03.004>
- Wan, Y., Fan, Y., Dan, J., Hong, C., Yang, S., & Yu, F. A review of recent advances in two-dimensional natural clay vermiculite-based nanomaterials. *Materials Research Express*. 2019; 6(10), 102002. <https://doi.org/10.1088/2053-1591/ab3c9e>
- Bellia G, Tosin M, Degli-Innocenti F. The test method of composting in vermiculite is unaffected by the priming effect. *Polymer Degradation & Stability*. 2000; 69(1):113-120. [https://doi.org/10.1016/S0141-3910\(00\)00048-3](https://doi.org/10.1016/S0141-3910(00)00048-3).
- Wei, X.-X., Zhang, M., Li, Q.-M., Liu, W.-L., Shen, S.-Y., & Dong, H.-F. Review of biodegradable plastics standards. *Standard Science*. 2016; 6(11), 58-64. <https://doi.org/10.3969/j.issn.1674-5698.2016.11.013>
- Loperena L , Ferrari M D ,Verónica Saravia,et al. Performance of a commercial inoculum for the aerobic biodegradation of a high fat content dairy wastewater. *Bioresource Technology*. 2007; 98(5):1045-1051. <https://doi.org/10.1016/j.biortech.2006.04.030>.
- Lora P O , Sjlund M , Tracol C ,et al. Adaptation of an inoculum to 2,4,6-trichlorophenol biodegradation in an activated-sludge bioreactor. *Water Science and Technology*. 2000; 42(5-6):179-183.
- Mezzanotte V, Bertani R, Degli Innocenti F, et al. Influence of inocula on the results of biodegradation tests. *Polymer degradation and stability*. 2005; 87(1): 51-56. <https://doi.org/10.1016/j.polyimdegstab.2004.06.009>
- Šašinková D, Serbruyns L, Julinová M, et al. Evaluation of the biodegradation of polymeric materials in the freshwater environment—An attempt to prolong and accelerate the biodegradation experiment. *Polymer Degradation and Stability*. 2022; 203: 110085. <https://doi.org/10.1016/j.polyimdegstab.2022.110085>
- Zhang G, Zhao Z, Yin X A, et al. Impacts of biochars on bacterial community shifts and biodegradation of antibiotics in an agricultural soil during short-term incubation. *Science of The Total Environment*. 2021; 771(6): 144751. <https://doi.org/10.1016/j.scitotenv.2020.144751>
- Ecosphere Resilience Research Center, Faculty of Applied Sciences & University of Sri Jayewardenepura. Biochar from municipal solid waste for resource recovery and pollution remediation. *Environmental Chemistry Letters*. 2019; 17(3), 1225-1235. <https://doi.org/10.1007/s10311-019-00866-0>
- Lanka S, Pydipalli M. Reduction of organic load from palm oil mill effluent (POME) using selected fungal strains isolated from POME dump sites. *African Journal of Biotechnology*. 2018; 17(36): 1138-1145. <https://doi.org/10.5897/AJB2016.15821>
- Nam K, Alexander M. Relationship between biodegradation rate and percentage of a compound that becomes sequestered in soil. *Soil Biology and Biochemistry*. 2001; 33(6): 787-792. [https://doi.org/10.1016/S0038-0717\(00\)00226-1](https://doi.org/10.1016/S0038-0717(00)00226-1)
- Alexander M, Scow K M. Kinetics of biodegradation in soil. Reactions and movement of organic chemicals in soils. 1989; 22: 243-269. <https://doi.org/10.2136/sssaspecpub22.c10>
- Waisner S, Hansen L, Fredrickson H, et al. Biodegradation of RDX within soil-water slurries using a combination of differing redox incubation conditions. *Journal of hazardous materials*. 2002; 95(1-2): 91-106. [https://doi.org/10.1016/S0304-3894\(02\)00052-3](https://doi.org/10.1016/S0304-3894(02)00052-3)
- Paixão S M, Sáágua M C, Tenreiro R, et al. Biodegradability testing using standardized microbial communities as inoculum. *Environmental Toxicology: An International Journal*. 2006; 21(2): 131-140. <https://doi.org/10.1002/tox.20165>
- Choi, C., Yoo, J.-C., Yang, S.-M., Lee, C.-G., Lee, S.-E., & Kang, S.-G. Characteristics of biodegradation under composting and vermiculite conditions. *Journal of Korea Technical Association of the Pulp and Paper Industry*. 2016; 48(1), 127-133. <https://doi.org/10.7584/ktappi.2016.48.1.127>
- Zhu, A.-P., Deng, H.-Y., Han, H., Zhao, H.-L., & Wu, Y.-W. Biodegradability of degradable plastic film bags comparison of composting method and vermiculite method. *Plastics Science and Technology*. 2022; 50(11), 97-103. <https://doi.org/10.15925/j.cnki.issn1005-3360.2022.11.018>
- Bamdad, H., Papari, S., Lazarovits, G., & Berruti, F. Soil amendments for sustainable agriculture: Microbial organic fertilizers. *Soil Use and Management*. 2021; 37(1), 94-120. <https://doi.org/10.1111/sum.12762>