Research on a New Green and Low Carbon Degradation Test Scheme

Jinfei Huang*, Kaiyue Liu, Xin Zhang, Qin Chou

Zhejiang Light Industry Products Inspection and Research Institute, Hangzhou, China

Abstract: With the rapid development of biodegradable plastics, the inspection and detection of degradable plastics is becoming more and more important, GB/T 19277.1 is the most commonly used degradable plastic compost detection method. However, there are some problems in this method, such as large differences among different decomposed composts, unstable compost between batches, subjective factors interfering greatly with the experimental results, and the nutrient composition of compost culture solution in vermiculite scheme is not clearly defined. In this paper, the effects of compost and vermiculite inoculants were compared by experiments. In addition, the formula of vermiculite culture solution was optimized, and the experimental results showed that the determination efficiency of product degradation rate could be effectively improved by adding specific microbial bacteria (potassium bacteria, bacillus group, lactic acid bacteria group, etc.). At the same time, the optimized culture solution formula can better control the nutrient composition, solve the problems of batch to batch instability, artificial factors, and improve the reproducibility and repeatability of detection. This is of great significance for promoting the application and development of biodegradable plastics testing standards. Through these improvements, the performance of biodegradable plastics can be more effectively assessed, the rational use of environmentally friendly materials and the control of plastic pollution can be promoted, the application of degradable materials in packaging materials, agriculture, medical care, textile and clothing, new energy and other fields can be promoted, the green transformation of the industry can also help "carbon peak" and "carbon neutral" realization.

1. Overview

With the promotion of policies such as the comprehensive promotion of plastic pollution control and the ban on single-use plastic products, biodegradable plastics have developed rapidly. The biodegradation of plastics is mainly carried out by microorganisms, including bacteria, fungi and certain algae [1,2], which secrete enzymes to break down the polymers in plastics, converting them into smaller molecules and eventually into environmentally friendly substances such as water, carbon dioxide and biomass [9,10].

According to the requirements of standard EN13432, the degradation of plastics is generally assessed from four aspects: first, disintegration performance, final fragmentation composting test, test material specimens and biological waste composting after 3 months, the quality of the test material residue must be less than 10% of the original quality; The second is biodegradable performance, which stipulates that more than 90% of degradable compost materials must be eventually converted into carbon dioxide, water and minerals within a maximum of 6 months under aerobic compost conditions; Third, the ecotoxicological properties, compost residues can not have a negative impact on the biological growth process; The content of heavy metals in compost residues is within the prescribed range, and must not have a toxicological effect on organisms. According to different sources of microorganisms, biodegradation tests are divided into three types: natural environment test, environmental microbial test and specific microbial test [11-14]. The current standard plastic biodegradation method can be divided into freshwater aerobic environment (GB/T 19276.2), seawater aerobic environment (IS0 22404), compost aerobic environment (GB/T 19277), soil aerobic environment (GB/T) according to the different oxygen conditions and degradation media 22047), aqueous medium anaerobic digestion environment (ISO 14853), controlled sludge anaerobic digestion environment (ISO 13975), etc. The measurement methods mainly include the measurement of CO2 production in the degradation process and the measurement of oxygen demand in the closed respiration meter. The mainstream international standards, including the American standard ASTM D5338, the European standard EN 14995 and the Chinese standard GB/T 19277, are used to decompose materials under aerobic composting conditions, and the method of measuring CO2 production is used to calculate the degradation rate [15,16].

GB/T 19277.1 specifies the method for determining the final aerobic biodecomposition capacity of materials under controlled compost conditions, which is also the most commonly used test method at present, and the method specifies two different inoculants of decomposed

* Corresponding author: hjf_fytest@163.com

compost and vermiculite. However, decomposed compost has the following problems: 1) Decomposed compost is a heterogeneous and extremely complex material, and it is difficult to quantify the polymer remaining in the solid bed at the end of the test; It is also difficult to determine the small molecules that may be released into the solid bed during polymer degradation, and it is difficult to evaluate biomass, so it is also difficult to calculate the complete carbon balance. 2) A large amount of organic matter mixed into the decomposed compost will suffer polymer-induced degradation, which will affect the determination of biodecomposition capacity. 3) The composition of microorganisms, minerals and organic matter in different compost is very different, and the compost from batch to batch is unstable [5-7]; Composting requires manual intervention, and subjective factors interfere greatly with the experimental results. Although vermiculite instead of decomposed compost as a solid bed medium can solve some problems caused by the complex composition of decomposed compost, there are still some defects: 1) compost extract is required, and there are also problems of instability between different compost batches; 2) The nutrient composition in the compost culture solution is not clearly defined, and there is no unified standard leading to large differences between different laboratories.

In this paper, the effects of compost and vermiculite inoculants in the standard were compared by a large number of experiments. It was innovatively proposed that the compost extract was prepared with despotic bacteria powder (including potassium bacteria, bacillus group, lactic acid bacteria group, etc.), which solved the problem of batch instability caused by different decomposed compost or decomposed compost extract liquid. At the same time, the formula of vermiculite culture medium was optimized, and the microbial composition and nutrients of vermiculite culture medium were fixed, which solved the problems of batch to batch instability and the influence of artificial factors, and improved the reproducibility and repeatability of detection, which was conducive to the promotion of test standards.

2. Experimental part

2.1 Experimental raw materials and equipment

Table 1: Experimental raw materials.

Name	Size	manufacturer
Compost	/	Shanghai Boshitong Electric Appliance Co., LTD
microcrystalline cellulose	/	Sinopharm Group Chemical reagent Co., LTD
carbamide	AR	Sinopharm Group Chemical reagent Co., LTD
corn starch	98%	Shanghai source leaf biological biology Co., LTD
H3BO3	AR	Jiangsu Qiangsheng Functional Chemical Co., LTD
KI	AR	Jiangsu Qiangsheng Functional Chemical Co., LTD
FeCl3	AR	MacLin
MnSO4	AR	Shanghai Lingfeng Chemical Reagent Co., LTD
(NH4)6Mo7O24	AR	MacLin
FeSO4	AR	Shanghai Lingfeng Chemical Reagent Co., LTD
KH2PO4	AR	MacLin
MgSO4	AR	Shanghai Lingfeng Chemical Reagent Co., LTD
CaCl2	AR	Jiangsu Qiangsheng Functional Chemical Co., LTD
NaCl	AR	Jiangsu Qiangsheng Functional Chemical Co., LTD

The composition of compost, reference and sample is shown in Table 2.

Table 2: Composition of compost, reference and sample.

	Total dry solids content %	Volatile solids content %	moisture content%	total content of organic carbon%
Compost 1	54.89	10.23	45.11	5.81
Compost 2	50.18	12.12	49.82	5.22
Compost 3	53.25	11.37	46.75	5.46
vermiculite	50.47	14.83	49.53	1.34
Reference (microcrystalline cellulose)	96.94	96.48	3.06	39.64
Straw 1	99.70	69.78	0.30	31.52
Straw 2	99.91	69.43	0.09	44.50
Membrane Bag 1	99.76	69.16	0.24	39.27
Membrane Bag 2	99.81	73.58	0.19	48.44

Table 3: Experimental equipment.

Device name	Model	manufacturer
Automatic compost degradation tester	ZHJJ-1	Suzhou Kangle Hui Medical Technology Co., LTD
analytical balance	FA1204 B	Shanghai Precision Scientific Instrument Co., LTD
electrothermal blowing dry box	DHG-9243A	Sand eagle instrument
Box Muffle furnace	SX2-5-12N	Shanghai Yiheng Technology Co., LTD
TOC	multiN/ C3100	analytikjena
high speed centrifuge	5430	eppendorf
ph-meter	PHS-3C	Shanghai Precision Scientific Instrument Co., LTD

2.2 Experimental methods

2.2.1 Composting

According to GB/T 19277.1-2011 test, blank results and reference results were respectively tested in the composting experiment. The volume of the compost container was 2.8L and the wet weight of the compost inoculum was 680g. In the reference experiment, the dry weight ratio of the inoculant to the reference material was 6:1. The inoculant and the reference material were thoroughly mixed and then put into the compost container. The experimental temperature was 58±2℃.

2.2.2 Vermiculite

Instead of compost, vermiculite is used in the culture medium containing organic matter, inorganic matter and decomposed compost, and the composition of the culture medium is configured according to the ratio of Table 4 to Table 6 (same as Table 1 to Table 3 in GB/T 19277.1-2011).

The compost extract was prepared by adding decomposed compost to 20%(mass/volume) of deionized water, mixing and placing for 30min, and then running with centrifuge at 1000r/min for 15 min to extract the supernatant[3].

The alternative formula of compost extract is prepared with special bactericide. 50g bactericide, 50g sucrose, 6 capsules of vitamin C6, 2 capsules of vitamin B1, B2 and B6 are dissolved in 5L distilled water and then filtered.

The alternative formula of compost extract is prepared by using special bactericide and adding certain nutrients.

Table 4: Components in a 1-liter inoculation solution.

ingredient	Mineral solution	NB*	carbamide	corn starch	cellulose	Compost extract
content	500mL	13g	5.8g	20g	20g	500mL

Table 5: Components in a 1 liter mineral solution.

ingredient	KH_2PO_4	$MgSO_4$	$CaCl_2$ (10% solution)	NaCl (10% solution)	trace element solution, See Table 6
content	1g	0.5g	1mL	1mL	1mL

Table 6: Components in a 1 liter solution of trace elements.

ingredient	H_3BO_3	KI	$FeCl_3$	$MnSO_4$	$(NH_4)_6Mo_7O_{24}$	$FeSO_4$
content	500mg	100mg	200mg	400mg	200mg	400mg

Activation of vermiculite: the ratio of vermiculite to culture medium is 1:3 (mass/volume), and it is placed in an activation reactor and activated at 50±2℃ for 3-4 days to ensure uniform aeration.

Composting of vermiculite: Put the activated vermiculite and the sample or reference into the compost container at 4:1 dry weight. The compost container is 3L, with 200g vermiculite (dry weight) and 50g test or reference material (dry weight), and the volatile solids content of activated vermiculite is 15%.

2.2.3 Data technology and analysis

Carbon dioxide emission (ThCO2) and biodecomposition percentage Dt (%) were calculated according to the calculation formula in Part 9 of standard GB/T 19277.1-2011.

$$ThCO2=M_{TOT} \times C_{TOT} \times 44/12 \quad (1)$$

In formula (1):

MTOT, total dry solid weight of the test material (g),

CTOT, the ratio of total organic carbon to total dry solids in the test material (g/g).

$$Dt(\%)=[(CO2)T-(CO2)B]/ThCO2 \times 100 \quad (2)$$

In formula (2)

(CO2) T, cumulative actual release of CO2 from the sample mixture, (g/ container),

(CO2) B, the cumulative amount of CO2 actually released from the blank container, (g/ container).

2.3 Results and discussion

2.3.1 Composting

Table 7 shows the results of blank test and reference test for different compost inoculants. It can be seen that there are great differences among different compost inoculants, and the success of the experiment largely depends on the properties of inoculants. Compared with DKB-3 and DKB-4, the CO2 release of inoculant compost 2 was too high at the beginning, and after aeration for 3 days, it could meet the test requirements, but this increased the workload of inspectors. DKB-5 and DKB-6 are operated by different personnel, and the CO2 release of the fertilizer compost 3 is relatively low. During the detection process, the inspectors oscillate the compost container, add water, and adjust the oxygen amount according to the real-time detection data. Subjective factors have great interference with the experimental results, which requires high experience and professionalism of the inspectors.

Table 7: shows the results of blank test and reference test for different compost inoculants.

blank experiment					Reference experiment		
Experimental lot number	inoculum	Total dry solids of inoculumg	Volatile solids content g	CO2 release from empty containers in the first 10 days mgCO2/g volatile solids	Experimental lot number	Reference material total dry solidsg	After 45 days, the biological decomposition rate of the reference material was %
DKB-1	compost 1	373	70	114	DCB-1	62	86.66
DKB-2	compost 1	373	70	116	DCB-2	62	90.24
DKB-3	compost 2	341	82	320	DCB-3	57	/
DKB-4	compost 2 (After aeration)	341	82	130	DCB-4	57	88.89
DKB-5	compost 3	362	77	55	DCB-5	60	73.14
DKB-6	compost 3	362	77	40	DCB-6	60	67.22

Table 8: Results of blank test and reference test of vermiculite inoculants.

blank test				Reference experiment	
Experimental lot number	NB*	Compost extract	CO2 release from empty containers in the first 10 days mgCO2/g volatile solids	Experimental lot number	After 45 days, the biological decomposition rate of the reference material was %
ZKB-1	/	Compost 1 extract	25	ZCB-1	/
ZKB-2	peptone	Compost 1 extract	52	ZCB-2	91.20
ZKB-3	nutrient broth	Compost 1 extract	85	ZCB-3	91.55
ZKB-4	nutrient broth	Compost 2 extract	92	ZCB-4	90.84
ZKB-5	nutrient broth	Compost 3 extract	46	ZCB-5	/

Table 9: Experimental results of alternative formula of compost extract.

Serial number	Formula composition (5L)				Blank test		Reference experiment	
	bactericide /g	Cane sugar /g	Vitamin C / tablet	B vitamin complex/tablet	Experimental lot number	CO2 release from empty containers in the first 10 days mgCO2/g volatile solids	Experimental lot number	After 45 days, the biological decomposition rate of the reference material was %
1	50	0			PKB-1	35.12	PCB-1	/
2	50	25			PKB-2	42.69	PCB-2	/
3	50	50			PKB-3	56.01	PCB-3	60.48
4	50	75			PKB-4	65.47	PCB-4	61.54
5	50	100			PKB-5	63.00	PCB-5	65.84
6	50	75	2		PKB-6	68.54	PCB-6	62.13
7	50	75	4		PKB-7	68.87	PCB-7	66.25
8	50	75	6		PKB-8	73.65	PCB-8	69.28
9	50	75	8		PKB-9	72.94	PCB-9	68.35
10	50	75	6	2	PKB-10	90.12	PCB-10	69.44
11	50	75	6	4	PKB-11	102.00	PCB-11	70.23
12	50	75	6	6	PKB-12	110.26	PCB-12	74.02
13	50	75	6	8	PKB-13	123.56	PCB-13	72.43

Table 10: Blank, reference and experimental material results of vermiculite optimization formula.

Blank test		Reference experiment		Sample experiment		
Experimental lot number	CO2 release from empty containers in the first 10 days mgCO2/g volatile solids	Experimental lot number	After 45 days, the biological decomposition rate of the reference material was %	Experimental lot number	Decomposition time/day	Biodecomposition rate %
HZKB-1	82.32	HZCB-1	70.29	Straw 1	62	96.13
HZKB-2	118.30	HZCB-2	74.61	Straw 2	70	91.23
HZKB-3	82.32	HZCB-3	70.29	Membrane Bag 1	76	90.43
HZKB-4	115.82	HZCB-4	71.55	Membrane Bag 2	70	91.63

2.3.2 Vermiculite

Table 8 shows the results of blank test and reference test for vermiculite inoculants. It can be seen that the choice of nutrient composition and compost extract have a decisive impact on the experimental results. The nutrient broth was selected as the nutrient component by experiment and comparison. At the same time, the instability between batches of compost extract will also lead to the failure of the experiment.

2.3.3 Optimization formula of vermiculite

In order to eliminate the fluctuation between batches of compost inoculants and compost extract, and to solve the uncontrollability caused by human factors during the experiment, this experiment is committed to developing a stable and easy to operate inoculant formula to help promote the standard. After a lot of experiments, we finally used vermiculite instead of compost as the inoculum, nutrient composition selected nutrient broth, and the compost extract was prepared with autoclavus powder (including potassium bacteria, bacillus group, lactic acid bacteria group, yeast group, 5406 bacteria group, etc.) + sucrose + vitamins.

The main function of compost extract is to provide bacteria and other microorganisms, and the compost extract also includes nitrogen, phosphorus, potassium and organic matter required for the growth of some microorganisms. The main role of nutrients is to provide nutrients needed for bacterial growth. Therefore, fixing the composition of compost extract, including microbial

species, content and organic matter and inorganic matter required for growth, is an effective means to solve the test difficulties. Compost 1-extract solution and compost 2-extract solution were analyzed and tested (including microorganisms, minerals, nutrients, etc.), and the alternative formula of compost extract solution was finally determined after a lot of experiments. As shown in Table 9, the CO2 release of the 5L solution cannot meet the requirements when only the bacteriostatic agent is added, and the addition of sucrose provides organic matter for the microorganisms, which significantly increases the CO2 release of the blank container in the first 10 days. When the amount of sucrose added reaches 75g, the CO2 release reaches the optimal level, and the continued addition has no obvious effect. Continued addition of vitamin C can further promote the decomposition of microorganisms on plastics, further increase the CO2 release of the blank container, and at the same time, the decomposition rate of the reference material is also increased[8]. The addition of B complex vitamin can further promote the decomposition ability of microorganisms to the material. After experimental comparison, 50g bactericide +75g sucrose +6 tablets vitamin C+6 tablets vitamin B complex +5L water was selected as the alternative formula of compost extract. In order to further verify the stability of the replacement formula, experiments were carried out, and the results were shown in Table 10. The experimental results between batches of the formula were stable, easy to operate, and the success rate reached 100%[17,18].

3 Conclusion

There is a large difference between different inoculants of decomposed compost, and the success of the test largely depends on the performance of inoculants. In addition, during the test process, inspectors need to oscillate the compost container, add water and adjust the amount of oxygen according to the real-time test data. Subjective factors have great interference with the experimental results, which requires high experience and professionalism of inspectors. The vermiculite program does not specify the nutritional composition in the standard, which increases the workload for inspectors to determine the appropriate nutritional composition after a large number of experimental data, and will bring differences between different testing institutions. In addition, because the compost extract in the vermiculite scheme comes from the decomposed compost, there are still differences in various microorganisms, organic matter and inorganic substances in different decomposed composts.

This paper innovatively prepared compost extract with despotic bacteria powder, optimized the composition of the culture medium, provided a suitable growth environment for microorganisms, improved the activity and stability of microorganisms, and improved the detection efficiency. In the optimized culture medium, nutrient broth, sucrose and corn starch together serve as carbon source, which is the basis of microbial cell material and the skeleton of organic macromolecules, while providing energy required for life activities. B-complex vitamins are small molecular nutrients required for microbial growth and play a crucial role in their growth and reproduction. For example, vitamin B9 (folate) is needed for cell growth, amino acid metabolism, red and white blood cell formation, and proper cell division. The cobalt in vitamin B12 is involved in the synthesis of folate and the synthesis of fatty acids. Vitamin C (ascorbic acid), which has a reductive effect, protects cells from the destruction of oxygen free radicals, can inhibit apoptosis in cell culture and promote the formation of stem cells[4]. At the same time, compared with the complicated operation of compost extract, the scheme used in this paper to prepare fertilizer extract is more simple, more stable, reproducible and stable, and the experimental success rate can reach 100%.

This is of great significance for promoting the application and development of biodegradable plastics testing standards. Through these improvements, the performance of biodegradable plastics can be evaluated more effectively, and it also provides a reference for the development of rapid detection methods for biodegradation. This study is helpful to develop more efficient culture solution formulations, increase degradation rate, reduce detection cycle and detection cost. At the same time, it can also promote the rational use of environmentally friendly materials and the control of plastic pollution, promote the application of degradable materials in packaging materials, agriculture, medical care, textile and clothing, new energy and other fields, help the green transformation of the industry, and help the realization of "carbon peak" and "carbon neutrality".

Acknowledgments

This research is supported by the Research Fund of Zhejiang Provincial Administration of Market Supervision (Serial number: CY2023214).

References

1. Chen Long, Cheng Hao, Wang Yi et al. Starch based degradable materials and their application in food industry [J]. Chinese Journal of Food Science, 22 (1), 2022.
2. Guo Bo, Yang Zhenxing, He Wenqing, Liu Jialei. Application effect and existing problems of biodegradable plastic film [J]. Chinese Journal of Agrometeorology, Volume 44, Issue 11, 2023.
3. Yuan Zhitong, Li Wenting, Li Qiwei. Research and application progress of plant fiber reinforced biodegradable plastic microcellular foam composite [J]. Plastics Industry, Volume 51, number 6, 2023.
4. Liu Jinhua, Fang Cunjiong, Jiang Heyang et al. Progress in preparation of biodegradable plastic foam by supercritical fluid foaming [J]. Engineering Plastics Applications, Volume 52, Number 4, 2024.
5. Su Feng, Zhang Hao, Liu Yuanrong et al. Research progress and application effect of biodegradable plastic film [J]. Plastic Science and Technology, Volume 51, Issue 4, 2023.

6. Wang Yunbing, Guo Gaoyang. Application of biodegradable polymer materials in interventional apparatus for cardiovascular therapy [J]. Journal of Tongji University (Natural Science Edition), Volume 51, Issue 11, 2023.
7. ZHANG K, HAMIDIAN A H, TUBIC A, et al. Understanding plastic degradation and microplastic formation in the environment: A review [J]. Environmental Pollution, 2021, 274: 116554.
8. LI Xinyue, Liu Zhuomiao, Xue Runze et al. Biodegradation of typical plastics and its mechanism [J]. Chinese Science Bulletin, 2019, 66(20): 2573-2589.
9. LIM B K H, THIAN E S. Biodegradation of polymers in managing plastic waste - A review [J]. Science of the Total Environment, 2022, 813: 151880.
10. KOTOVA I B, TAKTAROVA Y V, TSAVKELOVA E A, et al. Microbial degradation of plastics and approaches to make it more efficient [J]. Microbiology, 2021, 90(6): 671-701.
11. Zhou Jie, Su Tianyuan, Jiang Min, Qi Qingsheng. Preface to Special issue on Biodegradation and Transformation of plastics [J]. Chinese Journal of Bioengineering, 2023, 39(5): 1861-1866.
12. Zhao Wei, Li Yu, Zhang Wei et al. Comparison of detection methods for biodegradability of degradable materials [J]. Journal of East China Normal University (Natural Science Edition), 2023, Vol.2023, lssue (6):158-167.
13. Xu Wei, ZHAO Xinting, Yuli Wu, Wang Li, Guo Chunyun. Development status and testing methods of biodegradable plastics [J]. Aging and Application of Synthetic Materials, Volume 52, Number 4, 2023.
14. HUANG Kaisheng, Zhao Yan, Zhang Xihui, et al. Research progress of biodegradability detection methods for polymer materials [J]. Chinese Journal of Testing, 2019, 48(10): 16-24.
15. WANG Yuanbo, Zheng Shiyue, Wang Fan, et al. Research progress of detection methods for biodegradation of plastics [J]. Chinese Journal of Biological Engineering, 2023, 39(5): 1889-1911.
16. Diao Xiaoqian, Weng Yunxuan, Fu Ye, Zhou Yingxin. Review on application and performance evaluation methods of biodegradable plastics [J]. China Plastics, Volume 35, Issue 8, August 2021.
17. ZHAO Wei, LI Yu, Zhang Wei, ZHU Kehua, ZHOU Ke. Comparison of detection methods for biodegradability of degradable materials [J]. Journal of East China Normal University (Natural Science Edition), No.6, 2023.
18. Zhu Aiping, Deng Hongying, Han Hong, Zhao Hongliang, WU Yuwei. Comparative study on biodegradability of degradable plastic film bags by composting method and vermiculite method [J]. Plastic Science and Technology, Volume 50, number 11, 2022.