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Material Behaviour

Biodegradation behaviors of thermoplastic starch (TPS) and thermoplastic dialdehyde starch (TPDAS) under controlled composting conditions

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ABSTRACT

In this paper, the degradability of thermoplastic starch (TPS) and thermoplastic dialdehyde starch (TPDAS) under controlled composting conditions was investigated and a manual set-up was built according to ISO 14855 for this purpose. Chemical modification of starch can have a major impact on the biodegradation rate and final biodegradation percentage. The TPS degraded faster than TPDAS under controlled composting conditions. For the TPDAS, the degradation rate and final biodegradation percentage were closely related to the degree of oxidation of dialdehyde starch (DAS). The possible reason was also discussed. The biodegradation process of TPS and TPDAS exhibited three phases or stages with different degradation speeds. The biodegradation in the first phase was slow, accelerated in the second phase and leveled off in the third phase. Three kinds of actinomycete were isolated from the compost and identified as micromonospora, nocardia and streptomycete, which were degrading microorganisms of the starch tested. The abilities of these strains to decompose the TPS and TPDAS were also discussed.

1. Introduction

The large use of synthetic nondegradable polymer and plastic materials produced from petrochemicals has led to serious environmental pollution [1,2]. One answer to this problem is to use biodegradable polymers, and it is necessary to know their biodegradability in natural environments in order to extend their use. In the determination of biodegradation of polymer, composting has been accepted worldwide as one of the most promising technologies for the management of plastic waste. Due to the high microbial diversity of compost, it shows good potential degradation capacity for polymer materials [1].

Starch is a renewable and biodegradable macromolecule, and it can be one alternative material for replacement of many petroleum-based products. Unlike other synthetic thermoplastic polymers, starch can be processed into a thermoplastic material only in the presence of plasticizers and under the influence of heat and shear [3]. However, the major drawback of thermoplastic starch (TPS) is poor water resistance, which limits its application. To overcome this drawback, an effective method is chemical modification

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including esterification [4–11], etherization [12] or oxidation [13–15], etc.

The most valuable oxidized starch is dialdehyde starch (DAS). Dialdehyde starch (DAS) with different carbonyl contents was synthesized by our research group using sodium periodate as an oxidant [16]. It was found that when 25% glycerol was added into DAS, it had thermoplastic properties (designated TPDAS) and can be thermally processed into sheets with desirable properties. However, the biodegradable properties of TPDAS under controlled composting condition had not been investigated in detail, and it is necessary to study the biodegradation behavior of TPDAS and TPS in natural environments.

The isolation and screening of modified starch-degrading microorganisms from the compost are also good for further understanding the biodegradation of starch and its derivatives, and enlarging the application of biotechnology on biodegradable plastics. Therefore, in this paper a manual set-up has been built to appraise the biodegradation of TPS and TPDAS with different carbonyl content in controlled composting conditions according to ISO 14855. The effect of the degree of oxidation of TPDAS on the biodegradation was considered. In addition, the isolation and screening of modified starch-degrading microorganisms from the compost are also discussed.

2. Methods and materials

2.1. Principle of the test method

The biodegradability test by composting on a laboratory scale was conducted on the basis of ISO 14855 (1999) [17]: "Determination of the ultimate Aerobic Biodegradability and Disintegration of Plastic under Controlled Composting conditions".

In this test, mature compost was used as inoculum, which was derived from the organic fraction of municipal solid waste. A mixture of mature compost and the test material was introduced into a closed vessel and incubated under optimal oxygen, temperature and moisture conditions for a test period of, normally, 45 days. In parallel, blank vessels with compost only and controls with compost and a reference substance were investigated.

During the aerobic biodegradation of organic materials, carbon dioxide and water were the final decomposition products. The amount of carbon dioxide produced deriving from the biodegradation of the test material was measured, compared to the theoretical maximal amount and recorded as biodegradation percentage. The process of biodegradation was shown in a curve where carbon dioxide production was plotted as a function of time.

2.2. The test system to determine carbon dioxide evolved

The new test system was composed of three parts: I) a pressurized air control system, which was a pretreatment to remove the carbon dioxide from compressed air as a carrier gas to control aeration rate and to prepare saturated aqueous vapor; II) a composting system containing a mixture of test material and inoculum; III) a carbon dioxide trap system. The schematic diagram of the set-up is illustrated in Fig. 1.

2.3. Compost

The mature compost was obtained from a composting plant in Beijing. The compost had the following basic properties: total solids (TS, %) 49%; volatile solids (VS, % on TS) 28.38%; pH 7.2; C/N ratio 14.1.

2.4. Test materials

2.4.1. Raw material and reference substance

Corn starch (with 11.6 wt% moisture) was obtained from LangFang Starch Company (technical grade, LangFang, Heibei, China). Microcrystalline cellulose was used as reference substance in this biodegradation experiment

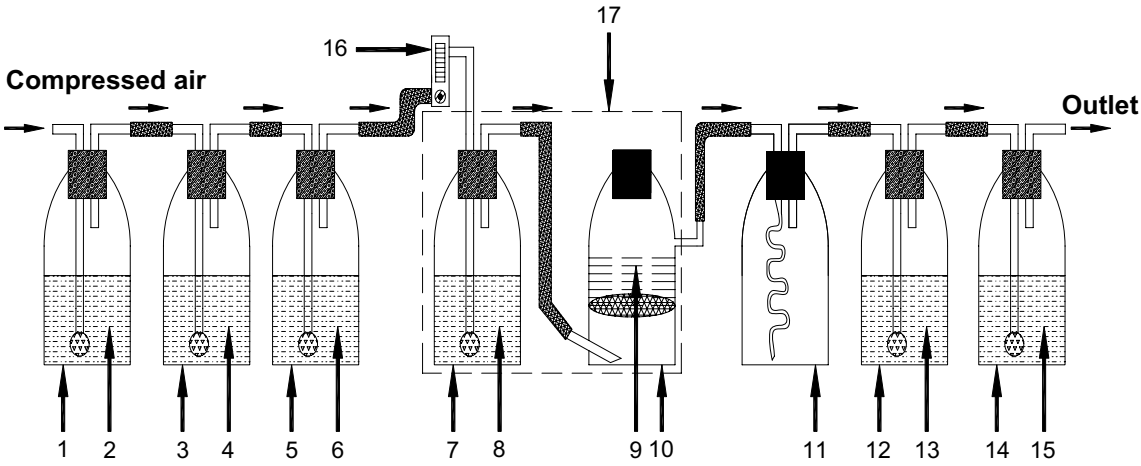

Fig. 1. The test system of biodegradation for polymer materials. **1,3,5**: carbon dioxide trap; **2,4**: 12 mol/L NaOH; **6**: 0.3 mol/L Ba(OH)2; 7: humidifier; **8**: water; **9**: mixture of compost, test material and sea sand; **10**: composting vessel; **11**: cooling unit; **12,14**: carbon dioxide trap system; **13,15**: 0.2 mol/L NaOH; **16**: flow meter with controller; **17**: thermostat.

(thin layer chromatography grade) with particle size of $<20\ \mu m$.

2.4.2. Preparation of dialdehyde starch (DAS) and thermoplastic dialdehyde starch (TPDAS) with different carbonyl content

The preparation and characterization of DAS had been reported previously by our laboratory [16]. The preparation process and molecular structure of DAS is shown in Fig. 2. Thermoplastic starch (TPS) and thermoplastic dialdehyde starch (TPDAS) were prepared by blending corn starch and DAS with 25 wt% glycerol in order to give them thermoplasticity. TPS and five TPDAS carbonyl contents were used in this study, which were TPDAS6, TPDAS30, TPDAS50, TPDAS70 and TPDAS95. The numbers 6, 30, 50, 70 and 95 stand for the DAS carbonyl content.

All materials were made into fine powder in advance. Two blank reactors and two reference reactors were included in this biodegradation testing system. The blank reactors contained only 120 g of inoculum without testing material. In the reference or test reactors, 20 g of reference material or test material was mixed with 120 g of inoculum. Microcrystalline cellulose was used as the positive reference material. The biodegradation of material was evaluated in duplicate.

2.5. Procedure

An amount of inoculum containing 120 g of total dry solids was weighed out and sufficient water was added to reach a moisture level of 65%. The compost was mixed well with sand with a moisture level of 15% which has previously been prepared by adding water into about 300 g of sea sand. 20 g of the dry solid test material was added to the compost and mixed well. The mixture was placed in the test environment at $58 \pm 2\ ^{\circ}C$. Three special vessels, which contained two replicates of each sample and a background control, were aerated at 30 mL/min throughout the experiment to ensure enough oxygen for the biodegradation process and the good absorption of CO_2 gas by sodium hydroxide solution. The CO_2 gas produced from biodegradation of test material or compost was absorbed by 500 mL 0.2 mol/L NaOH solution. The trapping reaction of CO_2 gas was done in a two step reaction shown below [18].

$$NaOH + CO_2 \rightarrow NaHCO_3 \quad (1)$$

then

$$NaHCO_3 + NaOH \rightarrow Na_2CO_3 \quad (2)$$

To determine amount of CO_2, 20 mL aliquots were removed as required from the mixture of 500 mL NaOH and Na_2CO_3 solution. Then, the absorbed CO_2 in the alkaline solution was titrated against 0.2000 mol/L HCl standard solution with the two end points detected by double-indicator, phenolphthalein and bromocresol green–methyl red mixture. During titration the following reactions took place:

$$NaOH + HCl \rightarrow NaCl + H_2O \quad (3)$$

$$Na_2CO_3 + HCl \rightarrow NaHCO_3 + NaCl \quad (4)$$

then

$$NaHCO_3 + HCl \rightarrow NaCl + H_2O + CO_2 \quad (5)$$

First, two drops of phenolphthalein indicator were added to the 20 mL mixture solution of NaOH and Na_2CO_3 and titration was carried out until the color of the solution turned from pink to colorless. At this first titration end point, reactions (3) and (4) had taken place and the used volume of HCl was noted as V_1. Secondly, two drops of bromocresol green–methyl red indictor were added and the titration continued until the solution turned from blue to red. At the second end point, reaction (5) had taken place and the used volume of HCl was V_2.

Because one mole Na_2CO_3 reacted with one mole HCl to produce one mole $NaHCO_3$ and then one mole $NaHCO_3$ reacted with one mole HCl to bring one mole CO_2, the molar quantity of CO_2 in the aggregate was $2 \times V_2 \times C_{HCl}$. Therefore, the quantity of CO_2 produced through biodegradation was counted by the following formula.

$$CO_2(g) = 2 \times V_2 \times C_{HCl} \times \frac{44}{2000} \times \frac{500.0\ mL}{20.00\ mL}$$

or

$$CO_2(g) = V_2 \times C_{HCl} \times 44 \times 0.5000/20.00$$

Here, C_{HCl} was the concentration of HCl standard solution and 44 was the molecular weight of CO_2.

The biodegradation percentage was calculated by the following equation.

$$D_t = \{(CO_2)_T - (CO_2)_B\}/ThCO_2 \times 100$$

Here, $(CO_2)_T(g)$ was the accumulated amount of carbon dioxide released by each composting vessel containing test material, $(CO_2)_B(g)$ was the accumulated amount of carbon dioxide released by the blank and $ThCO_2(g)$ was the

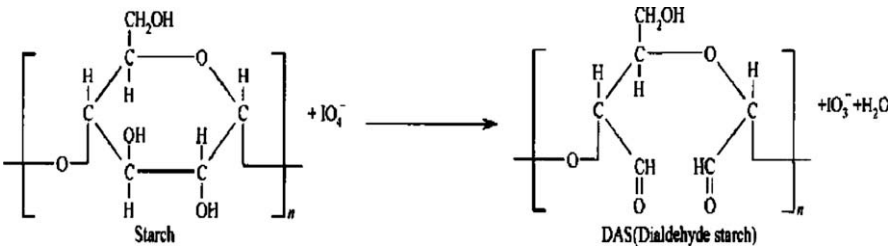

Fig. 2. The preparation process of dialdehyde starch.

theoretical amount of carbon dioxide of the test material in the test vessel. D_t was the biodegradable percentage of the test material under controlled composting conditions.

2.6. Isolation of compost microorganisms and screening of modified starch-degrading microorganisms

The compost sample was diluted with sterilized water by gradient-dilution. The 0.2 mL aliquot was spread on plates with modified starch medium, modified Gause medium and modified Martin medium, respectively. Modified starch medium (1000 mL) is composed of 10 g peptone, 3 g beef extract, 5 g NaCl, 20 g modified starch (TPDAS6) powder and 1000 mL water, and pH is 7.2–7.4. Modified Gause medium (1000 mL) is composed of 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g NaCl, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 20 g modified starch(TPDAS6) powder and 1000 mL water, and pH is 7.2–7.4. Modified Martin medium (1000 mL) is composed of 12.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1 mg vitamin B1, 10 g modified starch(TPDAS6) powder and 1000 mL water, and pH is neutral. A solid medium with agar (20 g/L) was added to the above media.

Then, the modified starch medium plate was incubated at 37 °C, while the modified Martin medium plate and modified Gause medium plate were incubated at 30 °C. Three or four days later strains were picked out. The strains were inoculated, respectively, into the above medium where modified starch powder was the sole carbon source for repeated screening.

2.7. The abilities of the strains to decompose the modified starch

Starch hydrolytic medium (1000 mL) is composed of 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g NaCl, Mg_2CO_3 1.0 g, agar 15–20 g, 10 g modified starch powder and 1000 mL water, and pH is 7.2–7.4; The hydrolytic abilities of the modified starch-degrading strains isolated for TPS, TPDAS6, TPDAS50 and TPDAS95 were examined by the clear-zone method. Each strain was inoculated in modified starch medium plate and incubated at 37 °C for 3 days. The hydrolytic ability was calculated by the following equation.

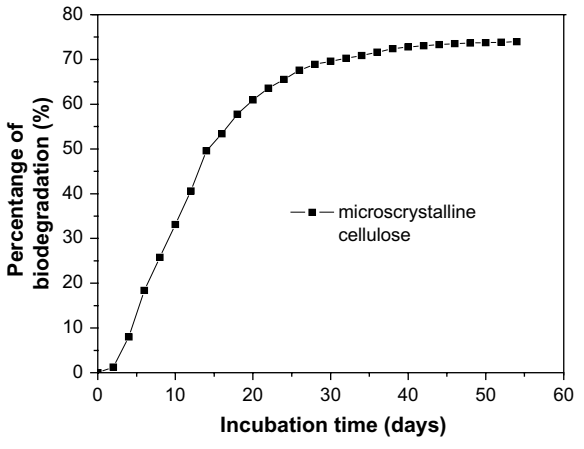

Fig. 3. Biodegradation of cellulose under controlled composting conditions.

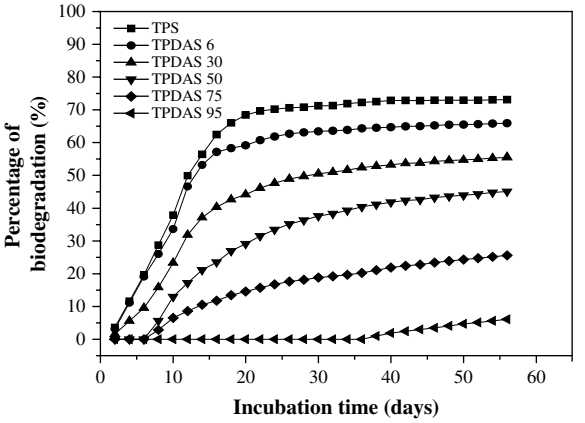

Fig. 4. Biodegradation of modified starch under controlled composting condition.

$$\mathrm{Up} = (D/d)^2$$

Here, Up was the hydrolytic ability, D was the diameter of the clear-zone and d was diameter of the colony.

3. Results and discussion

3.1. The biodegradation of TPS and TPDAS in compost

3.1.1. Validation of the quality of the inoculum used composting

An important criterion with regard to the quality of the inoculum is the biodegradation of the positive reference of cellulose. ISO 14855 prescribes that the degree of biodegradation of reference material is more than 70% after 45 days. The result of this experiment showed that the degree of biodegradation of the positive reference material (microcrystalline cellulose) was 74.05% in the compost after 45 days, as shown in Fig. 3.

3.1.2. The biodegradation behaviors of TPS and TPDAS

A convenient way to compare the biodegradation behavior of polymeric materials is to determine the converted carbon dioxide during the composting test. The biodegradation of TPS and TPDAS with different carbonyl content was tested by the manual set-up. The biodegradation curves of TPS and TPDAS with different carbonyl content are shown in Fig. 4. After 56 days for incubation in the composting system containing a mixture of test

Table 1
Three degradation stages of TPS and TPDAS under compost conditions

Sample	D_{56}/%	Lag phase/*d*	Biodegradation phase/*d*	Plateau phase/*d*
TPS	73.11	0–2	3–25	25–56
TPDAS6	65.91	0–2	3–25	25–56
TPDAS30	55.52	0–2	3–25	25–56
TPDAS50	45.12	0–6	7–45	45–56
TPDAS70	25.60	0–6	7–45	45–56
TPDAS95	6.079	0–36	37–52	52–56

D_{56} was the biodegradation percentage of the sample for 56 days under controlled composting conditions.

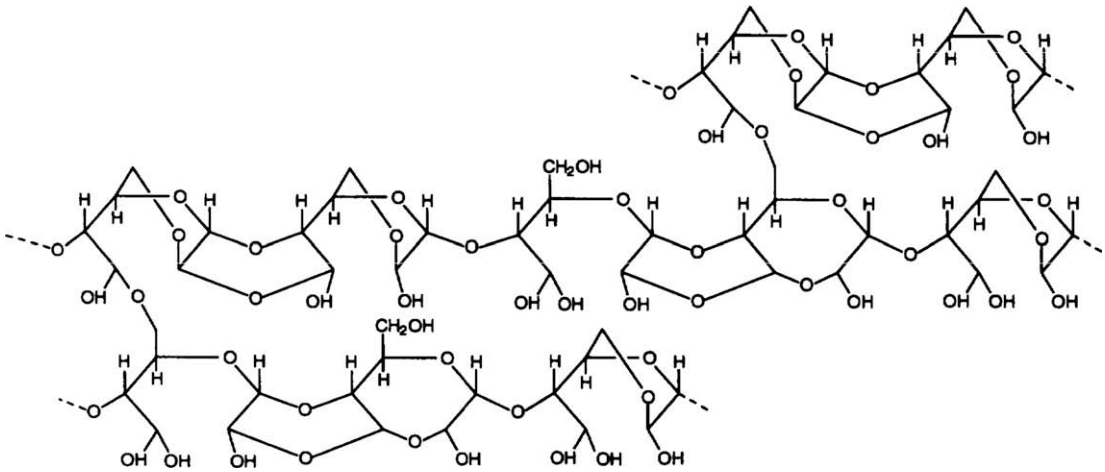

Fig. 5. The partially crosslink of DAS.

material and inoculum, the percentages of biodegradation were 73.11%, 65.91%, 55.52%, 45.12%, 25.60%, and 6.079%, for TPS, TPDAS6, TPDAS30, TPDAS50, TPDAS70 and TPDAS95, respectively. Obviously, as the degree of oxidation of TPDAS increased, its biodegradation rate decreased under controlled composting conditions. Three phases or stages were observed in the biodegradation curves of all samples, which can be defined as lag phase, biodegradation phase, and plateau phase, as listed in Table 1. From Fig. 4 and Table 1, it can be seen that the lag time was

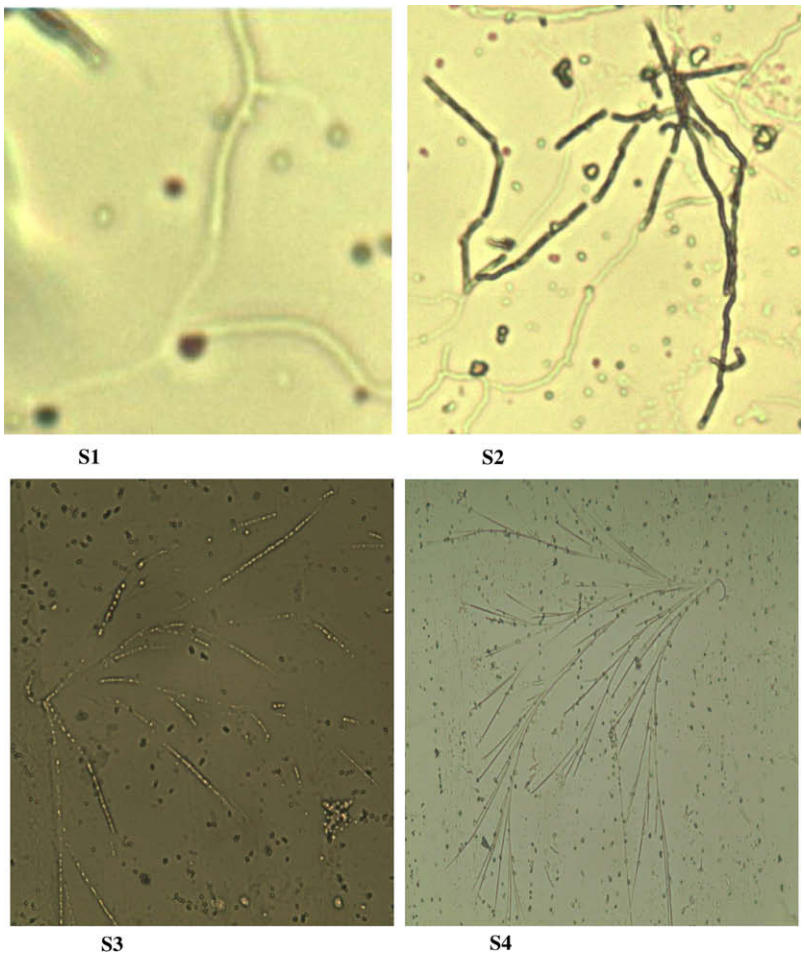

Fig. 6. Optical microscopic photographs of four strains isolated from compost.

Table 2
The abilities of the strains to decompose modified starch

Strains number	TPS			TPDAS6			TPDAS50			TPDAS95		
	D	*d*	Up	*D*	*d*	Up	*D*	*d*	Up	*D*	*d*	Up
1	3.5	2.0	3.0	3.0	2.0	2.3	2.6	1.8	2.0	2.1	1.6	1.7
2	5.2	1.6	10.6	4.6	1.5	9.4	3.8	1.3	8.5	2.6	1.1	5.76
3	1.7	1.4	1.5	2.2	2.0	1.2	2.0	1.9	1.16	1.7	1.7	1.0
4	4.5	1.6	7.9	4.0	1.5	7.1	3.4	1.3	6.8	2.5	1.2	4.33

different for TPDAS with varied carbonyl content, which indicated that the action of microorganisms in the compost to degrade starch had a close relation with the degree of modification and hydrophobic property of starch.

Through analysis of the biodegradation data of the TPDAS, it can be concluded that the degradation rate and final biodegradation percentage were closely related to the structure of DAS. This result is in agreement with the research of Van der Zee et al. [19]. The decreasing biodegradation rate of TPDAS may be ascribed to inter- and intramolecular cross-linking of DAS, its chemical scheme is shown in Fig. 5. The intramolecular cross-linking of DAS can result in the polymer being more hydrophobic, which causes conformational changes within the starch molecule, reducing the susceptibility to microorganisms [19]. The higher the content of carbonyl DAS, the more likely it was to form intramolecular cross-linking. Hence, as the oxidation degree of TPDAS increased, its biodegradation percentage decreased. Completely oxidized starch (TPDAS95) was only very slowly mineralized to CO_2. For example, after 56 days of incubation, only 6.079% of the TPDAS had been converted to carbon dioxide.

3.2. Isolation and screening of modified starch-degrading microorganisms

The biodegradation of modified starch under controlled composting conditions was the result of the synergic effect of the microorganisms in the compost. Screening of the best modified starch-degrading strains among them is helpful for further understanding the microbial degradation of starch and its plastic products. Four main strains, namely S1, S2, S3 and S4, were isolated from the compost. Strain S1 was a single spore-forming, branched and exuberant substrate mycelium, sparse aerial hyphae, aerobic and Gram positive micromonospora; Strain S2 was a branched and exuberant substrate mycelium, no eaerial hyphae, aerobic, producing catalase and Gram positive nocardia, and the colony morphology was wrinkled; Strain S3 was a long-chain spore-forming, exuberant substrate mycelium and aerial hyphae, aerobic and Gram positive streptomycete; Strain S4 was a exuberant substrate mycelium and aerial hyphae, aerobic and Gram positive streptomycete; according to their morphological characters and physiological tests respectively, S1 was identified as micromonospora. S2 was nocardia. Both S3 and S4 were streptomycete. The four strains all belong to actinomycete. It is difficult for bacteria to degrade starch in the initial stage, so fungi and actinomycete are mainly considered. There is a large quantity of actinomycete in the soil and the spores of actinomycete can resist harsh environments. The nutrition and gas mycelium of actinomyces can easily grow into the compounds. The optical microscope photographs of the four strains are shown in Fig. 6.

3.3. Four kinds of actinomycete degrading ability for TPS and TPDAS

To evaluate the abilities of the strains to assimilate modified starch powder, cultures were grown with modified powder as the substrate. As can be seen from Table 2, with the increase of the carbonyl content of the modified starch, the degrading ability of every actinomycete was restrained, which is in accordance with the result of TPDAS degraded in compost. Comparing the results, it is found that the degrading ability of nocardia was the best.

4. Conclusions

The biodegradation rate of TPDAS had been greatly influenced by the carbonyl content. As the oxidation degree of TPDAS increases, its biodegradation percentage decreased. Compared with TPDAS, TPS showed the fastest degradation. The biodegradation process of TPS and TPDAS exhibited three phases or stages with different degradation speeds. The biodegradation in the first phase was slow, accelerated in the second phase and leveled off in the third phase. Modified starch-degrading strains isolated from compost are mainly actinomycete. Only a little fungi and hardly any bacteria could be obtained. This is in accordance with other work [20]. Three kinds of actinomycete isolated from the compost were identified as micromonospora, nocardia and streptomycete. They showed different abilities to decompose TPS and TPDAS, with nocardia being the best modified starch-degrading microorganism.

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