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Process of rice straw degradation and dynamic trend of pH by the microbial community MC1

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Abstract: The process of the rice straw degradation in the fermentor with aeration at 290 ml/h was studied. The results of dissolved oxygen (DO) indicated that the optimum DO during cellulose degradation by microbial community MC1 ranged from 0.01 to 0.12 mg/L. The change model of pH values was as follows: irrespective of the initial pH of the medium, pH values decreased rapidly to approximate 6.0 after being inoculated within 48 h when cellulose was strongly degraded, and then increased slowly to 8.0—9.0 until cellulose was degraded completely. During the degradation process, 15 kinds of organic compounds were checked out by GC-MS. Most of them were organic acids. Quantity analysis was carried out, and the maximum content compound was ethyl acetate which reached 13.56 g/L on the day 4. The cellulose degradation quantity and ratio analyses showed that less quantity (under batch fermentation conditions) and longer interval (under semi-fermentation conditions) of rice straw added to fermentation system were contributed to matching the change model of pH, and increasing the quantity and ratio of rice straw degradation during cellulose degrading process. The highest degradation ratio was observed under the condition of rice straw added one time every five days (under semi-fermentation conditions).

Keywords: rice straw; cellulose degradation; the microbial community; MC1; pH

Introduction

Lignocellulose is one of the cheapest and most abundant resources in the world. The total worldwide production of cellulose and hemicellulose was 85×10^9 t/a and cereal straw was estimated to exceed 2.9×10^9 t/a (Sun *et al.*, 2004). In China, only the cereal straw total production was more than 600×10^6 t/a, which was higher than the total production of foodstuff. The forage grasses was just 10×10^6 t/a that was only 2% of the overall cereal straw production (Wang *et al.*, 1998). With the rapid increasing of the world population, the utilization of cellulose resource has aroused people's great attention and recognition all over the world in order to solve the food and energy crisis (Zhang *et al.*, 1991). Now, the exploitation of new energy and new food resource becomes such an important project that was studied all over the world. However, cellulose, embedded in the lignin matrix, have an insoluble high crystal structure framework, so it is difficult to be hydrolyzed into sugar or cell protein which is easy to use. The unsuitable treatment of cellulose not only results in huge amounts of resource wasting, but also brings a environmental problem.

The microbial community MC1 with lignocellulose degradation ability has been selected and constructed by our laboratory from overheated composting pile. It can degrade the rice straw completely within 8 d and the degradation ability is higher than the pure culture of single strain (Cui *et al.*, 2002; Haruta *et al.*, 2002). An anaerobic cellulolytic bacteria *Clostridium straminisolvens* CSK1 (Souichiro *et al.*, 2004a), an anaerobic non-cellulolytic bacteria *Clostridium thermocellum* and other five aerobic non-cellulolytic bacteria (*Bacillus licheniformis*; *Pseudoxanthomonas taiwanensis*; *Virgibacillus pantothenticus*; *Brevibacillus agri*; *Bordetella petrii*) were isolated from the MC1. Comparison of the cellulose degradation processes of the pure culture of *C. straminisolvens* CSK1 and the mixed-culture indicated that non-cellulolytic bacteria essentially contributed to cellulose degradation by supplying anaerobic environment, consuming metabolites, which otherwise deteriorated the cellulolytic activity, and neutralizing pH (Souichiro *et al.*, 2004b). This paper studied the following two aspects of MC1: (1) the requirement of oxygen and the change of fermentation products content during the cellulose degradation process; (2) the correlation between pH change and the ability of cellulose decomposition under different rice straw adding models.

1 Materials and methods

1.1 Materials

The microbial community MC1 capable of degrading cellulose effectively was constructed and characterized by our laboratory. The MC1 was maintained as frozen stock at -20℃ in 20% glycerol. After 3 d inoculation in PCS (peptone cellulose solution) medium containing 1% rice straw (w/v), the MC1 was prepared for the subsequent experiment as inoculants.

The rice straw (*Oryza sativa* L. cv.) after harvest

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was treated in 1.5% NaOH for 24 h, then was adjusted to pH 8.0 followed by drying at 80℃. The rice straw was chopped into appropriate length and was erected in flask just over the surface of the culture solution.

1.2 Medium and culture conditions

PCS medium was composed of the following: 5 g peptone, 10 g cellulose (rice straw or filter paper), 1 g yeast extract, 3 g $CaCO_3$, 5 g NaCl and 1 L H_2O (pH 8.0). The PCS was autoclaved at 121℃ for 15 min before use. After inoculation (seed volume of 5%, unless otherwise stated) the MC1 was cultured under static conditions at 50℃.

1.3 DO and pH value

The microbial community MC1, propagated as described above, was inoculated in the fermentor (ϕ10 ml, 1 L) in 400 ml PCS medium including 4 g rice straw with air flowing at the rate of 290 ml/h under static conditions at 50℃. The DO was determined using DO-meter (model OM-14, HORIBA, Japan) and pH values were determined using Compact pH meter (model B-212, HORIBA, Japan), respectively. The samples were obtained every 6 h during the first 24 h and then once every day until 12 d.

1.4 Product measurements

The samples, filtered with aperture of 0.20 μm, were determined by a combination of gas chromatography and mass spectrometry (GC-MS; model QP-5050, Shimadzu, Japan) with two capillary columns of CP-Chirasil-Dex CB and pora PLOT Q respectively. The determination conditions were as follows:

Conditions of column type CP-Chirasil-Dex CB (25 m×0.25 mm): the column temperature was 40℃ (2 min)→100℃, 4℃/min→200℃(2 min), 15℃/min; injector temperature 190℃; detector temperature 230℃; carrier gas: He (60 kPa); rate of flow: 30 ml/min; splitter ratio: 1/22; detector: 1.5 kV; sample volume: 1 μl.

Conditions of column type pora PLOT Q (25 m×0.25 mm): the column temperature was 80℃(1 min) →230℃ (1 min), 20℃/min; injector temperature 230℃; detector temperature 230℃; carrier gas: He (50 kPa); rate of flow: 50 ml/min; splitter ratio: 1/13; detector 1.8 kV; sample volume: 2 μl.

The final results were analyzed by NIST database for qualitative analysis. The standard samples were 100 times diluted for quantitative analysis.

1.5 Degradation quantity and pH value analysis when rice straw was added under different models

MC1, propagated as described in Section 1.1, was transferred to a 500-ml flasks in 400 ml PCS medium including rice straw. The flasks were sealed by foil and the cultures were incubated at 50℃. pH was deter- mined once a day throughout the culture process for 90 d and the dry weight of rice straw residue was determined in the end. Two groups of the experiment were carried out according to the different cellulose addition models.

The first group consisted of 5 kinds of batch fermentation tests: initial rice straw content of 1%, 2%, 3%, 4% and 5% (w/v) were added at the beginning and cultured for 90 d, respectively.

The second group consisted of 7 kinds of semi-continuous fermentation tests: 2 kinds of tests were primarily added rice straw 1%, and 2% on the day 1 and half of the initial quantity were added again on the day 29 and 51, respectively. Other 5 tests with rice straw content of 0.5% (w/v) were added at first and from then on the same quantity were added once every 3, 5, 8, 12 and 15 d, respectively. To obtain the degradation quantity and degradation ratio, the samples, filtered by fiberglass filter paper GF/D (Whatman, Japan) after cultivation for 90 d, were rinsed to get rid of bacteria with acetic-nitric reagent (Updegraff, 1969), and then they were filtered again and washed with water, and dried at 105℃, then finally weighed. All experiments were carried out in duplicate and the mean values of residual rice straw were obtained. Meanwhile, the degradation quantity and degradation ratio were calculated.

1.6 Degradation ratio analysis

Eighteen 500-ml flasks with 400 ml PCS including 1% rice straw inoculated MC1 were cultured at 50℃. With uninoculated medium as a control, 3 of the 18 flasks were chosen at random at a time at the day 1, 2, 3, 6, 9, and 12, respectively, during the degradation process. The degradation quantity and ratio were obtained as described in Section 1.5.

At the same time, pH was determined every 12 h during the 12 d.

2 Results and discussion

2.1 Changes of DO and pH value during the process of rice straw degradation

With air flowing into the fermentor at the rate of 290 ml/h, DO value decreased from initial 4.43 to 0.42 mg/L at the 6th hour and to 0.05 mg/L at the 12th hour, subsequently rose up appreciably to 0.44 mg/L at the 24th hour. After that, it dropped down gradually and remained in the range of 0.01—0.12 mg/L during the rapid straw-degradation from the day 3 to 8 (Fig. 1). MC1 could adapt to DO at such low level, however, it could not degrade cellulose under the anaerobic conditions.

The pH of the culture solution declined from initial 7.6 to 6.28 at the 24th hour and even to 5.94 at the 38th hour followed by ascending to around 8.8 after 10 d degradation (Fig.1). This rule was the characteristic of cellulose degradation of MC1. Otherwise, the pH would decrease slowly or would

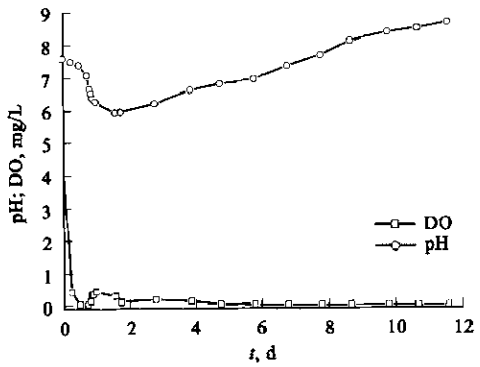

Fig.1 Dynamic trends of DO and pH value during the rice straw degradation by MC1

not rise up again when degradation ability disappeared or declined.

2.2 Products during the process of rice straw degradation

The samples of Section 1.3 were determined by GC-MS with two types of columns. Fig.2 shows the degradation products of MC1 after 5 d cultivation. With column CP-Chirasil-Dex, 12 peaks, besides air, were detected (Fig.2a). With column pora PLOT Q, four new peaks, 13. methanol, 14. formic acid, 15. ethyl acetate and 16. 1-butanol, were also examined (Fig.2b). Most of the products were organic acids or their ramifications besides ethanol and glycerol.

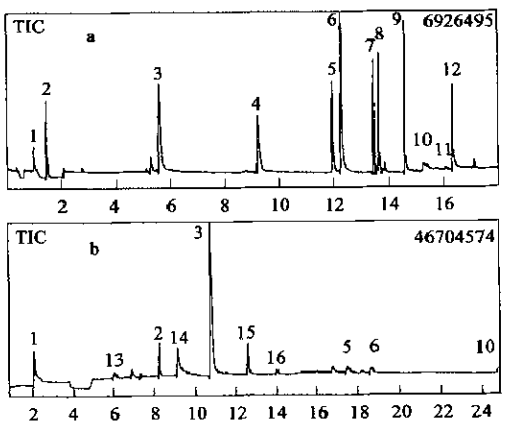

Fig.2 Products determined by GC-MS of MC1 after cultivated 5 d
a. with column of CP-Chirasil-Dex CB; b. with column of pora PLOT Q
1. air; 2. ethanol; 3. acetic acid; 4. propanoic acid; 5. 2-methyl-propanoic acid; 6. butanoic acid; 7. 3-methyl-butanoic acid; 8. 2-methyl-butanoic acid; 9. 4-methyl-pentanoic acid; 10. lactic acid; 11. glycerin1; 12. 2-piperidone; 13. methanol; 14. formic acid; 15. ethyl acetate; 16. 1-butanol

The concentration change of 7 kinds of substances (acetic acid, methanol, ethanol, formic acid, ethyl acetate, butanol, lactic acid) was carried out simultaneously (Fig.3). Acetic acid and formic acid were produced fastest among them, followed with ethanol and ethyl acetate, but the amount of ethyl acetate was so much more than the others that the concentration reached to maximum of 13.56 g/L on the day 4. Both the amounts of acetic acid and ethanol

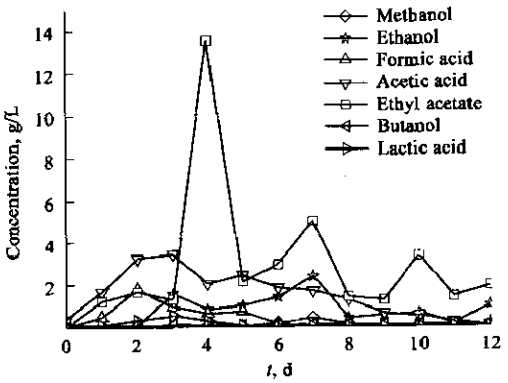

Fig.3 Dynamic trends of the products of rice straw

were decreasing sharply when the ethyl acetate accumulated excessively. Lactic acid appeared on the 1st day and extended to maximum of 1.6 g/L on the day 3. But it could not be checked out since the day 6. Methanol existed from the day 5 to 8. Butanol could be detected before 11 d whereas the concentration was rarely low.

2.3 Color, odor and morphological change of the rice straw

The batch fermentation medium adding 1% (w/v) rice straw, as described in Section 1.5, browned with strong acid odor after 3 d and rice straw was degraded rapidly. After 10 d, the tests adding 2% rice straw browned and there was less rice straw left. After 40 d, there was no rice straw left in the tests adding 1%, 2%, 3% and 4% rice straw, respectively, but the test with 5% rice straw was not degraded completely even after 90 d.

Rice straw added every 3 and 5 d, respectively, softened visibly in the preliminary 30 d and could not be degraded any more after 60 d under the semi-continuous fermentation conditions (the second group). The tests of addition interval of 8, 12 and 15 d were keeping degradation ability all along 90 d and the degradation speeds were much faster than that of 3 and 5 d.

2.4 Changes of pH of different batch fermentation tests

Changes of pH value with different rice straw addition quantity were different under batch fermentation (the first group). Fig.4 shows that: (1) the pH declined at first and then rose to alkalescence under the normal degradation condition; (2) the less rice straw addition contributed to the earlier re-increase of pH value. With the increasing of the addition quantity, the pH recovery process became slow. When 4% rice straw was once added, it required about 30 d for the pH to recover to the alkalescence, but when 5% was once added, it could not recover completely to the balance point even after 90 d cultivation. During the whole process of the fermentation, the pH declined with the degradation of rice straw, and then rose to the balance point at the

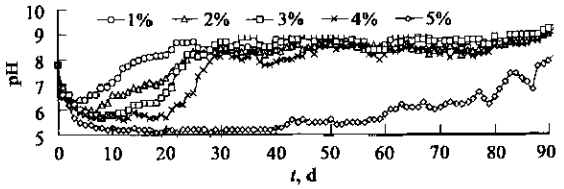

Fig.4 Changes of pH value with different rice straw addition under batch fermentation

end of the degradation process; when added excessively, the rice straw could not be degraded and the pH could not recover. It indicated that the pH value could significantly reflect the degradation process of MC1.

2.5 Changes of pH under semi-continuous fermentation

2.5.1 Changes of pH when added rice straw again on the day 29 and 51 respectively

In order to demonstrate the rule of pH change of MC1 experimentally, 1% and 2% rice straw were added respectively on the 1st day primarily. When they were degraded completely and the pH value recovered to the alkalescence stably, half of the initial addition quantity of rice straw was added on the day 29 and 51, respectively (the second group). As a result, the pH value repeated the fore-descending and after-ascending rule (Fig.5). Another rule was that the superaddition quantity took on the relativity with the declined extent and recovered speed of pH. The more the rice straw was added, the lower the pH declined and the longer time was needed to recover to the balance point.

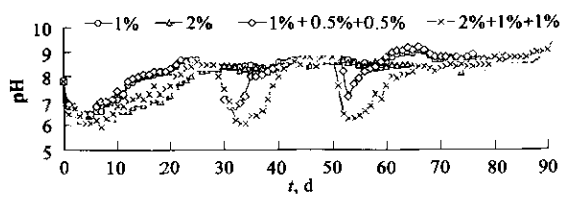

Fig.5 Changes of pH when added rice straw again on the day 29 and 51, respectively

2.5.2 Rule of pH under different semi-continuous fermentation conditions

In order to investigate the degradation potential of MC1, the rice straw content of 0.5% was added primarily and from then on the same quantity was added every 3, 5, 8, 12 and 15 d, respectively. At the same time the rule of pH change was observed (the second group). The results were as follows: the tests of adding rice straw every 3 d could only remain the pH change rule about 20 d (Fig.6), and then the rice straw could not be degraded at all; the tests of adding every 5 d and 8 d could keep the pH change rule 30 and 50 d, respectively, and then lost the fore-descending and after-ascending rule; the tests that the rice straw was added every 12 d and 15 d could remain the rule 90 d

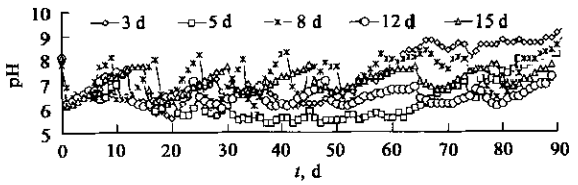

Fig.6 Rule of pH change when rice straw was added under different semi-continuous fermentation conditions

and the rice straw could be degraded all the time. It indicated that MC1 could consume the products (such as the organic acids) with adding rice straw at the same interval. Meanwhile, it could recover to the pH balance point and recover the degradation ability. Therefore, it could keep the degradation ability for a long time if the rice straw was added appropriately.

2.6 Changes of pH and degradation ratio

As shown in Fig.7, pH declined when the cellulose began to degrade and recovered to about 8.8 with the rice straw degraded completely and then remained this balance point for a long time. When pH recovered to the alkalescence, the degradation ratio became very slow. So the rule of fore-descending and after-ascending could reflect the degree of the rice straw degradation and the alkalescence of pH could represent the accomplishment of the degradation.

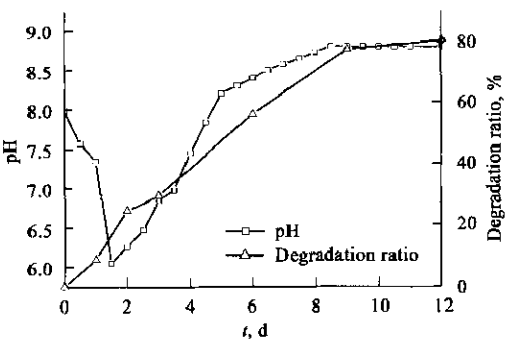

Fig.7 Dynamic trends of pH and degradation ratio during the process of rice straw degradation

2.7 Degradation ratio and degradation quantity under different addition conditions

The degradation ratio and degradation quantity were the important indices reflecting the degradation ability of MC1. The results of the degradation ratio and degradation quantity, which was designed in Section 1.5, are shown in Table 1.

The less rice straw addition under the batch fermentation conditions and the longer addition interval under the semi-continuous fermentation conditions contributed to the higher degradation ratio. On the other hand, the more rice straw addition under the batch fermentation conditions contributed to the higher degradation quantity. However, the longer addition interval did not always bring the higher degradation quantity under the semi-continuous fermentation conditions. From Table 1, the test by

Table 1 Degradation ratio and degradation quantity under different addition models

Addition model	Batch fermentation tests					Semi-continuous fermentation tests				
Addition quantity	1%	2%	3%	4%	5%	0.5% (every 3 d)	0.5% (every 5 d)	0.5% (every 8 d)	0.5% (every 12 d)	0.5% (every 15 d)
Total addition times	1	1	1	1	1	12	12	10	7	6
Total addition quantity, g	4	8	12	16	20	24	24	20	14	12
Residual straw, g	0.6	2.1	5.5	9.5	13.2	8.5	7.8	6.2	2.4	1.6
Degradation quantity, g	3.4	5.9	6.1	6.5	6.8	15.5	16.2	13.8	11.6	10.4
Degradation ratio, %	85.0	73.7	50.8	40.5	34.0	64.6	67.4	69.0	82.8	86.7

adding rice straw every 5 days had the highest degradation quantity.

3 Conclusions

For optimization of rice straw degradation conditions for MC1, the present study showed that the optimum DO was in the rang of 0.01 to 0.12 mg/L, namely an microaerobic condition (Cui *et al.*, 2004).

15 kinds of degradation products were determined by GC-MS. Most of them were organic acids. Quantity analysis was carried out, and concentration of ethyl acetate was the highest among them that reached 13.56 g/L on the day 4.

When the cellulose was degraded by MC1, an obvious rule was observed under the batch fermentation conditions that the pH declined when the cellulose began to degrade and then recovered to about 8.8 with the rice straw degraded completely, finally remained this balance point for a long time. If the rice straw was superadded again, the pH could also repeat the rule of fore-descending and after-ascending.

During the semi-continuous fermentation, the tests of addition rice straw every 3 d could only remain the pH change rule 30 d. The longer addition interval contributed to the longer maintenance of the pH rule and the higher degradation ratio, but the longer interval did not always accompany the higher degradation quantity and the tests of addition rice straw every 5 d had the highest degradation quantity. Compared with the batch fermentation, the degradation ratio under semi-continuous fermentation was higher when the rice straw was added with the same total quantity.

References:

Cui Z J, Li M D, Piao Z *et al.*, 2002. Selection of a composite microbial system MC1 with efficient and stability cellulose degradation bacteria and its function[J]. Environmental Science, 23: 36—39.

Cui Z J, Piao Z, Wang W D *et al.*, 2004. Conditions of cellulase production in the presence of microbial consortia MC1 capable of degrading cellulose with high efficiency [J]. Journal of Agro-Environment Science, 23(2): 296—299.

Haruta S, Cui Z, Huang Z *et al.*, 2002. Construction of a stable microbial community with high cellulose-degradation ability[J]. Appl Microbiol Biotechnol, 59: 529—534.

Souichiro Kato, Shin Haruta, Cui Z J *et al.*, 2004a. *Clostridium straminisolvens* sp. nov., a moderately thermophilic, aerotolerant and cellulolytic bacterium isolated from a cellulose-degrading bacterial community [J]. International Journal of Systematic and Evolutionary Microbiology, 54: 2043—2047.

Souichiro Kato, Shin Haruta, Cui Z J *et al.*, 2004b. Effective cellulose degradation by a mixed-culture system composed of a cellulolytic *Clostridium* and aerobic non-cellulolytic bacteria [J]. FEMS Microbiology Ecology, 51: 133—142.

Sun X F, Sun R C, Tomkinson J *et al.*, 2004. Degradation of wheat straw lignin and hemicellulosic polymers by a totally chlorine-free method [J]. Polymer Degradation and Stability, 83: 47—57.

Updegraff D M, 1969. Semimicro determination of cellulose in biological materials[J]. Analytical Biochemistry, 32 : 420—424.

Wang W Y, Zhu J H, Wu S Y, 1998. Research progress of cellulose science and cellulos [J]. JiangSu University Science Technology in China, 19: 20—28.

Zhang F Q, Shu Y C, Hao J *et al.*, 1991. The change of sugar cane pulpa to sugar and produce of SCP by trichoderma koning and cellulase [J]. Microbiology Report, 18(4): 81—88.

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