International Scholars Journals

International Journal of Diseases and Disorders ISSN 2329-9835 Vol. 11 (7), pp. 001-011, July, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Metabolic control of lignocellulose degradation by lignocellulose-degradation microbial community MC1

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Accepted 28 March, 2023

This study was to reveal the limiting factors of lignocellulose degradation and further improve the potential degradation of microbial community MC1, by pH manual adjustment (pHMA) and culture medium partial replacement (CMPR) during the process of rice straw degradation. The results indicated degradation rate in control (5% rice straw) increased, ranging from 33.3 to 67.5% via pHMA method, and for treatment with CMPR which was elevated to 83.3%. Meanwhile, pH in control dropped to 5.4 and remained after 20 days of incubation, while pH increased to 6.9 in sample with pHMA and 6.5 in sample with CMPR. With molecular footprint technology, 9 bands undetected in previous work were discovered in MC1 from the DGGE profiles. The results of soluble products with two treatments demonstrated that CMPR technology could not only facilitate the degradation but also accumulated quantities of soluble products compared to pHMA method which decreased quantities of soluble products.

Key words: Lignocelluloses degradation, microbial community, pH manual adjustment, culture medium partial replacement.

INTRODUCTION

The amount of energy from the sun which is stored as carbon via photosynthesis is 10 times than the world usage (Kumar et al., 2008). On a worldwide basis, terrestrial plants produce 1.3×10^{10} metric tons (dry weight basis) of wood per year, which has the energy equivalent of 7×10^{9} metric tons of coal or about two-third of the world's energy requirement (Kumar et al., 2008). In the products from photosynthesis, lignocelluloses matter occupies 80% of tree wood which also contains cellulose, hemicelluloses and lignin. In 2006, it was estimated that in China, approximately 86 million tons were destroyed by fire or lost (Cui et al., 2008). From above data, tons of lignocelluloses resources were lost indicating a substantial waste of resources and increased environmental pollution. Although, extensive studies have been carried out to explore lignocelluloses, there is no process or available technology to utilize this natural compound because of the complicated chemical structure. Utilization of lignocelluloses to produce ethanol or other fuels is expected to be an important energy source to replace rock oil (Lynd et al., 2008). Therefore, the development of efficient biodegradation of lignocelluloses techniques not only has significant commercial value but also facilitates the resolution of environment problems.

Physical, physic-chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials. However, biological pretreatment is considered the most economically promising approach owing to the advantages of low energy requirement and mild environmental conditions (Balat, 2011).

With the precondition of maintaining natural relationship among microbes, a stable microbial community with high effective performance marked MC1 had been successfully selected and cultured to degrade rice straw lignocelluloses (Cui et al., 2002). MC1 exhibited satisfied degradation ability on rice straw, cassava residue and corn stalk at the maximum degradation rate up to 60, 47

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Abbreviations: CMPR, Culture medium partial replacement; **pHMA,** pH manual adjustment.

and 70%, respectively (Guo et al., 2008a; Guo et al., 2008b; Haruta et al., 2002, 2004). Denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene sequence analyses indicated the coexistence of aerobic and anaerobic bacteria in the microbial community (Haruta et al., 2002; Kato et al., 2004a).

Previous research had suggested that pH value is an important indicator in the degradation by MC1, which indicated the ability of lignocellulose degradation (Liu et al., 2006). At the beginning, the pH value dropped gradually during the degradation process and then rose back to 8.0 at the end of process. With the increasing of additional rice straw, the degradation became difficult to be continued and the pH value also could not to be raised back to the balance point. There was a positive correlation between the amounts of rice straw added with the decrease of pH, while a negative correlation with the ability of degradation. Therefore, this process of adding rice straw during the degradation process limited MC1 effectiveness in treating large quantities of rice straw. So factors as to why the degradation process was inhibited are important, especially for the improvement of degradation rate and the development of technology.

From above, the target of this study was to optimize the degradation conditions by pH manual adjustment (pHMA) and culture medium partial replacement (CMPR) in rice straw fermenting system starting with the products of microbial metabolites, and to study the impact of artificial control on degradation ability and microbial community. Furthermore, the effect of artificial control to the way of improving degradation efficiency was also explored.

MATERIALS AND METHODS

Preparation for cultivation

The original bacterial source in this experiment for lignocelluloses degradation was a complex microbe community which had been denominated as MC1. Freezing storage microbial strains were activated for the purpose of fermentation in a peptone cellulose cultured solution (PCS) (Haruta et al., 2002). Rice straw was dipped into 1.5% (W/V) NaOH solution for 48 h, then flushed with tap-water until the pH value reached pH 7.0, dried at 105°C, and finally cut into 8 cm in length to facilitate placement in a 300 ml flask.

A 2% NaOH solution for subsequent pHMA experiments and fresh PCS for following CMPR experiment were prepared.

Growth conditions

A 300 ml-Erlenmeyer flask, containing 238 ml PCS medium as well as 3 and 5% (W/V) rice straw respectively, inoculated with 12 ml MC1 suspension after 3 days activation (seed volume of 5%), and was incubated at static cultivation at 50°C.

In the experiment of pHMA, pH was determined every 8 h during fermentation process. For samples of 3%-pH (3% w/v rice straw and pH adjusting treatment) and 5%-pH (5% w/v rice straw and pH adjusting treatment), A 2% NaOH solution was used to adjust pH to 7.0 when the readings were lower than 6.5.

In the experiment of CMPR, two kinds of different replacing frequency was set up during the period of 20 days fermentation as follows:

Treatment 1

Four times replacement for “3%-M-4” (3% rice straw feed and 4 times of CMPR), half of fermentation liquid was exchanged by fresh culture medium at equal volume with a sterilized injector at 3, 6, 9 and 12 day, respectively.

Treatment 2

Seven times replacement for “5%-M-7” (5% rice straw feed and 7 times of CMPR); the same operation described above was conducted at 2, 4, 6, 8, 11, 14 and 17 day, respectively.

Samples without pHMA or CMPR were used as controls. “3%-control” and “5%-control” were marked for 3 and 5% rice straw addition, respectively. Samples without rice straw addition at the presence of inoculation only were used as controls for the biomass balance calculations.

Determination of pH value

The pH value was measured every 8 and 24 h in the pHMA and CMPR method by using a method described previously (Yang et al., 2007).

Degradation rate of rice straw

The degradation solution was filtered with weighted filter paper after sampling respectively at 0, 3, 6, 12 and 20 day, then the residue was dried at 105°C and weighted subsequently. The treatment in the absence of rice straw was used as a control to remove the impact from bacteria and culture medium. Degradation rate was calculated according to the residual rice straw. Water-soluble substance inside the culture medium was also confirmed after dried and weighted.

Chemical composition analysis

Determination of the components of straw after degradation

The residual rice straw was milled after drying, screened through a 1 mm sieve and a 0.5 g sample was transferred into a filter bag (Model F57, ANKOM Technology, USA). Components of soluble matter in rice straw, cellulose, hemicellulose, lignin and ash were analyzed using a fiber analyzer (Model ANKOM^{200}, ANKOM Technology, USA), according to the method described by Goering and van Soest (1970). Three replicates were carried out.

Determination of soluble sugar

The soluble sugar in the culture medium was determined calorimetrically by the anthrone method (Thomas, 1977). Three replicates of all samples were measured.

Microbial communities analysis

For analyzing the complexity of the microbial communities during fermentation process, the DNA of each treatment was extracted by the benzyl chloride method (Zhu et al., 1993). The DNA

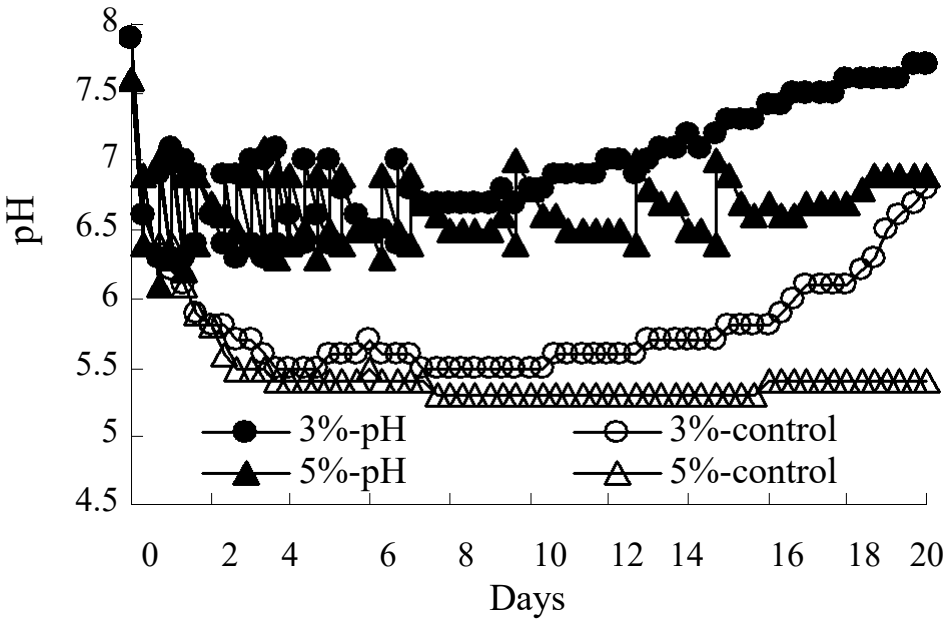

Figure 1. The pH changes in the experiment of pH manual adjustment. Solid circle, 3%-pH: 3% rice straw (w/v) and pH manual adjusted treatment; open circle, 3%-control: 3% rice straw (w/v) and non-treatment; Solid triangle, 5%-pH: 5% rice straw (w/v) and pH manual adjusted treatment; open triangle, 5%-control: 5% rice straw (w/v) and non-treatment.

concentration was determined with a DNA/RNA/protein analyzer (Biospecmini, Shimadzu, Japan), and was diluted to the same concentration (10 ng/uL) for the template in PCR amplification. PCR amplification was performed using Gradient Delivery System (Bio-Red Model 475, Applied Biosystems, USA). The primers used for DGGE were the following: 357F-GC, 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′ (*Escherichia coli* positions, 341 to 357), and 517R, 5′-ATTACCGCGGCTGCTGG-3′ (*Escherichia coli* positions, 517 to 534) to amplify the V_3 region of 16S rDNA (Wang et al., 2006). The temperature cycle consisted of 30 s of denaturing at 93°C and 30 s of annealing at 65°C for the first 10 cycles, 60°C for the second 10 cycles, and 55°C for the last 10 cycles, with 1 min of primer extension at 72°C. An initial denaturing at 95°C for 10 min and a final 5-min primer extension were also carried out (Haruta et al., 2002). The products were examined by electrophoresis on 2% agarose gel. The PCR products were used for DGGE analysis (Muyzer et al., 1993; Wang et al., 2006). DGGE was performed with the Dcode system (Bio-Rad Laboratories, Hercules, Calif.) 35-50% denaturant gradient (where 100% is defined as 7 M urea with 40% formamide), and an electrophoresis. The gels were stained with SYBR Green I (Molecular Probes, Eugene, Ore) and photographed, as described by Wang et al. (2006). The V_3 region bands of 16S rDNA on the gel were recovered and reamplified with the primers 357F (5′-CCTACGGGAGGCAGCAG-3′) and 517R (5′-ATTACCGCGGCTGCTGG-3′) (Haruta et al., 2002). The PCR products were sequenced by Sangon Biotech Company (Beijing, China). The sequence similarity searches were performed in the GenBank data library using the BLAST Program.

Nucleotide accession number

The sequences obtained in this study were deposited in GenBank under accession numbers HM193900 to HM193908.

Statistical analysis

Each trial was performed in triplicates including appropriate control. The data was statistically analyzed as average value of different trials for each experiment. All data was subjected to ANOVA for significant differences by the general linear models procedure of Statistic Analysis System (Ver.6.12; SAS Inst., Cary, NC, USA).

RESULTS AND DISCUSSION

pH changes in the process of degradation with rice straw

pH changes in the experiment of pHMA

Changes of pH value corresponded with the capability of MC1 in lignocelluloses degradation. At the beginning of the degradation, pH decreased rapidly and then increased with the quantity decrease in rice straw. The slow increase indicated the weak degradation capability of MC1. If pH did not increase, the degrading capability of MC1 was unavailable (Liu et al., 2006). In the pHMA experiment, the pH was obviously higher in control no matter how much rice straw was added in the system (Figure 1). The pH of 3%-control decreased to about 5.5 at the second day and increased after 10 days incubation, maintaining this low level until the end of experiment for 5%-control. At the early stage in the pHMA treatment, frequent changes in pH were serrated in curve. 7 days later, the change of the pH decrease was reduced and the frequency of the adjusting treatment was

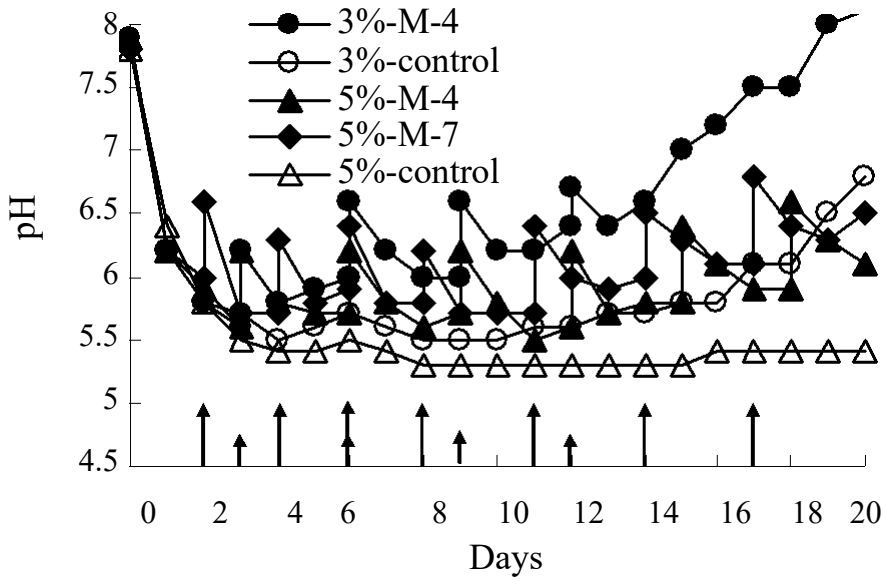

Figure 2. The pH changes in the experiment of culture medium partial replacement. Solid circle, 3%-M-4: 3% rice straw (w/v) and culture medium partial replacement for four times; open circle, 3%-control: 3% rice straw (w/v) and non-treatment; Solid triangle, 5%-M-4: 5% rice straw (w/v) and culture medium partial replacement for four times; solid diamond, 5%-M-7: 5% rice straw (w/v) and culture medium partial replacement for seven times; open triangle, 5%-control: 5% rice straw (W/V) and non-treatment.

also decreased. Moreover, there was an obvious difference between 3%-pH and 5%-pH, such that the pH value increased after 7 days fermentation and reached 7.7 after 20 days for 3%-pH while the treatment of pHMA was continued until 15 days and returned to 6.9 after 20 days.

pH changes in the experiment of CMPR

The trend of pH changes in the experiment of CMPR (Figure 2) was similar with the experiment of pHMA. The pH increased as the culture medium was replaced. The fluctuation in pH values ranged from 5.5 to 7 in total and increased gradually. For 3%-M-4 treatment, the pH was 7.0 until after 15 days and reached 8.0 after 20 days. For 5%-M-4 and 5%-M-7 treatments, the pH values were 6.1 and 6.5, respectively after 20 days.

Based on the results described above, it was demonstrated that the quantity of rice straw addition visibly influenced the lignocelluloses degradation with MC1. The pH with 3% rice straw addition rallied more early than the treatment of 5% rice straw feed, which had been summarized by the less rice straw addition promoting the pH increase (Liu et al., 2006). The pH increase and degradation capability of recovery were both earlier than the control in these tests. For 5%-control, pH did not increase 20 days later, which means that the long-term accumulated organic acid from degradation products has some effect on pH and the degrading process after the precipitates increased. According to the characteristics of MC1, it was necessary to perform the manual adjustment.

Degradation of rice straw

Whereas the degradation rate of control sample increased significantly at the first 6 days, there were only 50.0 and 33.3% for samples of 3%-control and 5%-control 20 days later. For samples with pHMA and CMPR, MC1 degraded rice straw efficiently during the whole process. The overall mass losses were reaching 79.2, 83.3, 67.5 and 78.9% for 3%-pH, 3%-M-4, 5%-pH and 5%-M-4 in respectively after 20 days of incubation (Figure 3A and B).

As shown in Figure 3, the degradation rate of 3% rice straw-containing cultures were significantly higher than in cultures containing 5% rice straw over the first 12 days of incubation. Interesting but not surprising, our results also showed rice straw addition quantity displayed positive correlation with the degradation rate under the manual treatment condition. For instance, the degradation rate of 3%-M-4 was higher than 3%-control, improving 33.3% after 20 days, while that which was 45.6% for 5%-M-4 compared to 5%-control. MC1 degraded rice straw more efficiently in CMPR compared to pHMA. The total degradation rate of 5%-M-4 (78.9%) was significantly higher than 5%-pH (67.5%) after 20 days. In particular, rice straw loss was very high, with a final loss of 83.3%

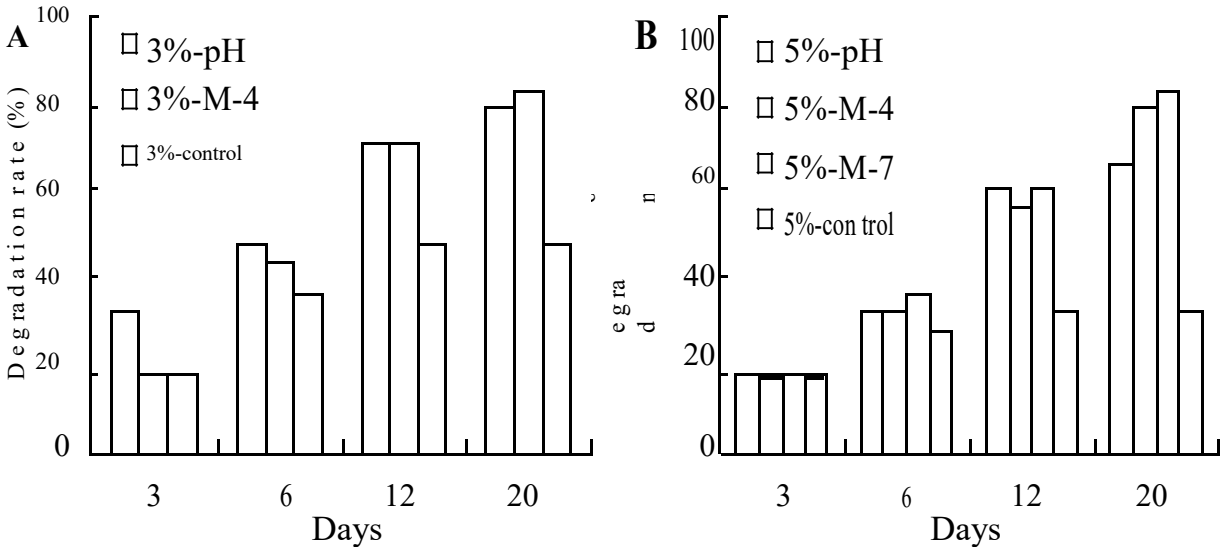

Figure 3. Degradation rates in the experiment of pH manual adjustment and culture medium partial replacement for A) 3% rice straw B) 5% rice straw.

after 20 days.

It has been reported that pH change in rice straw fermentation indicated the degradation capability of MC1 in previous research (Cui et al., 2002; Liu et al., 2006). If pH increased after a rapid drop because of the rice straw degradation, the degrading capability of this system would continue. Otherwise, if pH maintained a low level and would not increase for an extended period of time, the degrading capability of this system would be limited. The results from this study indicated that manual adjustment of the pH could improve degradation rate when pH did not increase naturally. The method of CMPR had less effect on the pH increase than pHMA. However, the degradation rate of the former was higher than the later, especially for the high frequency of CMPR. It also has been reported that the key strain for rice straw degradation by MC1 was CSK-1 (*Clostridium straminisolvens* sp.nov.), which produced small molecular sugars and organic acid (Kato et al., 2004b). Other strains (M1-3, M1-5, M1-6 etc.) would degrade the product produced by CSK-1 with final conversion to CO_2 (Kato et al., 2004a). Eventually, the decreasing pH and the excessive accumulating products would be relieved. These network relationships among microbes promoted by the metabolic cycle from cellulose to CO_2 ensured high-efficiency degradation by MC1 (Kato et al., 2005; Kato et al., 2008). The stability of this metabolic cycle was influenced by the concentration of rice straw addition. pH rose back to the initial value in 10 days and recovered the degrading capability of MC1 for 1% rice straw addition, while pH partly rose back after 80 days and did not recover the degrading capability (Liu et al., 2006). In this study, it was indicated that pH could rise back quickly and promote degradation consistently in tests with 5% rice straw addition using the pHMA or CMPR. Comparing these two treatments, the degradation rate in CMPR was higher. Generally speaking, pHMA relieved the survival environment for the microbes and continued the degradation. The CMPR was fundamentally a measure for consecutive degrading because of reducing production concentration in fermentation system. Meanwhile, the broth obtained from CMPR contained many small molecular substances (for instance, ethanol, acetic acid, glycol, propionic acid, butyric acid, glycerol etc.), which could be utilized for biogas fermentation or further application (Liu et al., 2006). On the other hand, the pHMA treatment was also an efficient method with the simple operation and low cost for batch fermentation. Those characteristics of MC1 facilitated a large of quantity rice straw degradation and enhanced its development.

At present, research on the saccharification of lignocellulose to produce ethanol is conducted worldwide (Kumar et al., 2008). However, other avenues should be explored. The use of lignocelluloses for methane fermentation should be studied to determine if small molecular soluble could be produced. Thus, researches on the development of these small molecular soluble components accumulated during the MC1 degradation process are necessary and valuable.

Degradation of rice straw components

Components (ligin, cellulose, hemicellulose, soluble) of residue after degradation by MC1 with 3 and 5% rice straw addition were listed in Figure 4A and B, respectively.

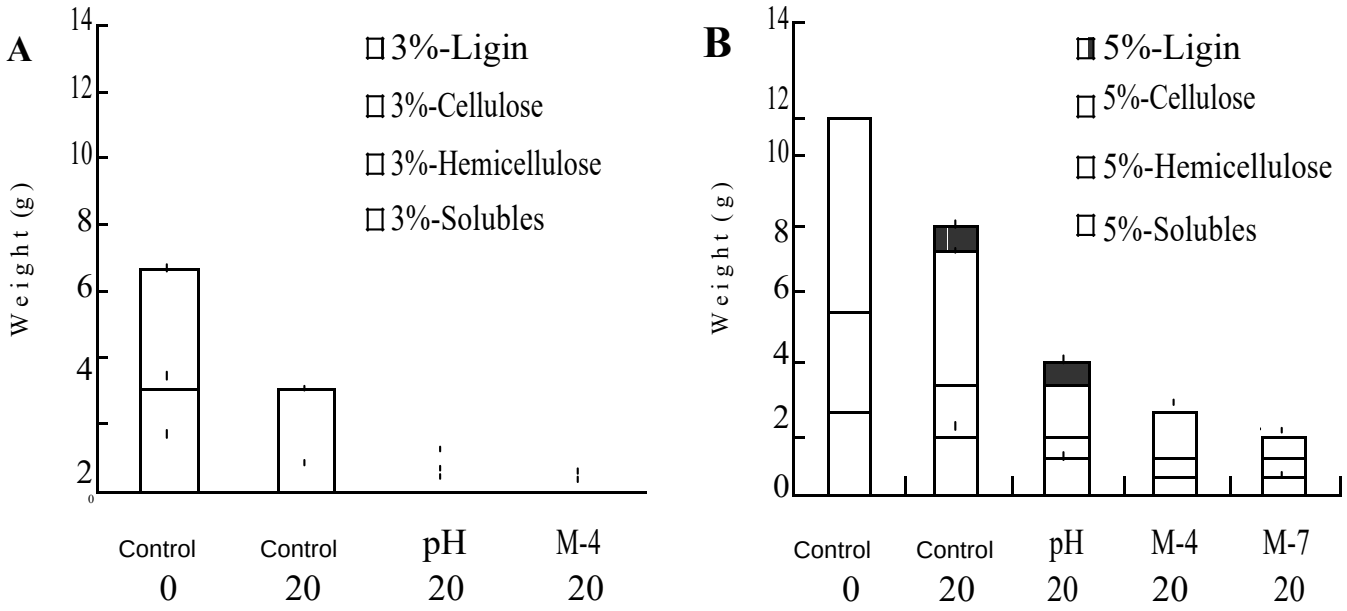

Figure 4. Analysis of major components of rice straw in the experiment of pH manual adjustment and culture medium partial replacement for A) 3% rice straw B) 5% rice straw; control 0: The first zero day sample of non-treatment; control 20: The 20th day sample of non-treatment; pH 20: The 20th day sample of pH manual adjusted treatment.; M-4 20: The 20th day sample of culture medium partial replacement for four times; M-7 20: The 20th day sample of culture medium partial replacement for seven times.

The total content of straw decreased substantially after 20 days, with the hemicellulose showing the greatest decrease, the cellulose decrease was intermediate and the lignin decrease was the least. The cellulose decrease with the 5% rice straw addition was less than 3%, which elucidated that excessive rice straw added to the degradation system result in the cellulose degradation rate sharply decreased.

After 20 days, the degradation rate of cellulose and hemicelluloses in samples with pHMA and CMPR was considerably higher than the control. As described in Figure 4B, degradation rate of cellulose and hemicelluloses in 5%-control were only 33.6 and 53.2%, respectively. However, in 5%-pH, the rate increased greatly to 72.9 and 77.8%, respectively, which is up to 24.7 and 39.3% higher than the control. In 5%-M-4, degradation rate of cellulose and hemicelluloses was 79.9 and 77.6%, respectively, which is 46.3 and 24.4% higher than the control. In 5%-M-7, degradation rate of cellulose and hemicelluloses was 86.5 and 80.5%, respectively, which is 52.9 and 27.3% higher than the control. Depending on the results obtained above, the following observations are possible:

1. Measurement of pHMA and CMPR would greatly improve the degradation capability of cellulose and hemicelluloses of MC1. The method of CMPR exhibited more available influence than pHMA
2. Whereas the hemicellulose degradation rates were almost equal with different treatments, the total mass losses in cellulose were very different in these experiments. Thus, treatments for MC1 degrading improvement generally function in cellulose degrading. Cellulose was the maximum content in rice straw, and it could be converted into glucose monomers which was major product in microbial carbon metabolism (Kumar et al., 2008). Therefore, it is very important to promote the cellulose degradation in lignocellulose degradation. The research on the method of improving the degradation was necessary. In particular, the study on continuous fermentation by CMPR, which with improving the degradation rate and collecting the decomposition products, has an important value for the technology development of cellulose degradation.

Concentration of soluble sugar

In the controls, the concentration of soluble sugars in the system dropped rapidly at the beginning and then gradually rose as the process continued (Table 1). This pattern was similar for 3 and 5% rice straw addition. However, for samples with pHMA or CMPR, the concentration of soluble sugars declined consistently over 20 days. For the treatment of CMPR, the concentration decrease was the greatest. Comparing this result with the degradation rates of different treatments, the concentration of soluble sugars in the control with low degradation rates accumulated gradually during the whole process. For the treatment of CMPR, although it

Table 1. The concentration of soluble sugar in the experiment of pH adjustement and culture medium partial replacement.

Time (days)	3%-pH	3%-M-4	3%-control	5%-pH	5%-M-4	5%-M-7	5%-control
	mg/ml (3%)			mg/ml (5%)			
0	0.88±0.04^{a}	0.88±0.04^{a}	0.88±0.04^{a}	1.7±0.06^{a}	1.7±0.06^{a}	1.7±0.06^{a}	1.7±0.06^{a}
3	0.46±0.02^{b}	0.47±0.02^{ab}	0.5±0.02^{a}	0.67±0.06^{b}	0.92±0.03^{a}	0.51±0.03^{c}	0.91±0.01^{a}
6	0.54±0.02^{b}	0.38±0.01^{c}	0.61±0.03^{a}	0.78±0.06^{b}	0.72±0.01^{b}	0.33±0.02^{c}	1.04±0.05^{a}
12	0.52±0.01^{b}	0.18±0.01^{c}	0.65±0.07^{a}	0.67±0.07^{b}	0.25±0.02^{c}	0.16±0.01^{c}	0.92±0.03^{a}
20	0.52±0.03^{b}	0.14±0.01^{c}	0.71±0.04^{a}	0.78±0.02^{b}	0.14±0.01^{c}	0.12±0.01^{c}	1.58±0.11^{a}

Data are presented as mean ± standard deviation (n = 3). ^{a,b,c,d} Within a row, means followed by different superscripts are significantly different (P＜0.05).3%, 3% rice straw treatment, 5%, 5% rice straw treatment.

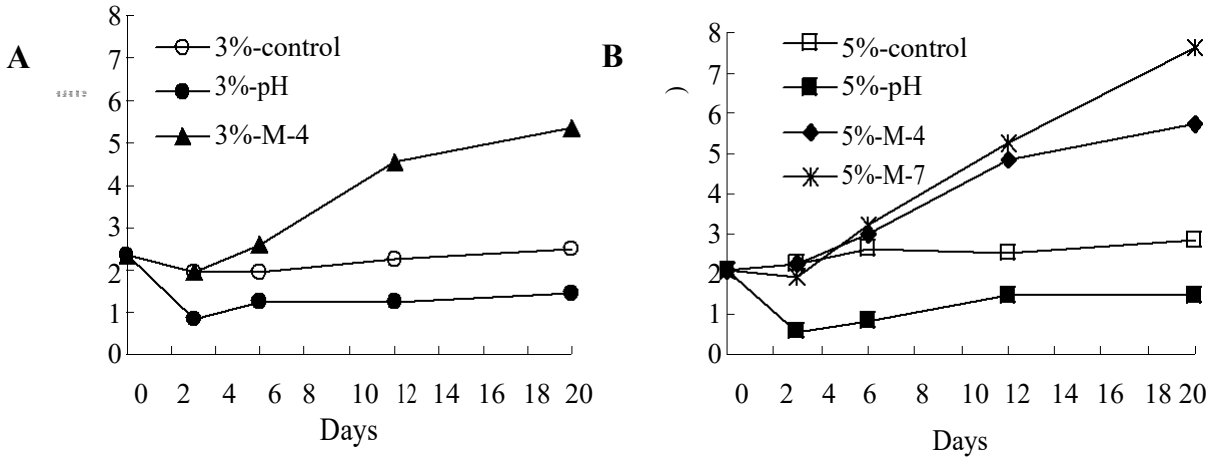

Figure 5. Weights of water-soluble substances in the experiment of pH manual adjustment and culture medium partial replacement for A) 3% rice straw B) 5% rice straw.

had the highest degradation rate, the concentration of soluble sugar declined the most. So the relation between degradation rates and soluble sugar accumulation was negative correlation in this fermentation system.

Concentration of water-soluble substances

The concentration of water-soluble substances in the control declined at the beginning and then gradually increased. For samples with pHMA or CMPR, the concentration declined throughout the whole process. Considering the outcome from CMPR, the total accumulation of water-soluble substances in samples was calculated (Figure 5A and B). The concentration of water-soluble substances with pHMA was always lower than the control. However, for samples with CMPR, the total accumulation of water-soluble substances gradually rose and was highest at the end of the experiment. The trend of water-soluble substances with 3% rice straw addition was similar to those in 5%. Our results also showed that rice straw addition quantity displayed positive correlation with the accumulation of water-soluble substances just as the relationship with degradation rate by the manual treatments (Figure 5). The result demonstrated that the major reason of the improvement of degradation was the removal of water-soluble substances inside the system. It was considered that pHMA improved the growing environment of microbe inside the fermentation system, which would facilitate MC1 to remove water-soluble substances continuously during degrading process (Kato et al., 2004a), with remission of the pH reduction and product accumulation (Kato et al., 2005, 2008). Comparing it with CMPR, the latter not only reduced the concentration of water-soluble outcome to facilitate degradation, but also collected the decomposition products by replacement operation. It should be available for the further discussion and utilization.

Analysis of DNA concentration

The amount of DNA was detected in the unit fermentation

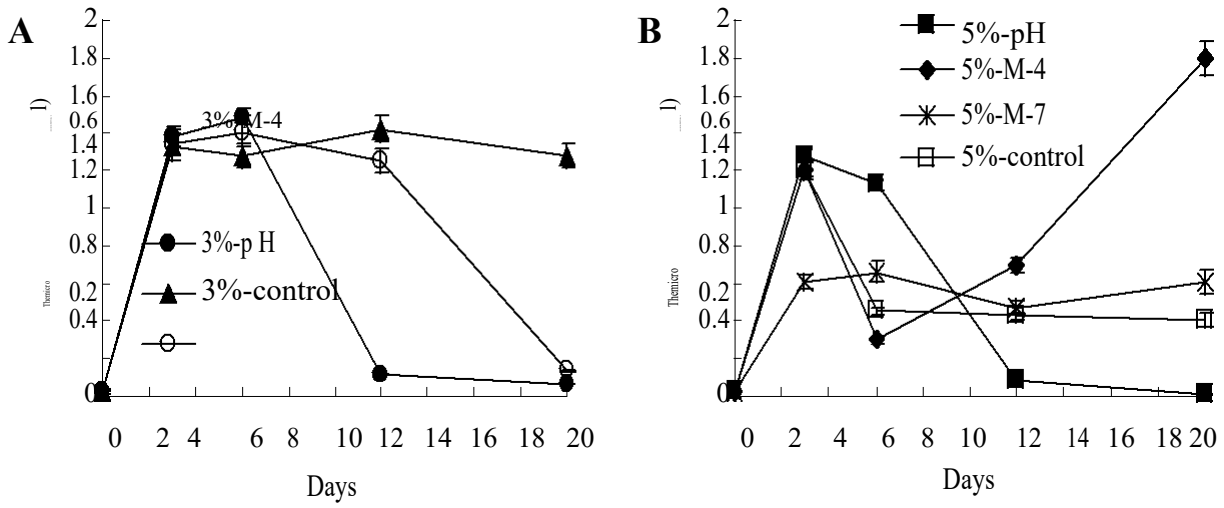

Figure 6. Concentrations of microbial DNA in the experiment of pH manual adjustment and culture medium partial replacement for A) 3% rice straw B) 5% rice straw.

solution and clearly expressed the microbe quantities during the whole degradation process (Yang et al. 2007). For treatments with 3% rice straw addition, the amount of DNA increased rapidly in the first 2 days (Figure 6A) to 1.2-1.4 mg/ml and then slowed considerably. After 6 days, the amount of DNA in the control declined and reached 0.2 mg/ml after 20 days. In the pHMA experiment, it declined earlier to 0.2 mg/ml after 12 days of incubation. For samples with CMPR, the DNA amount always maintained a high concentration. However, the DNA amount in treatments with 5% rice straw addition (Figure 6B) was different from the former. A peak of DNA amount appeared after 3 days and then declined sharply. For the control and 5%-M-4, its DNA concentration was stable at 0.5 mg/ml. For 5%-pH, the DNA concentration dropped to below 0.1 mg/ml after 12 days, while for 5%-M-7 rose to 1.8 mg/ml.

From the above changes in DNA concentration, it was indicated that microbe quantities with pHMA declined to a low level. Whereas the treatments of pHMA relieved the pH reduction and improved the rice straw degradation rate, it did not improve the growing environment for microbe propagation. In contrast, the treatment with CMPR removed many strains from the fermentation system, which greatly promoted microbe propagation and maintained the stability of the strains. Therefore, the results demonstrated that the method of CMPR could guarantee the degradation of rice straw consistently by MC1.

Changes of microbial community on rice straw during the degradation

PCR-DGGE was conducted to analyze changes of microbial community during 20 days of degradation by MC1 (Figures 7 and 8). The appearance and disappearance of bands in the DGGE pattern indicated important shifts in the microbial community structure. The bands on the profile were excised and amplified by PCR. The DGGE profile of microbial community in control was the same as the previous results after sequencing DNA fragments (Haruta et al., 2002). Band A was stably associated with rice straw during the degradation process which had a 99.5% similarity with *C. thermosuccinogenes* (Kato et al., 2004a). It was reported that this bacterium utilizes cellobiose, xylose, glucose, and sucrose and produces acetate, lactate, and H_2 by fermentation (Haruta et al., 2002). Bands B, C-E, and F were derived from bacteria belonging to the genera *Bordetella*, *Brevibacillus*, and *Pseudoxanthomonas*, respectively (Haruta et al., 2002).

From the comparison of DGGE bands in Figure 7 between 5%-pH and 5%-control, 1-9 new bands previously absent were found in the profile in samples with pHMA during the fermentation process by MC1. Those 9 new bands on the profile were excised and amplified by PCR. The DNA fragments were sequenced and analyzed during BLAST. The phylogenetic tree was constructed (Figure 9). The result showed that the band 8 had a higher identity (99.2%) to rumen bacterium (EU124834.1) and uncultured *Clostridium* sp. (EF710221.1). It has been reported in a previous study that rumen bacteria were considered to be primarily responsible for the biological degradation of plant fiber, due to their high fibrocystic activity and large biomass in the rumen (Koike et al., 2010). The other bans were not found to have any identity to cellulose bacteria. There exists also another possibility that these bacteria only utilize the products released from rice straw degradation.

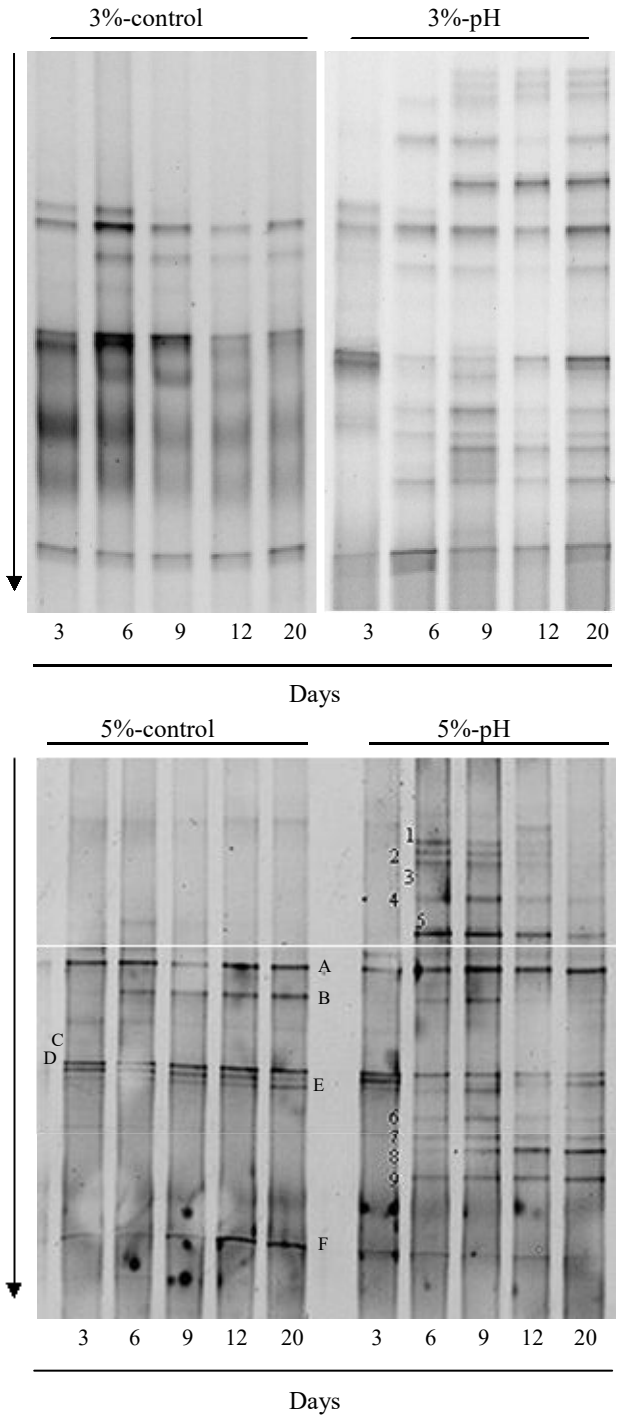

Figure 7. The denaturing gradient gel electrophoresis patterns of the 16S rDNA fragments in the experiment of pH manual adjustment. The arrow represents the direction of the denaturant (35–50%) and the polyacrylamide (6–12%) gradients.

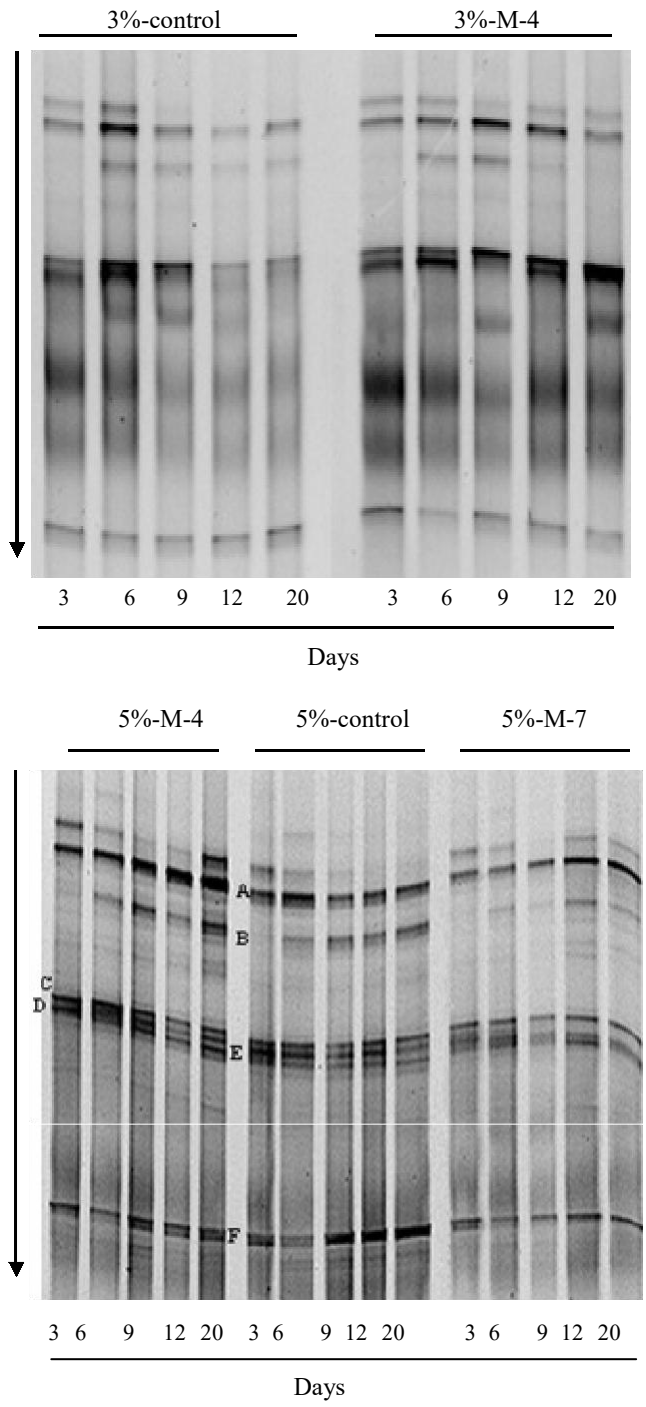

Figure 8. The denaturing gradient gel electrophoresis patterns of the 16S rDNA fragments in the experiment of culture medium partial replacement. The arrow represents the direction of the denaturant (35–50%) and the polyacrylamide (6–12%) gradients.

Therefore, experiments of pHMA could promote the growth of these microbes inside MC1, especially for rumen bacterium and *Clostridiaceae* bacterium which have the capability for cellulose degradation. In Figure 8, the DGGE dynamic profiles of each treatment were similar to others. It also indicated that major structure of microbial community was stable and bands of preponderant microbes were consistent during the

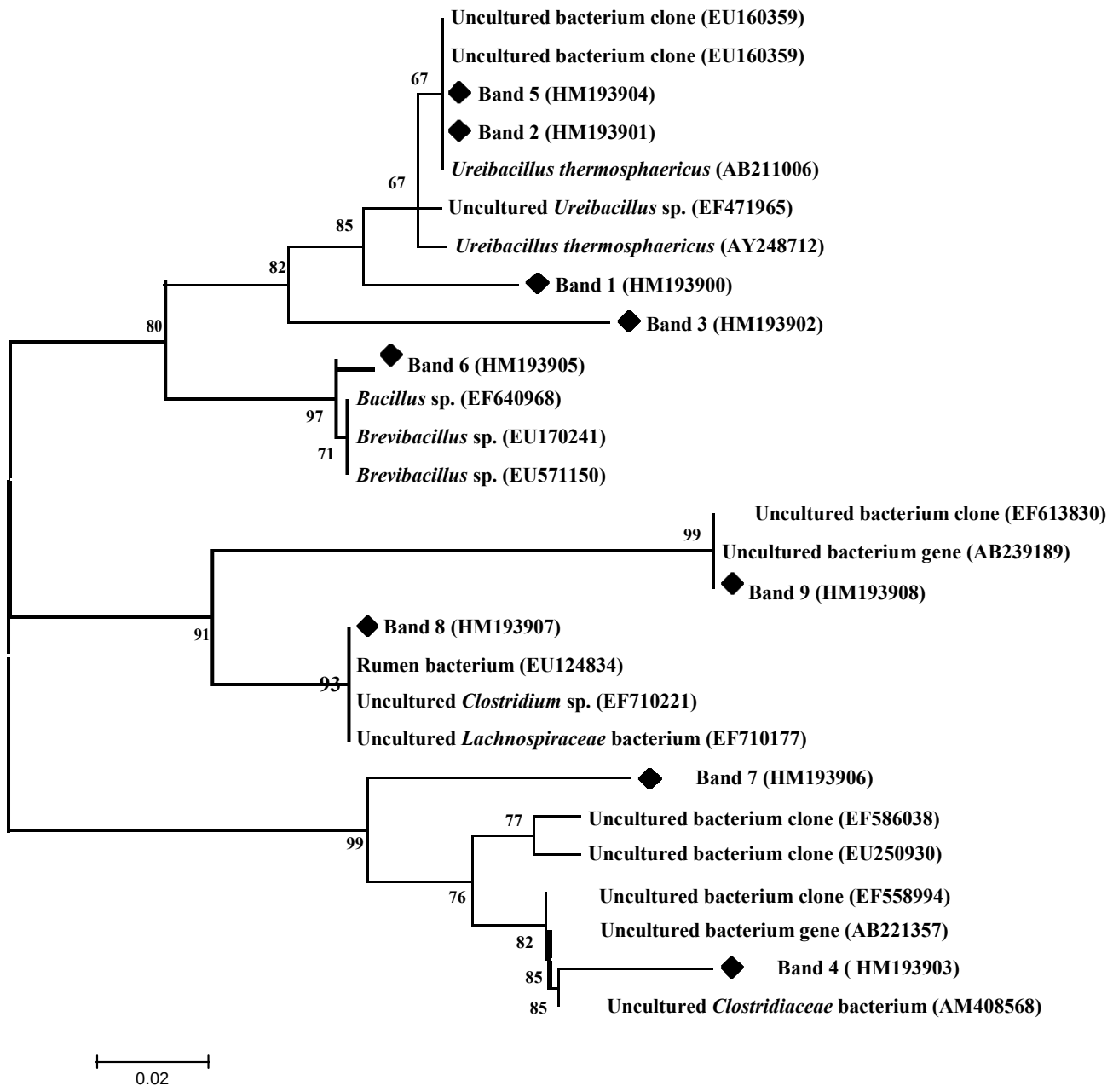

Figure 9. Phylogenetic tree derived from partial 16S rDNA sequence of 9 new bands on the profiles. The tree was constructed by the neighbor joining method.

incubation by CMPR.

Cellulose is the main polymeric component of the plant cell wall and the cellulolytic microorganisms include protozoa, fungi and bacteria and are ubiquitous in nature. So far, there were most information about the degradation of lignocellulose as a result of fungal strains and their extracelluar enzymes. For examples, *Trichoderma reesei* is the most widely employed fungus for the production of cellulolytic enzymes and has been extensively studied (Stockton et al., 1991). Nevertheless, the significant importance of the bacteria should be noticed. This work demonstrated that MC1, which consisted of bacteria, was able to efficiently degrade rice straw. Meanwhile, the degradation rate of rice straw was improved significantly by CMPR, which was in agreement with previous studies. It was reported that removal of the excess of sugars promotes further cellulose degradation by the primary species because cellobiose induced inhibition of cellulase action and repression of cellulase synthesis were precluded (Bayer et al., 2006).

Conclusion

With improving the potential degradation of lignocellulose-degradation community MC1, the treatment of pHMA and CMPR offered an alternative during rice straw fermenting system. The degradation rate in control (5% rice straw feed) increased, ranging from 33.3 to 67.5% via pHMA method, and for treatment with CMPR which was elevated to 83.3%. With molecular footprint technology, 9 bands undetected in the previous work were discovered in MC1 from the DGGE profile, especially for related species of rumen and *clostridiaceae* bacterium which could degrade cellulose. In the treatment with CMPR, microbial composition was stable and the band of dominant microbe was legible in the absence of new bands. Relying on the analysis of soluble products from the two treatments, it was demonstrated that CMPR technology could not only facilitate the degradation but also accumulated quantities of soluble products.

ACKNOWLEDGMENTS

This work was supported by the National Commonwealth Industrial (Agriculture) Specific Research (No. 200803033) and Development Program of China during the 11th Five-Year Plan Period (No. 2006BAD07A01).

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