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Bioconversion of corn straw by coupling ensiling and solid-state fermentation

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Abstract

A two-stage process that combined solid-state fermentation (SSF) and ensiling was used for bioconversion of corn straw, in order to increase nutritional value and palatability for animal feed. SSF of corn straw increased the level of protein from 6.7% to 14.7% and decreased the cellulose by 38.0% and hemicellulose by 21.2%. Cellulase and xylanase were produced during SSF. After SSF, the fermented substrate was directly ensiled by inoculating with lactic acid bacteria (LAB). In situ produced enzymes and bacterial inoculation resulted in a rapid drop in pH, a high level of lactic acid production, partial degradation of cell wall components and generation of reducing sugars (RSs). Efficiency of ensiling at 25°C, 30°C, 35°C, 40°C was evaluated. Temperature influenced the effect of ensiling; the higher the temperature, the shorter the ensiling period. The combined fermentation upgraded the nutritional value, enhanced the efficiency of ensiling and reduced bioprocessing costs.

Keywords: Corn straw; Solid-state fermentation; Ensiling; Bioconversion

1. Introduction

Agricultural straw consists mainly of cellulose, hemicellulose and lignin: it is the most abundant renewable biopolymer. Much of this material is disposed of by burning, which results in environmental pollution; only a little is used as feed for ruminants. Unfortunately, its low energy, low digestibility and protein content prevent its use in feedlots. Various methods have been adopted for improving the nutritive value of straw, bioconversion of straw by solid-state fermentation (SSF) is often used due to its low effluent generation, requirement for simple fermentation equipment and the direct applicability of the fermented product for feeding. During SSF, microorganisms produce cellulase, degrade components of the cell wall and synthesize microbial protein. A number of microorganisms and straws have been tried for producing microbial protein and cellulase by SSF (Gao et al., 1997; Bisaria et al., 1997; Sermanni et al., 1994). By SSF, nutritive values and digestibility of straw are increased, but the fermented product has poor palatability and preservation; on the other hand, SSF is carried out in a simple way, fermentation proceeds for a long time (at least a week), contamination often occurs, especially on scale up, so further treatment is necessary for the product of SSF.

Ensiling is a forage crop preservation method which has been in use for centuries, a combination of anaerobic conditions and acidity protects the forage from proliferation of deleterious bacteria and fungi, it also increases the palatability of the forage due to the lactic acid produced, but straw used directly ensiles only poorly, due to the low content of soluble carbohydrates. Addition of cellulases to straw at ensiling should increase the availability of readily fermentable carbohydrates and increase organic acid content (Singh et al., 1990). In recent years, a biological technology, simultaneous lactic acid fermentation and enzyme hydrolysis by cell wall degrading enzymes (ENLAC) has been used widely in plant processing to increase fiber digestibility of forages (Tengerdy et al., 1991) and to increase the recovery of cell content from plants (Tengerdy et al., 1992; Weinberg et al., 1990). This technology evolves from attempts to improve the ensiling process with silage additives that contain both lactic acid bacteria

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(LAB) and cell wall hydrolyzing enzymes. When combinations of cell wall degrading enzymes and microbial inoculants are used, enzymatic degradation of fiber should provide more sugars to the LAB leading to a more rapid achievement of a stable pH, thus improving the silages. Addition of enzymes is capable of increasing the amount of substrate available for fermentation, but the use of enzymes is limited by the high price of commercial enzymes. A possible alternative is the production of the necessary enzymes in situ in plant residues by SSF (Tengerdy et al., 1996), the fermented substrate may be used as in situ source of plant cell wall degrading enzymes for agro-biotechnological processes such as ensiling and animal feed up-grading (Tengerdy et al., 1991). In this paper, a process is given which combines SSF with ensiling to bioprocess corn straw.

2. Methods

2.1. Microorganisms and inocula

Penicillium decumbens NO.1 used in SSF was obtained from the Institute of Microbiology, Shandong University, China. The stock culture was grown on potato dextrose agar slants for 8 d at 28°C, then spores were washed off with sterile water, and spore suspension were used for inoculating the substrate. *Lactobacillus plantarum* used in ENLAC was obtained from the China Committee for Culture Collection of Microorganisms (CCCCM). *L. plantarum* was prepared by growing it in MRS broth at 37°C for 8 h.

2.2. Solid-state fermentation

SSF was carried out in Petri dishes. Each Petri dish was loaded with 10 g dry weight substrate containing 90% corn straw (milled to 0.5–2.5 mm particle size) and 10% wheat bran, 25 ml feed solution containing 0.5 g $(NH_4)_2SO_4$, 0.2 g KH_2PO_4, 0.16 g K_2HPO_4, 0.02 g $MgSO_4 \cdot 7H_2O$ was added and mixed well, the pH was adjusted to 5.5. After sterilization (121°C, 30 min) and cooling to room temperature, 0.5 ml (6.43×10^7 spores/ml) spore suspension was aseptically sprayed over the substrate and mixed well. Petri dishes were incubated at 28°C for 4 d.

2.3. ENLAC treatment

After SSF, the fermented substrate was inoculated with molasses, at the 5% level on dry fermented substrate, and a culture of *L. plantarum* containing 1.5×10^9 cfu/ml to give an initial count of 3×10^8 cfu/g dry substrate. The inoculated substrate was mixed well and packed into glass jars and ensiled at different temperatures. Each treatment had three replicates, and each sample was assayed in duplicate.

2.4. Analytical methods

Cellulose, hemicellulose were estimated according to the procedures of Van Soest (Goering and Van Soest, 1970). Nitrogen was estimated by a micro-Kjeldahl method. Crude protein was determined by multiplying the nitrogen content by 6.25. The samples of bioconverted residues were washed with water to remove soluble nitrogenous compounds before analysis. In vitro dry matter digestibility (IVDMD) was determined as described by Jones and Hayward (1975). For estimation of enzyme activities, 4 g fermented residues (dry weight bases) were suspended in 100 ml citrate buffer (pH 4.8, 0.02 M) and shaken gently for 2 h and filtered, the supernatant was used for the enzyme activities determination. Filter paper activity (FPA), endoglucanase activity (CMCase). β-glucosidase activity (βGA), and xylanase activities were measured as described by Ghose (1987). One international unit (IU) of enzyme activity was defined as the amount of enzyme that released 1 μmol product per min.

For reducing sugars (RSs), lactic acid and pH determination, 1 g wet sample was diluted with 9 ml distilled water, blended for 2 min, then centrifuged. The supernatant was used for the assay. RS was determined by the dinitrosalicylic acid method (Miller, 1959). Lactic acid was determined according to Steinsholt and Calbert (1960). The pH was determined with a pH meter (pHS-25 model).

3. Results and discussion

3.1. SSF

Economical utilization of lignocellulosic material is hindered because of the high cost of cellulase production. One effective approach to reduce the cost of enzyme production is to replace pure cellulose as substrate with relatively cheaper materials, such as lignocellulosics. There have been reports of successful attempts to produce cellulase on lignocellulosics (Gupte and Madamwar, 1997; Chahal and Chahal, 1996); the development of technology with minimum capital investment is another approach to reduce the cost of cellulase production: an SSF process is suitable. In this work, SSF of corn straw was carried out; cellulase, xylanase and β-glucosidase were produced. The FPA, CMCase activity, β-glucosidase and xylanase activities attained 2.88, 9.05, 27.66, 13.59 IU/g, respectively, after 4 d of culture. A high level of β-glucosidase was produced by *Penicillium decumbens*. β-Glucosidase provides a key catalytic activity to cellulase preparations and complete

saccharification (Pushalkar et al., 1995). Several attempts have been made to increase β-glucosidase amount either by improving the strain or by supplementing with β-glucosidase externally (Gupte and Madamwar, 1997). Enzyme activities are still low when compared with results obtained by other authors (Duenas et al., 1995), there is still considerable scope to improve cellulase yield by optimization of the fermentation and by pretreatment of substrate.

Fermentation changed the composition of the straw. The protein content increased from 6.7% in unfermented straw to 14.7% in fermented straw. Hemicellulose and cellulose content decreased from 33.1% and 25.8% to 26.1% and 16%, respectively. In order to improve performance of SSF, optimization of the conditions of fermentation and pretreatment of substrate are necessary.

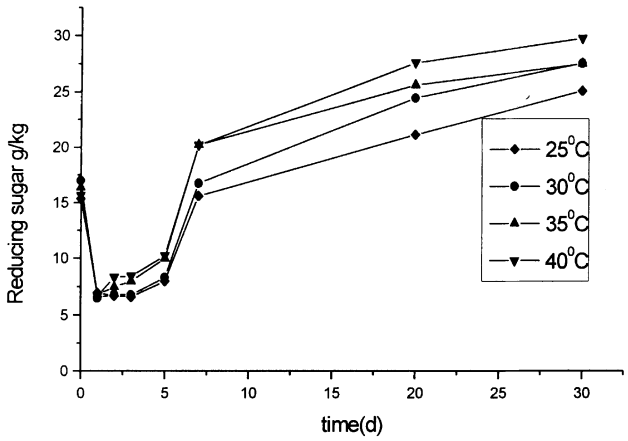

Fig. 1. RS production during ENLAC as a function of time.

3.2. ENLAC

After SSF, fermented substrates were ensiled by inoculating with LAB. Effects of different temperatures were studied. In a 30 d ENLAC treatment the ensiling fermentation proceeded normally, based on changes of pH in each treatment. An initially rapid decrease in pH (6.3–4.5) was observed from all temperatures during the first day, then the pH values exhibited a gradual decline. The pH values depended on the length of the ensiling period and temperature, higher the temperature and longer the period, lower the pH. Most of the pathogenic bacteria are killed in silage with pH lower than 5.0 (Kamra and Svivastava, 1991). In the present study, a pH lower than 4.2 could be reached during a week at 40°C, which could inhibit contamination.

Lactic acid production was rapid in the first week, then increased slowly. High temperature resulted in active microbial metabolism, especially at 35°C, lactic acid was produced at approximately 24 g per kilogram dry matter at 20 d of ensiling.

RS extracted from the ensilage sample showed conversion of the polymeric carbohydrates into soluble sugars during ensiling. Fig. 1 shows extractable RS as a function of ensiling time for the four treatments. The initial rapid decline in RS was due to fermentation of RSs to organic acids, but subsequent gradual accumulation of RS showed conversion of the polymeric carbohydrates into soluble sugars (Henk and Linda, 1992). The RS content varied, depending on the length of the ensiling period and temperature. The maximum RS yield was observed at 40°C. This temperature was more suitable for cellulase activity.

ENLAC increased the digestibility of corn straw. At 25°C, 30°C, 35°C, IVDMD increased with time, but at 40°C IVDMD reached the maximum in 7 d ensiling, IVDMD increasing from 43.4% to 49.7%. After 7 d, IVDMD did not increase. This might have been because conversion of the digestible carbohydrates into volatile acids left more material resistant to digestion; or lignocellulolytic activity decreased quickly at high temperature. It might also be that digestible material was used for respiration by silage microorganisms when ensiled for longer time.

Data obtained by fiber analysis are shown in Table 1. Contents of hemicellulose and cellulose in corn straw ensilage decreased.

In conclusion, corn straw has low nutritional values and poor palatability. The combined SSF-ensiling fermentation process overcame some of these limitations. SSF increased nutritional value and enhanced the effect of ensiling by in situ enzyme production.

Table 1
Changes in cellulose and hemicellulose during ENLAC (% DW)

Time (d)	25°C[a]		30°C		35°C		40°C	
	HC	C	HC	C	HC	C	HC	C
0	20.9	15.5	19.3	16.2	20.7	12.4	20.0	10.1
7	20.9	15.4	18.8	13.9	20.5	11.3	19.6	8.9
20	18.7	14.2	17.8	15.1	17.4	11.3	17.7	9.1
30	17.6	14.6	18.6	15.7	17.9	10.4	19.1	8.8

[a] Temperature of ENLAC; HC: hemicellulose; C: cellulose.

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