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Microbial and enzymatic improvement of anaerobic digestion of waste biomass

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Abstract

Metabolic activities of different microorganisms (*Bacillus subtilis*, *B. licheniformis* and *Aspergillus niger*) and hydrolytic enzymes (concentrations: 1 to 200 mg enzyme solids g^{-1} feed) were studied individually and in combinations with respect to H_2 and methane production from damaged wheat grains. *Bacillus subtilis*, *B. licheniformis* and pre-existing hydrogen producers (control) produced 45 to 64 l H_2 kg^{-1} total solids and subsequently, with the help of added methanogens, 155 to 220 l methane kg^{-1} total solids could be produced. H_2 production from damaged wheat grains could be decreased to 28% or enhanced up to 152% with respect to control, by employing various microbial and enzymatic treatments. Similarly, it has been made possible to vary methane production capacities from as low as 17% to as high as 110% with respect to control.

Introduction

Anaerobic digestion is gaining importance as a process to treat and recover energy from biological wastes rich in organic matter (Dean 1998). Metabolically, diverse microorganisms interact to degrade complex organic matter to methane and CO_2. The production and/or accumulation of H_2 and volatile fatty acids such as propionic and butyric acids (Mackie & Bryant 1995) can adversely affect this process. These conditions can result in the failure of anaerobic digesters. Some of the techniques employed to revive such adversely affected digesters are decreased loading rates, increased retention of substrates (Callander & Barford 1983), prolonged hydraulic retention periods (Ramakrishna & Desai 1997), use of micro-nutrients (Raju *et al*. 1991, Rao & Seenayya 1994), stirring of the digester components (Joubert *et al*. 1985), etc. In this paper, *Aspergillus* and *Bacillus* and hydrolytic enzymes have been employed to improve this process.

Materials and methods

Feed stock material

Damaged wheat grains (dumping type) were procured from the Food Corporation of India (Kalia *et al*. 1994).

Organisms and growth conditions

Bacillus licheniformis strain JK1 (Kalia *et al*. 1994) and *B. subtilis* strain VC2 were isolated in our laboratory (identified by Microcheck, USA). These were grown and maintained on nutrient agar. *Aspergillus niger* was obtained from the Botany Department, Delhi University, Delhi, and grown on Czapek Dox medium at 28 °C for 10 days.

Enriched methanogens

Enrichment of methanogens was accomplished by incubation of cattle dung slurry (3% w/v total solids) at 37 to 40 °C for 16 to 20 days.

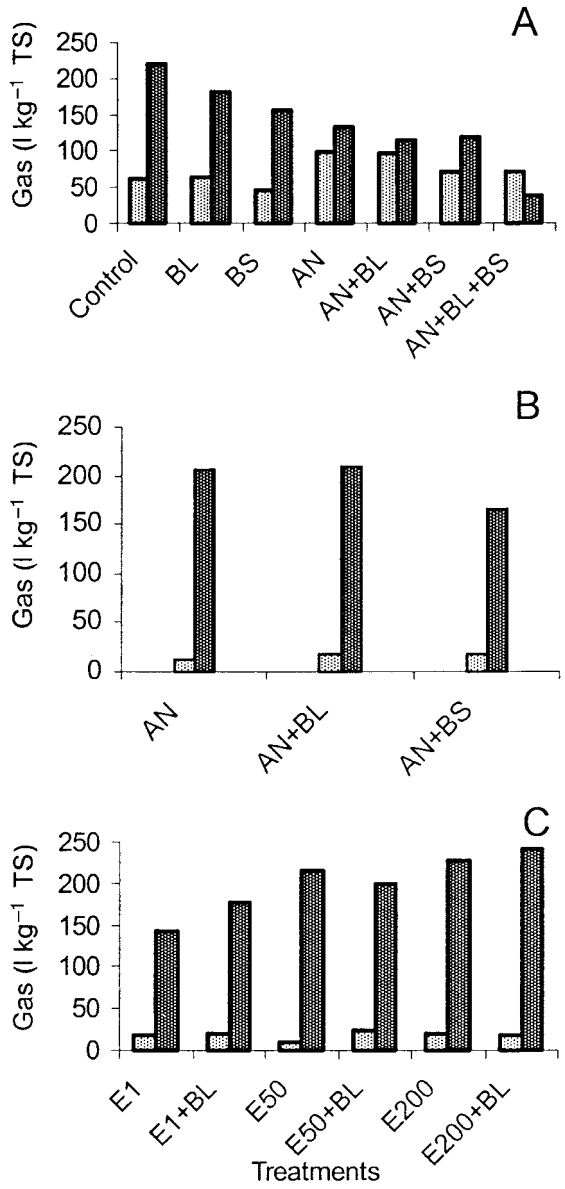

Fig. 1. H_2 and methane production from damaged wheat grains. Effect of 1 h (A) and 72 h (B) *Aspergillus niger* (AN) pretreatment and enzymatic pretreatment (24 h) (C). Microorganisms and enzymes were used individually and in combinations, where pretreatments were followed by inoculation with hydrogen producers: *Bacillus licheniformis* (BL); *B. subtilis* (BS); and with pre-existing bacteria (control). ▩, H_2; ▨, methane. E1, E50 and E200 are 1, 50 and 200 mg enzyme solids g^{-1} feed, respectively. TS: total solids.

Feed preparation

Seventeen g (dry wt) damaged wheat grains and 20 ml distilled water were placed in 300 ml bottles. The assembly with feed was autoclaved at 1 kg cm^{-2} steam pressure for 45 min, at 24-h intervals for 3 days. The various microbial and enzymatic treatments were given at this stage, individually and in combinations. In combination treatments, *Aspergillus niger* and enzymatic pretreatments always preceded inoculation with hydrogen producers. Methanogens were added at the end of the H_2 production phase. The final slurry was made up to 280 ml with distilled water.

Treatment

The following treatments were given to the feed:

(1) Feed was pretreated with 10 ml (10.3 mg protein) *Aspergillus niger* culture for 1 h and 72 h. The specific activity of cellulase and pectinase were 7 and 83 U mg^{-1} protein, respectively.

(2) Pretreatment with hydrolytic enzymes (Trizyme, a commercially available enzyme mixture containing cellulase – 0.98 U mg^{-1} solids, α-amylase – 0.8 U mg^{-1} solids and protease – 0.013 U mg^{-1} solids), at the rate of 1, 50 and 200 mg solids g^{-1} feed material at 37 °C for 24 h.

(3) Inoculation with 10 ml (50 mg protein) *Bacillus licheniformis* or *B. subtilis* culture.

(4) Uninoculated controls for H_2 production. The unsterilized feed material evolves H_2 with pre-existing hydrogen producers. This H_2 production decreased on repeated autoclaving of the feed. Even after three consecutive autoclaving at 24 h interval, 47% of the H_2 production could still be observed in comparison to unsterilized feed material. It indicates a decrease of population of pre-existing hydrogen producers.

(5) Inoculation with enriched methanogens for biomethanation.

(6) Protein and enzyme activities were done by standard procedures.

Digestion

Twohundred-eighty ml damaged wheat grain slurry containing 6% (w/v) total solids (TS) and 5.8% (w/v) organic solids, were prepared in distilled water. These were inoculated as described above (treatments) and incubated at 37 °C for 4 days for H_2 production. The pH was adjusted to 7 at the beginning and daily thereafter. Argon was flushed to make the inner atmosphere

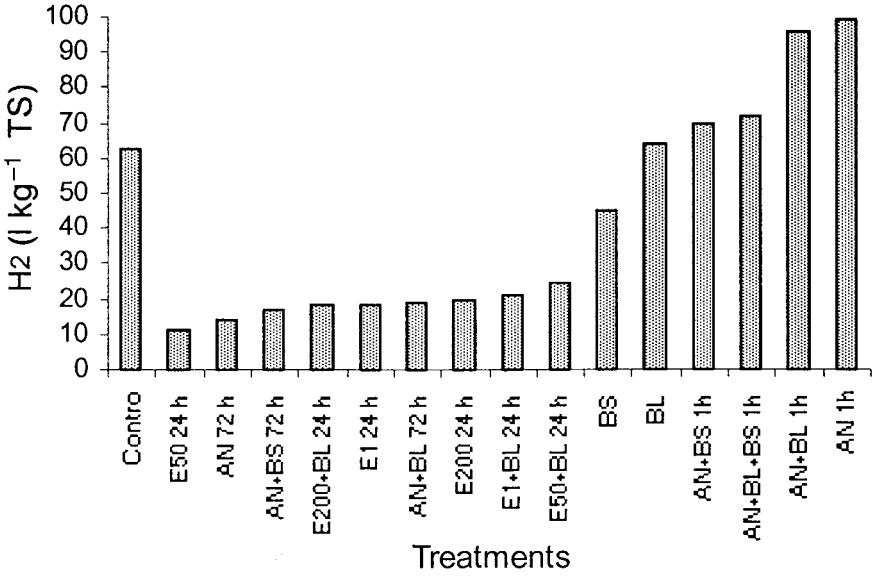

Fig. 2. Comparison of change in H_2 production by damaged wheat grains due to different treatments (microbial and enzymatic). Microorganisms and enzymes were used individually and in combinations, where pretreatments were followed by inoculation with hydrogen producers: *Bacillus licheniformis* (BL); *B. subtilis* (BS); and with pre-existing bacteria (control). E1, E50 and E200 are 1, 50 and 200 mg enzyme solids g^{-1} feed, respectively. TS: total solids.

O_2 free. The slurry was not stirred during incubation. At the end of the H_2 production phase, the slurry was further inoculated with enriched methanogens at 9:1 (v/v) ratio and incubation continued at 37 to 40 °C for a period till gas production ceased, which varied from 60 to 120 days. Gases were collected over water (pH 2) in graduated aspirator bottles. Gases, H_2, methane and CO_2 were analysed daily by standard GC procedures. The observations are based on two sets of experiments. The variations in the values were in the range of 5 to 10%.

Result and discussion

Hydrogen metabolism

From 45 to 64 l H_2 kg^{-1} TS could be generated from damaged wheat grains with the help of pre-existing hydrogen producers (control) or with *Bacillus licheniformis* or *B. subtilis* as inoculum. Short-term (1 h) pretreatment of damaged wheat grains with *Aspergillus niger* gave a 1.5-fold higher H_2 yield in all three cases, respectively (Figure 1A). However, *Bacillus subtilis* in combination with *Aspergillus niger* caused 30% decrease of the gains recorded in H_2 yield due to *Aspergillus niger*. Long-term (72 h) pretreatment of damaged wheat grains with *Aspergillus niger* inhibited H_2 generation (Figure 1B). Here, only 14 to 24% H_2 production was recorded as compared to 1 h pretreatment. Similarly, 18 to 32% decrease of H_2 production was recorded in pretreatment (24 h) with different hydrolytic enzyme concentrations (Figure 1C).

Methane metabolism

After the H_2 production phase, digestion of damaged wheat grains with enriched methanogens gave 220 l of methane kg^{-1} TS. Pretreatment (1 h) of damaged wheat grains with *Aspergillus niger* resulted in 23 to 38% decrease of methane yield in comparison to their respective controls (Figure 1A). This inhibitory effect on methane production was further enhanced by the combined effect of *Aspergillus niger*, *Bacillus licheniformis* and *B. subtilis* such that only 17% methane was produced in comparison to control (Figure 1A).

A metabolic shift in H_2 production towards methane production can also be achieved with the help of long-term (72 h) pretreatment of damaged wheat grains with *Aspergillus niger*. It gave 2.6–4.5-fold decrease of H_2 production and concomittantly there has been a switch over to recovery in methane production to the extent of 39 to 81%.

Aspergillus niger played a clear cut role in decreasing H_2 production and thus shifting the metabolism towards methane production, e.g., in 72 h pretreatment

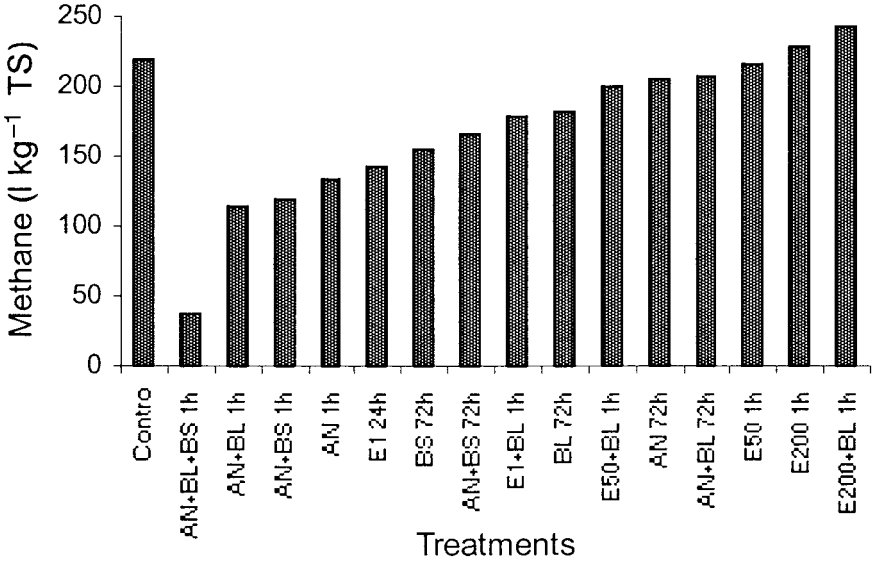

Fig. 3. Comparison of change in methane production by damaged wheat grains due to different treatments (microbial and enzymatic). Microorganisms and enzymes were used individually and in combinations, where pretreatments were followed by inoculation with hydrogen producers: *Bacillus licheniformis* (BL); *B. subtilis* (BS); and with pre-existing bacteria (control). E1, E50 and E200 are 1, 50 and 200 mg enzyme solids g^{-1} feed, respectively. TS: total solids.

of feed. Hence under certain stress conditions where the feed could produce H_2, sickening of digesters can occur. This situation can be averted by pre-treating the feed with *Aspergillus niger* for 72 h.

Similarly hydrolytic enzymes also have the tendency to decrease H_2 production and thus a shift towards methane production can be achieved (Figure 1C). Their role in supporting methane metabolism becomes evident due to 7 to 14% enhancement in its production in comparison to control.

Although recent studies have provided some solutions to the problems related to biogas digester failure, the use of microbes has not been much emphasized. In this study, H_2 production could be either decreased (72% inhibition) or enhanced (52% higher) with respect to control (Figure 2). Similarly, methane production could be decreased by 73%, recovered completely or even enhanced, though marginally above the control values (Figure 3). Hence, the overall metabolism can be improved to a large extent by employing microorganisms and enzymes.

Acknowledgement

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