Appl Microbiol Biotechnol (1990) 33:607-609

Applied and Microbiology Biotechnology

Short contribution

Utilization of spent agro-residues from mushroom cultivation for biogas production

Ragini Bisaria[1], **Padma Vasudevan**[1], **and V. S. Bisaria**[2]

[1] Centre for Rural Development and Appropriate Technology, Indian Institute of Technology, Hauz Khas, New Delhi - 110 016, India
[2] Biochemical Engineering Research Centre, Indian Institute of Technology, Hauz Khas, New Delhi - 110 016, India

Received 15 February 1990/Accepted 23 February 1990

Summary. Various spent agro-residues obtained after cultivation of the edible mushroom *Pleurotus sajor-caju* were used in anaerobic digestors for production of biogas. The changes that take place in the residues during bioconversion were quantified in terms of composition of cellulose, hemicellulose, lignin, carbon and nitrogen. These "mycostraws" resulted in increased biogas production over the untreated ones, which varied from 21.5% in the case of spent bagasse to 38.8% in the case of spent paddy straw. The increased biogas generation by the spent residues seems to be due to the increased susceptibility to digestion and more favourable C/N ratio of the residues.

Introduction

The efficiency of utilization of agro-residues in an anaerobic digestion process is determined by their overall digestibility. Lignin, which remains unutilized during biogas generation, determines to a large extent the digestibility of the residues. Single residues such as cow manure and water hyacinth provide suboptimal carbon/nitrogen ratios, restricting the availability of several enzyme systems responsible for the degradation of a variety of components present in the residues. The use of mixed residues with favourable C/N ratios as substrates has been reported to result in higher production of biogas (Ghose and Das 1982).

Biologically pretreated straws with white rot fungi such as *Pleurotus* sp. "florida" have been found to significantly enhance the biogas yield (Mueller and Troesch 1986). However the advantage gained in biogas production seems to be offset by the reduction in the amount of original straw on biological pretreatment. Spent residues such as those obtained after cultivation of edible mushrooms could be a better source of biologically pretreated substrates for biogas production (Bisaria et al. 1987a). Preliminary studies conducted in the authors' laboratory (Bisaria et al. 1983) have shown increased production of biogas from spent paddy and guar straws obtained after cultivation of the edible mushroom *Pleurotus sajor-caju*. The present work reports detailed investigations on biogas generation from a number of spent agro-residues and from those residues that were harvested during different stages of mushroom growth.

Offprint requests to: V. S. Bisaria

Materials and methods

Culture. Pleurotus sajor-caju (Fr.) Singer, obtained form Forest Research Institute, Dehradun, India, was used.

Substrates. Agricultural residues such as paddy straw (*Oryza sativa*), wheat straw (*Triticum aestivum*), bagasse (*Saccharum officinarum*), guar straw (*Cyamopsis tetragonoloba*), bajra straw (*Pennisetum typhoideum*), sarkanda leaves (*Saccharum munja*), maize straw (*Zea mays*) and jowar straw (*Sorghum vulgare*) were used.

Spawn. Spawn was prepared on unbroken wheat grains as described by Pal and Thapa (1979).

Cultivation. The cultivation of *P. sajor-caju* on agro-residues was carried out as described by Pal and Thapa (1979). The yield of fruit bodies on dry and wet weight bases is reported elsewhere (Bisaria et al. 1987b). The first flush of fruit bodies was harvested after 23 days and that of the second and third flushes after 28 and 36 days, respectively.

Biogas production. Anaerobic digestion of residues for biogas production was carried out in a 500-ml batch fermentor with small side ports at 37° C and an initial pH of 6.8-6.9. The gas outlet port was connected to a trap and a graduated cylinder. The bottles were tightly sealed. Fifteen-day-old digested cattle manure (N content = 1.9%) was used as inoculum at the 10% level. The total initial dry matter in the fermentor was 4%. Analysis of biogas was done by a gas chromatograph using a Porapak column (Nucon Engineers, New Delhi).

Analyses. Cellulose, hemicellulose and lignin content in the residues before and after mushroom growth were estimated by the method of Datta (1981). Carbon and nitrogen were determined by using a Perkin Elmer (Norwalk, Conn, USA) CHN Analyzer model 2400.

Reproducibility. The results reported are the average of five replicates. The variation in individual values was not more than 12% from the average value.

Results and discussion

After 36 days of mushroom growth, when the third flush of fruit bodies had been harvested, the residues were dried to determine the loss of organic matter and analysed for their cellulose, hemicellulose and lignin content (Table 1). These spent residues were used for biogas production in the anaerobic digestors as described in Materials and methods. The biogas produced from untreated and spent residues during 15 days is also given in Table 1.

It was found that maximum biogas production took place on spent paddy straw (108 l/kg solids), presumably because of its highest delignification (41.7%). This was followed by spent wheat straw on which biogas production was 97.3 l/kg solids in spite of low delignification. Probably, spent wheat straw was rendered more susceptible to digestion due to structural modifications in the residue after growth of *P. sajor-caju*.

Sarkanda leaves, with minimum delignification (13.4%) amongst the residues used, was found to yield the lowest amount of biogas (65.0 l/kg solids). However delignification is not the only criterion on which the usefulness of biological pretreatment of different residues can be assessed. The reorganization of the physical structure of the residues as a result of fungal growth could also contribute to the susceptibility or resistance to the action of hydrolytic enzymes in the digestor. Similar results have also been reported by Mueller and Troesch (1986) on the use of biologically delignified wheat straw for biogas production.

The carbon and nitrogen content of the residues (untreated and spent) were determined to see if the C/N ratios had been altered in the spent residues during the bioconversion process. The results indicate that the C/N ratios had changed from about 80 to 20 as a result of mushroom growth during 36 days (Table 1), which was partly due to the loss of organic matter. Another factor which contributed to the lower C/N ratios was an increase in the absolute amount of nitrogen as a result of bioconversion of residues due to mushroom growth. The total amount of nitrogen in the paddy straw, for example, just after inoculation with wheat spawn was 0.8%, which included the nitrogen contributed by the spawn (nitrogen content of wheat spawn = 3.68%). The nitrogen content of the bioconverted paddy straw was 1.46% after taking into account the weight loss (36%) of the substrate (or 2.08% without

Table 1. Change in composition of agroresidues as a result of mushroom growth and production of biogas on untreated and spent residues

Agro-residue	Composition of			C/N[a] ratio	Biogas[b] produced in 15 days (l/kg total solids)	Increase in biogas production (%)
	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)			
Paddy straw						
Untreated	34.5	25.0	16.8	80.8	77.5	—
Spent	27.0	21.1	9.8	19.8	107.8	38.8
Wheat straw						
Untreated	30.7	26.1	12.9	84.1	72.3	—
Spent	21.8	21.9	10.2	20.9	97.3	34.7
Guar straw						
Untreated	28.1	23.1	16.2	88.3	70.4	—
Spent	19.9	18.1	11.9	21.6	92.3	31.1
Bajra straw						
Untreated	30.0	26.0	15.1	76.5	65.4	—
Spent	19.5	19.0	10.1	21.4	82.5	26.1
Jowar straw						
Untreated	28.2	22.0	15.1	77.5	61.4	—
Spent	17.1	16.0	11.0	21.3	81.3	32.4
Maize straw						
Untreated	31.8	28.1	13.0	83.4	61.3	—
Spent	23.1	22.0	10.0	22.8	82.0	33.7
Bagasse						
Untreated	32.7	22.0	14.0	89.5	58.7	—
Spent	26.1	18.0	11.0	22.0	76.2	29.8
Sarkanda leaves						
Untreated	30.0	28.6	20.9	86.7	53.5	—
Spent	22.8	20.3	18.1	24.5	65.0	21.5

[a] Carbon content of untreated and spent paddy straw was 38.8% and 41.3%, respectively, and the nitrogen content 0.48% and 2.08%, respectively

[b] Methane content of biogas produced from untreated paddy straw was 60% and that from spent paddy straw was 64%

accounting for weight loss). There was thus an increase in nitrogen content to the extent of 18.3 mg/g loss of organic matter.

The increase in nitrogen content of the bioconverted residues was found to be due to the presence of certain bacteria such as those belonging to Group III of *Clostridium* spp. *Pleurotus sajor-caju* did not fix nitrogen, as confirmed by the acetylene reduction test for nitrogenase activity. The increase in nitrogen content of cellulosic residues due to participation of nitrogen-fixing bacteria is substantiated by the results of several workers (Lynch and Harper 1983; Halsall and Gibson 1985) who have shown that co-cultures of cellulolytic fungi such as *Penicillium corylophilum* and *Cellulomonas geladia* and nitrogen-fixing bacteria such as *Clostridium butyricum* and *Azospirillum brasilense* can coexist and effect substantial increases in the rate of substrate decomposition.

The effect of C/N ratio on biogas production was also studied by supplementing the untreated paddy- and wheat-straws with ammonium nitrate and using them for biogas generation. It was observed that an optimum C/N ratio of about 15–20 and 20–25 is required for paddy- and wheat-straw, respectively, for maximum generation of biogas (results not shown). Since the C/N ratios reached in spent paddy- and wheat-straws as a result of mushroom growth were close to these optimum ranges, it is clear that these two spent residues were better substrates for biogas generation, although the nature of the nitrogen in spent residues is different from that of ammonium nitrate.

Further experiments were conducted to investigate whether the residues from different stages of growth of the mushroom could result in higher production of biogas. Thus the residues obtained after harvesting the first, second and third flush of fruit bodies were used for biogas generation. For all the residues used, it was observed that the biogas production increased with the time of bioconversion. However, the rate of increase of biogas production decreased when spent residues after 36 days of mushroom growth were used compared to those used after 28 days of growth. It therefore seems that the maximum changes in terms of overall increased susceptibility of residues took place during the first 28 days of mushroom growth, and especially between the first and second harvests of fruit bodies.

There seems to be only one report on the use of biologically pretreated residues for biogas production (Mueller and Troesch 1986). Using a mixture of wheat straw pretreated by the oyster mushroom *Pleurotus* sp. *"florida"* and manure in a ratio of 1:2, the biogas yield increased by about 30%. These results were extended to conclude that the "myco-straws" can yield double the biogas compared with that on untreated straw on the basis of its content in the total substrate (i.e. 33% of total dry matter) (Mueller and Troesch 1986). However, if one takes into account the loss of dry matter during a long 90-day bioconversion of wheat straw by *Pleurotus* sp. *"florida"* (which was 42.5%), there was hardly any net gain in the production of biogas by using this "myco-straw" over that of untreated straw. Thus, the specific application of fungal treatment to get more susceptible straw for biogas production does not seem to be a viable proposition.

The results reported in this paper are significant in the sense that one can use the straws for mushroom cultivation, which is already an economically viable process, and in addition dispose of the spent residues in an environmentally safe way to obtain a useful by-product in the form of biogas.

Acknowledgement. The first author (Ragini Bisaria) thanks the Council of Scientific and Industrial Research, New Delhi, for awarding a Research Associateship to carry out this piece of work.

References

Bisaria R, Gujaral GS, Bisaria VS (1987a) An integrated approach to utilization of agro-residues through *Pleurotus* cultivation. Crit Rev Biotechnol 7:17–41

Bisaria R, Madan M, Bisaria VS (1987b) Biological efficiency and nutritive value of *Pleurotus sajor-caju* cultivated on different agro-wastes. Biol Wastes 19:239–255

Bisaria R, Madan M, Mukhopadhyay SN (1983) Production of biogas from residues from mushroom cultivation. Biotechnol Lett 5:811–812

Datta R (1981) Acidogenic fermentation of lignocellulose: acid yield and conversion of components. Biotechnol Bioeng 23:2167–2170

Ghose TK, Das D (1982) Maximization of energy recovery in biomethanation process – Part II: use of mixed residues in batch system. Process Biochem 17:39–43

Halsall MD, Gibson AH (1985) Cellulose decomposition and associated nitrogen fixation by mixed cultures of *Cellulomonas gelida* and *Azospirillum* species or *Bacillus macerans*. Appl Environ Microbiol 50:1021–1026

Lynch JM, Harper SHT (1983) Straw as a substrate for cooperative nitrogen fixation. J Gen Microbiol 129:251–253

Mueller HW, Troesch W (1986) Screening of white rot fungi for biological pretreatment of wheat straw for biogas production. Appl Microbiol Biotechnol 24:180–185

Pal J, Thapa CD (1979) Cultivation of dhingri (*Pleurotus sajor-caju*) made easy. Indian J Mushroom 5:17–20