55

Biotechnology Techniques Vol 6 No 1 87-90 (1992)
Received 14th October

POTENTIAL OF ENSILING FOR EFFICIENT MANAGEMENT OF SPENT RESIDUE FROM SOLID STATE FERMENTATION SYSTEM

S. ROUSSOS, [1*] M. RAIMBAULT, [1] F.GEOFFROY,[2]
G. SAUCEDO-CASTANEDA [3] and B.K. LONSANE [4]

1 ORSTOM Montpellier, Biotechnologie PMC, 911, Avenue Agropolis, BP 5045, 34032 Montpellier, France
2 INRA Petit Bourg, Laboratory of Animal Sciences, BP 1232, 97184 Pointe à Pitre, Guadeloupe, France
3 Dept. Biotecnología, Universidad Autonoma Metropolitana, AP 55-535, CP 9340, Mexico DF, Mexico
4 Fermentation Technology & Bioengineering Discipline, Central Food Technological Research Institute, Mysore 570 013, India

SUMMARY

Studies on ensiling of spent solids from solid state fermentation process for production of cellulases by *Trichoderma harzianum* showed that good quality ensiled solids can be obtained by using about 43% initial substrate dry matter with 0.3% ensiling additive.

INTRODUCTION

Research and development efforts on waste management in solid state fermentation (SSF) system are conspicuously absent (Lonsane and Krishnaiah, 1991) inspite of the recent resurgence of interest in the system throughout the world (Steinkraus, 1984). The involvement of lower expenses on treatment of liquid effluent, however, has been pointed out as one of the most attractive advantage of SSF system over submerged fermentation processes (Lonsane and Ramesh, 1990). The spent solid residue, another stream of waste generated in SSF system during product leaching from fermented solids, however, may prove problematic due to its high organic matter content, moist condition and heavy microbial load. A number of modes have been suggested by many workers (Budiatman and Lonsane, 1987; Lonsane and Krishnaiah, 1991; Toyama, 1976) and include its use for biogas production, landfilling, ethanol production after enzymic saccharification, nucleic acid recovery from spores present in the residue and in manufacture of bricks, boards and paper. It can also be used as fuel, antagonistic agents to phytopathological fungi in soil and cattle feed after protein enrichment. Almost all of these modes are based on speculations without studies on standardization, practicability and commercial viability. It is well known that the waste treatment involves heavy capital and operating expenses with no financial returns (Lonsane and Ahmed, 1989). The selection of strategy for waste management, therefore, assumes critical importance. The absence of information on these modes may even form a stumbling block in commercialization of the SSF system.

Ensiling of waste solids and byproducts has been practised extensively (Kamra, 1987) and may offer potential in management of spent solids from SSF system. It yields a product which is needed in large quantity for animal feeding (Saucedo-Castaneda *et al.* 1990). The potential of ensiling spent bagasse residue from SSF system for production of cellulases (Roussos, 1987) was, therefore, investigated and the results are presented in this paper. No work appears to has been done on ensiling of spent solids from SSF system.

MATERIALS AND METHODS

Source of the spent solids. The spent solid residue was from the pilot plant experiments carried out for urea 2.4 , KH_2PO_4 5.0 and tap water 117. The medium (50% moisture) was charged in cloth sacs, autoclaved at 110°C for 1 h, cooled to about 30°C and inoculated with spore suspension at a rate of $3x10^7$ conidiospores/g SDM. The moisture content of the medium at this stage was 74%. The inoculated medium was then charged at 12 kg SDM level in Zymotis. The fermentation was carried out at 29°C for 60 h. The temperature of the fermenting solids was controlled by combined action of forced aeration by humidified air and water circulation through the heat exchangers of the fermenter. Other experimental details, design of Zymotis and methodology for preparation of conidiospore inoculum in disc fermenter D2 were as reported elsewhere (Roussos, 1987; Roussos *et al.*, 1989, 1991; Blanco-Gonzalez *et al.*, 1990). The fermented solids, at the end of fermentation, were pressed in hydraulic press (type 45 T, Pinette Emidecau S.A. 71, Chalon/Saone, France) at 220-230 bar pressure for 1 min to recover the enzymes.

Effect of SDM content on ensiling. SDM of the spent solid residue was adjusted to 32.7 and 44.9% by adding appropriate quantities of tap water. The resulting mass was mixed throroughly in a stirred tank for 5 min to impart homogeneity, charged in 5 kg moist weight quantities in plastic bags, pressed firmly to expel air and sealed for ensiling at ambient temperature (23°-28°C) for 6 months.

Effect of additives on ensiling. Various additives were mixed with tap water at different concentrations before using it for adjusting the initial SDM content of the spent solids to 32.6± 1.9% and the subsequent ensiling as per the procedure described above. The additives used and their concentration based on initial SDM content of the cake include: a) Proteinor (BP, Paris, France), a commercially available inoculum of lactic acid bacteria, at 0.2, 0.3 and 0.4% , b) an ensiling additive, consisting of 30% formic acid and 70% formaldehyde , at 0.3 and 0.4%; c) A.I.V. (Virtanen, 1952), a mixture of HCl and H_2SO_4 (7:1 on volume basis) at 1% and d) black-strap cane sugar molasses (50% sugar) at 0.5%. In another experiment, the effect of these additives at the same levels was evaluated for ensiling the spent solids with 43.0±1.7% initial SDM content by using exactly similar methodology.

Analytical aspects. The bags were opened after 6 months and the ensiled solids were homogenized by mixing in the bread kneader for determination of total DM and total nitrogen contents (A.O.A.C., 1980). The homogenized ensiled solids were subjected to hydraulic pressing to obtain juice for use in determination of pH, NH_3 (A.O.A.C. 1980) and lactic acid by colorimetric method of Barnett (1951). Ethanol and volatile fatty acids were determined as per the method of Jouany (1982). The quality of the ensiled solids was determined by calculating % lactic acid formed with respect to the total fermentation products (lactic acid, volatile fatty acids and ethanol) formed during ensiling.

RESULTS AND DISCUSSION

Spent solids from SSF system. The fermented solid substrate medium, after recovery of cellulases by hydraulic pressing at 220-230 bar pressure for 1 min, yielded a moist cake amounting to 40-45 % of the moist weight of the fermented solids. Its pH was in the range of 5.5-5.9 and it contained 52.4-55-7% DM. It is thus apparent that large quantity of moist spent solids will be generated in industrial SSF plant. For example, the plant of the size of 100 tons of moist solids per day will generate 40-50 tons of the spent solids, with about 50% DM content, per day. It will also occupy a larger space due to its lower bulk density. The solid waste of this magnitude will constitute a serious problem to the industry as the spent solids will need to be properly treated because of its high organic matter content and heavy environmental pollution potential, if discharged as such in the natural streams. It the absence of the viable and efficient waste management techniques, the SSF plant may not even get clearance from the agencies dealing with environmental pollution and protection. Hence, the development of simple , efficient, economical and practical means of its management is vitally essential for promoting industrialization of the SSF system.

The ensiling of the spent solids is one of the potential means which may prove economical at least to some extent because of the asssured return from the sales of the ensiled solids. It is emphasized that the ensiling of innumerable agro-industrial residues is the widely practised technique (Woolford 1984) and the absence of information on ensiling of spent solids from SSF system is difficult to explain except for the reasons of apathy on this aspect of the SSF and limited commercialization of the system in Western and European countries

Table 1: Effect of initial dry matter content of the spent solid residue on the quality of ensiled product.

Attribute	Initial dry matter %	
	32.7	42.9
Final dry matter,%	29.6	42.6
pH of the ensiled solids	4.2	4.1
Total nitrogen *	14.6	12.9

Table 2: Effect of additives on ensiling of the spent solids with 32.6±1.9 and 43.0±1.7% initial dry matter.

Initial Dry Matter (%)	**Additive**		**Product formed, % of dry matter**				**Lactic acid formed, % of total fermentation products**
	Name	Addition (%)	Lactic acid	Acetic acid	Volatile fatty acids	Ethanol	
32.6±1.9	**Proteinor**	0.2	42.8	4.8	5.7	2.1	84.6
		0.3	37.0	4.5	5.4	1.3	84.6
[illegible]							
	additive	0.4	30.4	6.2	7.2	2.5	76.0
	Molasses	0.5	49.6	8.6	9.6	30.0	55.4
43.0±1.7	**Proteinor**	0.2	35.6	5.8	6.9	0.3	83.1
		0.3	33.2	4.7	5.7	0.5	84.4
		0.4	35.4	6.9	8.0	0.8	80.0
	Ensiling additive	0.3	45.3	4.1	5.2	0.5	88.8
		0.4	12.1	3.1	3.7	0.8	72.6
	A.I.V.	1.0	5.5	1.8	2.1	0.0	72.0
	Molasses	0.5	42.8	3.4	4.0	19.0	65.0

ACKNOWLEDGEMENTS

The autors thanks Mme Laure Hannibal for excellent assistance. B.K.L. thanks Department of Biotechnology, Government of India, New Delhi for the award of Overseas Associateship. Council of Scientific and Indus- [illegible]