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

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## 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 the production of cellulases by *Trichoderma harzianum* CCM F-470 by SSF technique using Zymotis, the fermenter designed at ORSTOM (Roussos, 1987; Roussos *et al.*, 1989). The culture medium (pH 4.4) contained (g/100 g substrate dry matter; SDM): bagasse from sugar cane mill 80, wheat bran 20, $(NH_4)_2SO_4$ 9.7, 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 thr oroughly 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 (Lonsane, 1991).

**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 |
| Soluble nitrogen * | 1.6 | ND |
| Lactic acid formed * | 42.8 | 34.3 |
| Acetic acid formed * | 4.8 | 4.2 |
| Volatile fatty acids formed* | 5.7 | 48.5 |
| Ethanol formed * | 2.1 | 1.3 |
| Lactic acid formed, % of total fermentation products | 84.6 | 42.8 |

* = expressed as % of dry matter; ND = Not done

**Effect of initial dry matter content.** Data in Table 1 indicate that the ensiling with 32.7% initial DM gives much better product as compared to that with 44.9%. The product from the latter was extremly poor due to very high volatile fatty acids content. Such poor quality of product is probably due to the role of higher initial DM in influencing the rate of fermentation (McDonald, 1981), promoting secondary fermentation by clostridia which ferment lactic acid to butyric acid (Seale *et al.*, 1982) and the ability to effect growth of desirable and undesirable microorganisms in different ways (Woolford 1984). The high DM content also imposes a type of barrier to free diffusion of lactic acid in the ensiling solids which ultimately leads to the inhibition of the metabolism of lactic acid bacteria due to gradient in lactic acid concentrations (Saucedo-Castaneda *et al.*, 1990).

**Effect of different additives.** The inclusion of three different additives at various concentrations in the spent solids with 32.6±1.9% initial DM has resulted in either no improvements or decreased quality of the product (Table 2). The volatile fatty acid content was higher in most cases though the lactic acid formed was lower in case of the addition of ensiling additive and with higher doses of Proteinor. In spite of higher level of lactic acid in case of molasses addition, the product quality was poor due to extremly higher level of ethanol.

In contrast, the additives at lower levels improved the quality of the product to significant extent in case of spent solids with 43.0±1.7% initial DM. For example, % lactic acid formed, with respect to total fermentation product, was nearly double in case of all the levels of Proteinor and 0.3% ensiling additive (Table 2) as compared to the control without any additive (Table 1). The increase in other cases was however of lower magnitude. The volatile fatty acids formed were lower in all the cases and ranged between 2.1-8.0%. The ethanol formed was 19% in case of molasses addition (Table 2). The trend of the results is similar to that reported by many workers for ensiling of agro-industrial residues with high initial DM and the additives (O'Leary and Hemken, 1982; Kung *et al.*, 1984; Woolford, 1984).

The use of spent solids with higer initial DM is advantageous as it avoids losses of DM in the effluent during ensiling and lessen the weight of water to be transported (Seale, 1986). It also helps in obtaining better product due to relatively greater tolerance of lactic acid bacteria to low moisture availability (resulting due to use of solids with higher initial DM content) than the vegetative forms of clostridia ((Woolford, 1984). The ensiled product obtained in the present studies by using 43.0±1.7% initial DM with addition of 0.3% ensiling additive is best among all the different conditions evaluated and may prove of high potential in management of spent solids from SSF system.

**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 |
| | | 0.4 | 36.0 | 12.2 | 13.0 | 1.3 | 71.6 |
| | **Ensiling additive** | 0.3 | 29.6 | 11.7 | 12.8 | 3.3 | 64.9 |
| | | 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 |

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