Design of Lab Scale Solid State Fermenter for the Production of Value Added Products

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ABSTRACT

The study is concerned with the design and fabrication of laboratory scale solid state fermenter for the production of value added products using *Aspergillus flavus* and *Aspergillus oryzae* with very low cost effective requirements, for the biosynthesis of protease using solid state fermentation. The solid state fermenter that is designed and fabricated is a tray type fermenter, so that the fungal species can get the maximum surface area for the growth and yield the maximum product. The protease that is produced is extracellular, so the cost of downstream processing is reduced. Total four types of fungal strains was utilized, NCIM no. 646 and 1212 of *Aspergillus oryzae* and 535 and 549 of *Aspergillus flavus*. Out of these four, *Aspergillus oryzae* 1212 gave the maximum yield of protease, that is 8.6 g/L. Vegetable waste of cauliflower and cabbage were used as media, as it contains the enough amount of carbohydrates as carbon source and other nutritional ingredients. The cost of fermentation was reduced by using carbon source as agriculture waste .The fabrication and design of the solid state fermenter is done for carrying out the fermentation process under specified optimal conditions. The different parameters are optimized for studying enzyme activity, such as effect of pH and temperature. At pH of 7.2 & temperature 32^{0}C, maximum yield of protease were obtained.

Keywords: ***Design of solid state fermenter, Aspergillus flavus, Aspergillus oryzae, solid-state fermentation, vegetable waste.***

Introduction:

Solid-state fermentation (SSF) has been defined as the fermentation process occurring in the absence or near-absence of free water. Solid State Fermentation processes generally employ a natural raw material as carbon and energy source. Solid State Fermentation can also employ an inert material as solid matrix, which requires supplementing a nutrient solution containing necessary nutrients as well as a carbon source. Solid substrate (matrix), however, must contain enough moisture. Depending upon the nature of the substrate, the amount of water absorbed could be one or several times more than its dry weight, which leads relatively high water activity (a_w) on the solid/gas interface in order to allow higher rate of biochemical process. Low diffusion of nutrients and metabolites takes place in lower water activity conditions whereas compaction of substrate occurs at higher water activity. Hence, maintenance of adequate moisture level in the solid matrix along with suitable water activity is essential element for Solid State Fermentation processes. Solid substrates should have generally large surface area per unit volume (say in the range of 10^3-10^6 m^2/cm^3 for the ready growth on the solid/gas interface). Smaller substrate particles provide larger surface area for microbial attack but pose difficulty in aeration/respiration due to limitation in inter-particle space availability. Larger particles provide better aeration/respiration opportunities but provide lesser surface area. In bioprocess optimisation, sometimes it may be necessary to use a compromised size of particles (usually a mixed range) for the reason of cost effectiveness. For example, wheat bran, which is the most commonly used substrate in SSF, is obtained in two forms, fine and coarse. Former contains particles of smaller size (mostly smaller than 500-600 µ) and the latter mostly larger than these. Most of SSF processes use a mix of these two forms at different ratios for optimal production. A **protease** (also termed **peptidase** or **proteinase**) is any enzyme that performs proteolysis, that is, begins proteincatabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the

protein. Proteases have evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms. Proteases can be found in animals, plants, bacteria, archea and viruses. Bacteria secrete proteases to hydrolyze (digest) the peptide bonds in proteins and therefore break the proteins down into their constituent monomers(amino acids). Bacterial and fungal proteases are particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated by nutritional signals in these organisms. The net impact of nutritional regulation of protease activity among the thousands of species present in soil can be observed at the overall microbial community level as proteins are broken down in response to carbon, nitrogen or sulphur limitations.

Design And Fabrication of A Bioreactor

Tray bioreactors represent the simplest technology for SSF. As we are going to use *Aspergillus* species for the project, there is no requirement of mixing. Therefore, tray bioreactor is convenient and more efficient for the desired process.

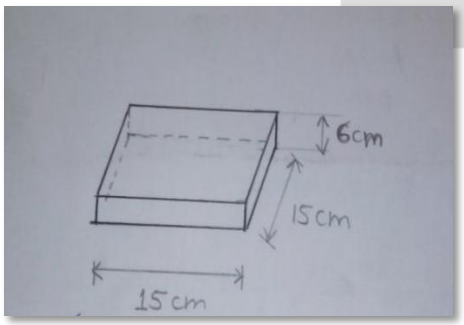
We designed the Tray fermenter according to the needs. The fermenter can carry 3 Kg of the dry substrate at a time. Its material of construction and the dimensions are given as follows-

Construction materials

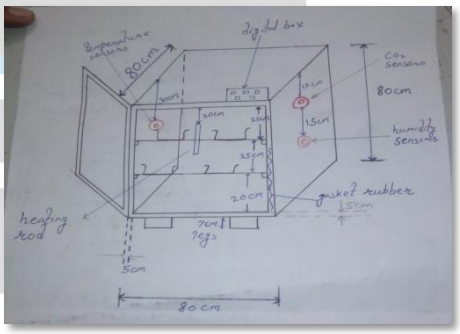
1. Outer surface of reactor : metal steel (ms)
2. Inner surface of reactor : stainless steel (ss316)
3. Insulation material of reactor : glass wool
4. Vertically arrange heating rod inside reactor
5. Three trays made of stainless steel (ss316)

Dimensions of reactor

1. Outer side dimension of reactor -80x80x80 cm
2. Inner side dimension of reactor -60x60x60 cm.
3. Tray dimension inside the reactor-15x15x6 cm (4 trays) + 15×15×6 inch (1 tray)



Tray



Solid State Fermenter for Fabrication

The sensors were also placed in the fermenter. The manufacturing company of Temperature sensor is **SELEC,** whereas that of Humidity sensor is **Leutron** (foreign company). This bioreactor was given to the Vikas Workshop for its fabrication and construction.

Aeration was provided by connecting a silicon pipe to a 1 HP compressor. The pipe was inserted in the tray and was fixed inside it. Small pores were made in the pipe with a pin and its one end was closed. This was done to allow uniform aeration throughout with the minimum pressure. The trays do not have lids, so they need to be packed properly to avoid contamination. So, we packed the trays with the autoclavable bags and put the trays inside the fermenter; and the silicon pipes were then inserted through the packing by making a suitable hole (for aeration).

Fabricated Solid State Fermenter

Materials and Methods

Chemicals

Sodium potassium tartarate, DNSA reagent, sodium hydroxide pellets, standard glucose solution, sodium phosphate buffer (of pH 6.0, 6.4, 7.2 and 7.6), Na_2HPO_4, NaH_2PO_4, Caesin, Bovine Albumin, $CuSO_4$, Carbonate buffer (pH 10), trichloroacetic acid (TCA), FC reagent, HCl, NaCl and Potato Dextrose Agar were used.

Microorganisms

Microorganisms were collected from National Center for Industrial Microorganisms (NCIM), a division at National Chemical Laboratory (NCL), Pune.

Aspergillus oryzae NCIM no. 649 and 1212

Aspergillus flavus NCIM no. 549 and 535

Substrate

The substrate used was vegetable waste, mainly constituting of cabbage and cauliflower waste, since it is available all the year around and is free of cost. We collected the vegetable waste from the girls' and boys' mess of our college and also from the vegetable market. We collected around 5 to 6 Kg of the substrate.

Preparation of the Substrate

The substrate collected was first chopped into small pieces and then sun dried for about 4 days. Then, the dried substrate was grinded into very fine particles in a mixer. The above grinded substrate was collected in 250 ml conical flasks and moistened with a salt solution containing gm/l : KH_2PO_4 2, NaCl 1, $MgSO_4.7H_2O$ 1. The substrate was moistened till saturation. Like this, 4 flasks were made and then autoclaved for 15min at 121°C for proper cooking of the substrate and to increase the amenability of the microorganisms.

Inoculum Preparation:

1. *Aspergillus oryzae*

   Commercially prepared spores of *Aspergillus oryzae* was stored at 4^0C in the refrigerator. The inoculum was prepared by streaking loop full of the spore in Potato Dextrose Agar. The test tube containing the above was kept at 37^0 C for 2 days.

2. *Aspergillus flavus*

The same was done for *Aspergillus flavus*, but the only difference is that, the inoculum was kept at 40^0 C for 3 days.

Fermentative production of Protease:

100 gm substrate was mixed with the salt solution (mentioned in the substrate preparation) in each tray.The autoclaveable packed trays were then autoclaved at 15 psi for 1 hour. Then the autoclaved trays were transfered to the laminar air flow. The required inoculums (strain no. 1212, 549, 535 and 569), autoclaved saline solution (0.2%) and phosphate buffer solutions (pH 6.4 to 8) were also brought in the laminar air flow. Now, spores as such cannot be scratched and sprinkled on the substrate as the growth will not be uniform. Therefore, 0.2% saline solution was put in the test tubes containing the inoculums and then a sterile loop was put inside it and then the spores were scratched. This was done till the saline water became turbid. After this, the four strains were put in the four different trays, labeling them correctly. Now, buffer solutions of different pH with initial pH 6.4 was added to the each trays with *A.oryzae* species and *A.flavus* species. The buffers were added till the saturation point was achieved. All the things were mixed properly and the trays were kept in the Solid State Fermenter for fermentation process. *A.flavus* labeled tray was kept at 37^0C and *A.oryzae* labeled trays were kept at 32^0C.

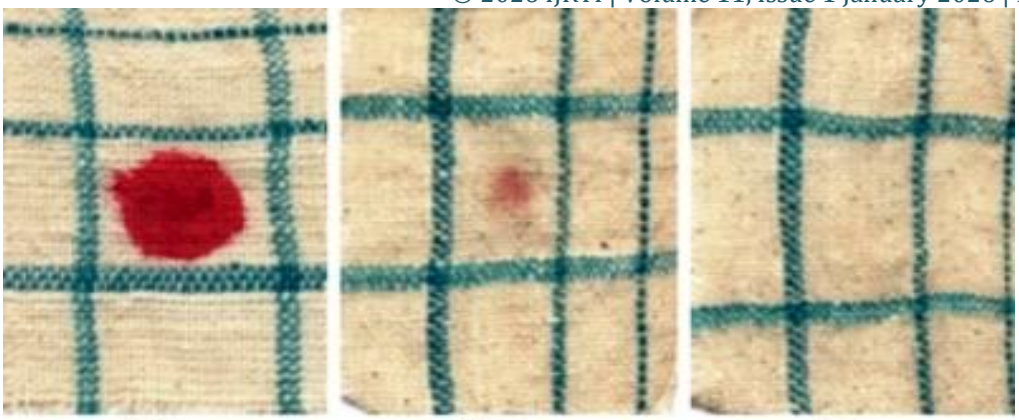
Quantitative Analysis

1. 10mL of distilled water was mixed with 1gm of the sample taken from each flask
2. Resulting slurry agitated on a rotary shaker at 180 rpm for 30 min.
3. Then it was centrifuged at 1000 rpm for 10 min.
4. Supernatant was recovered and held at 4^0C until the analysis.
5. Storage period of the supernatant should not be more than 5 days.
6. 1mL of suitably diluted supernatant was mixed with 5mL solution of 2% (gm per 100 mL) casein dissolved in 0.5 mol/L carbonate buffer (pH 10)
7. Resulting solution was incubated at 40^0C on gyratory shaker (300 rpm for 30 min.)
8. 0.5mL of the reaction mixture was withdrawn and the reaction was quenched by adding 1.5mL pre chilled trichloroacetic acid (10%)
9. Reaction tube was immersed in the ice bath for 5 min to completely precipitate the protein.
10. Supernatant was recovered by centrifugation again, at 1000rpm for 10min.
11. Tyrosine liberated during casein hydrolysis was measured in the supernatant using the method of Lowry et al.

Identification Porteolytic Activity of Enzyme:

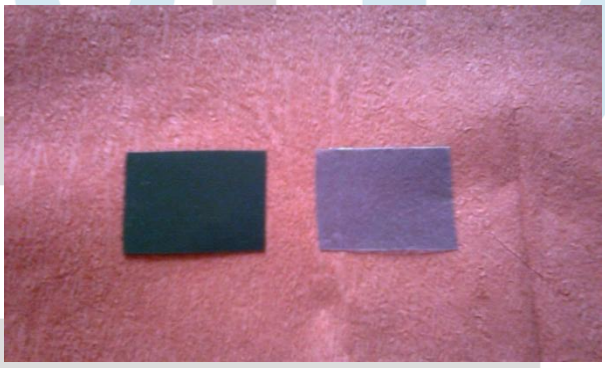
1. Destaining of blood:

A clean piece of white cloth (5x5) was stained with blood and allowed to dry the cloth. The cloth was incubated with the purified protease at 37^0C incubation. After incubation time, cloth was rinsed with water for 0 min, 1 min,2 min and then dried. The same procedure was done for the control except incubation with the enzyme solution.



Degradation of gelatin layer from used x-ray film:

- The used X-ray films were washed with distilled water and wiped with cotton impregnated with ethanol, and were cut into 4 × 4 cm^2 pieces after drying in an oven at 40°C for 30 Minutes.
- Each of the film was mixed in series 100 mL of stock enzyme solution.
- the flask containing film and enzyme extract were stirred at 50 rpm for 24 hrs.
- After completion of time period the film get completely decolorized and grey-brown colored slurry was obtained that contains the silver metal.



Decolonization of X-ray Film

Results:

From the studies maximum yield of the enzyme were obtained at temperature 32^0C, that is of 0.78 gm/L.

Conclusion:

From the work we concluded that, tray type solid state fermenter is best suited for the growth of *Aspergillus oryzae*, as its maximum growth occurred at 32^0C and pH 7.2. In solid State Fermentation Process, the concentration of the protease was obtained as 0.78 gm/L under optimal conditions. Growth of the micro-organism on a static bed of waste material showed the desirable result.

References:

1. Jarun Chutmanop, Sinsupha Chuichulcherm, Yusuf Chisti and Penjit Srinophakun , Protease production by Aspergillus oryzae in Solid state fermentation using Agro-indutrial subtrate ,chem Technol biotechnol 83:1012-1018(2008)
2. Chinnasamy Muthulakshmi, Duraisamy Gomathi, Duggaanaboyana Guru kumar, Ganesan Ravikumar and Chandrasekar Uma, Production, purification and Characterization of protease by Aspergillus flavus under Solid State Fermentation, Volume 4,number 3,September 2011

3. H. Kh. Q. ALI and M. M. D. ZULKALI, Design Aspects of Bioreactors for solid state fermentation: Review, *Chem. Biochem. Eng. Q.* **25** (2) 255–266 (2011)
4. Idouraine A, Khan MJ and Weber CW, *In vitro* binding capacity of wheat bran, rice bran and oat fiber for Ca, Mg, Cu and Zn alone and in different combinations. *J Agric Food Chem* **44**:2067–2072 (1966).
5. Impoolsup A, Bhumiratna A and Flegel T W. Appl EnvironMicrobio, 42(4) (1981) 619
6. Kunitz M J Gen physiol, 340(1947) 291.
7. Lonsane B K, in Solid State Fermentation, edited by p. Ashok (Wiley Eastern Ltd. New Delhi) 1994,11.
8. Naivikul O, *Wheat: Science and Technology*. Kasetsart Univ as early initiation enzyme production.
9. Ogawa A, Yasuhara A, Tanaka T, Sakiyama T and Nakanishi K, Production of neutral protease by membrane-surface liquid culture of Aspergillus oryzae IAM2704. J Ferment Bioeng **80**:35–40 (1995).
10. Sandhya C, Sumantha A, Szakacs G and Pandey A, Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. *Process Biochem* **40**:2689–2694 (2005).
11. Sangsurasak P and Mitchell DA, The investigation of transient multi dimensional heat transfer in solid-state fermentation. *Chem Eng J* **60**:199–204 (1995).
12. Wang R, Chau Sing Law R and Webb C, Protease production and conidiation by *Aspergillus oryzae* in flour fermentation. *Process Biochem* **40**:217–227 (2005).
13. Aikat K and Bhattacharyya BC, Protease production in solid state fermentation with liquid medium recycling in a stacked plate reactor and in a packed bed reactor by a local strain of Rhizopus oryzae. Process Biochem **36**:1059–1068 (2001).Srinivas M R S and Lonsane B K, Microb Neotrope, 6(1993) 1.
14. Barnett H L and Berry B H, in illustrated genera of imperfect fungi (Burgess Publishing co., Minnesota)1972.
15. Battaglino R A, Huergo M, Pilosof AMR and Bartholomai G. B, Appl. Microbial Biotechnol, 35 (1991) 292.
16. Gerhardt AL and Gallo NB, Full-fat rice bran and oat bran similarly reduce hypercholesterolemia in humans. *J Nutrition* **128**:865–869 (1998).
17. Gilbert M, Xiao X, Chaitaweesub P, Kalpravidh W, Premashthira S, Boles S, *et al.*, Avian influenza, domestic ducks and rice agriculture in Thailand. *Agric Ecosyst Environ* **119**:409–415 (2007).