ABSTRACT

The environmental factors pertinent to the production of protease by the solid state fermentation of wheat bran by *Rhizopus oryzae* RO IIT RB-13 NRRL No. 21498, a strain locally isolated and characterized previously, were optimized. The optimum conditions found were an inoculum age of 7 days, incubation time of 9 days, re-incubation time of 3 days, amount of liquid medium of 60 ml per 10 g of dry wheat bran, and incubation temperature of 33°C. Initial pH of the liquid medium and substrate did not have much effect. Studies were also conducted on the extraction of the enzyme from the fermented biomass. The optimum amount of water for extraction was 50 ml per 10 g of dry wheat bran with one extraction being sufficient. Extractant did not have much effect and modified Czapek dox medium could be used as extractant as effectively as distilled water. Investigations carried out on two types of reactors-the stacked plate reactor and the packed bed reactor-showed that the latter gave a four times yield compared to the former. Reactor operations involved the extractive fermentation process with intermittent removal of part product followed by fresh feed yielding good results. Decolorization (90%) and purification (3-fold) of crude extract were accomplished using activated charcoal. A protease hyperproducing mutant (1.6 times production compared to wild type strain) was isolated following mutagenesis of *Rhizopus oryzae* RO IIT RB-13 with MNNG. A novel substrate for protease was developed by incorporating Coomassie Brilliant Blue R 250 into BSA cross-linked by glutaraldehyde at low temperature. The substrate was tested with *Rhizopus oryzae* protease and trypsin and found to be satisfactory.