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Techno-economic analysis of processes for *Aspergillus carbonarius* polygalacturonase production

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A techno-economic analysis of submerged (SmF) and solid state fermentation (SSF) processes for *Aspergillus carbonarius* polygalacturonase production was performed to make an appropriate process selection. The downstream processing involved integrated membrane process (IMP) and alginate affinity precipitation (AAP). For a production scale of 30 kL purified polygalacturonase concentrate per year, the total upstream cost of SmF was 14% lower than the SSF process. Downstream processing cost by IMP was 47% lower than AAP. The SmF-IMP process required a total capital investment that was 15–24% lower than the SmF-AAP and SSF-AAP processes. The corresponding unitary product cost was also lower by 24–36% in SmF-IMP process. Thus the SmF-IMP process proved to be very attractive from an economic point of view.

[**Key words:** Economic analysis; *Aspergillus carbonarius*; Polygalacturonase; Submerged fermentation; Solid-state fermentation; Integrated membrane process; Alginate affinity precipitation]

Pectinase is a major food enzyme contributing to almost 25% of the global food enzyme sales (1). Polygalacturonase (PG; EC 3.2.1.15), is a depolymerizing pectinase, which catalyzes the hydrolysis of α-1,4 glycosidic linkages in homopolygalacturonan backbone. Since the enzyme secreted by *Aspergillus niger* has been used commercially, a number of studies describing its properties have been reported in literature (2–4). However, literature information on fermentation processes used for enzyme production and economic analyses of the processes are scanty.

Enzymes from fungi can be industrially produced by submerged (SmF) and solid-state fermentations (SSF). Though both the processes have claimed merits, about 90% of all industrial enzymes are produced by SmF using genetically manipulated microorganisms owing to several process advantages over SSF (5). The major problems encountered with SSF are difficulties in scale-up and control of process parameters such as pH, temperature, oxygen transfer and moisture. Besides, SSF process suffers from low mixing efficiency and higher impurity product increasing the product recovery costs (6). Still, SSF gained some attention in the last three decades due to the possibility of using cheap agro-industrial wastes as substrates.

There are few reports that compared the pectinases production from *A. niger* by SmF and SSF. Solis-Pereira et al., comparing the production of endo- and exo-pectinases, concluded that the overall productivities of the enzymes by SSF were 18.8 and 4.9 times higher than in SmF (7). They attributed the lower productivity in SmF to longer fermentation time and catabolic repression by free sugars released during fermentation. Patil and Dayanand showed higher productivity of endo pectinase (12.6 U/mL) in SmF and exo pectinase (34.2 U/g) in SSF and increased productivities by supplementing the medium with green gram husk (8,9). However the above process comparisons have not taken into consideration the efficiencies of downstream processing of the fermented substrates for enzyme recovery. Likewise, higher productivity claims in SSF based on enzyme units per gram dry substrate as against units per millilitre broth in SmF cannot reflect enzyme yields by itself. Hence there is a need for realistic comparisons, probably by normalizing the enzyme activity in terms of starch present in the substrate, for process and cost assessments as described by Ghildyal et al. while reporting the economics of amyloglucosidase production from *A. niger* by SmF and SSF (10).

A mutant strain *A. carbonarius* secreted different forms of polygalacturonases when grown in SmF and SSF cultures (11). Porous membranes have been employed generally for the concentration of enzymes (12,13). In our earlier study, polymeric membranes, when used for downstream processing, resulted in high recovery and purification as evidenced by the improved specific activities of PG (14,15). The enzyme activities, recovered after downstream processing, when compared with commercial polygalacturonases of *A. niger* for apple juice clarification showed similarity in performance (16). Hence, comparative economic analysis for industrial production of *A.*

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carbonarius PG was performed and the details are described in this paper.

MATERIALS AND METHODS

Organism The constitutive mutant *A. carbonarius,* deposited at the culture collection centre of the Food Microbiology Department, CFTRI under the accession number UV 10046, was used for enzyme production in both SmF and SSF processes.

Process description For SmF, the fungus was grown in a medium made of 2.5% (w/v) corn flour and 0.313% (w/v) each of di-ammonium hydrogen phosphate and ammonium di-hydrogen phosphate (14). SmF was carried out in a 75 L fermentor (M/s Bioengineering AG, Wald, Switzerland) using an inoculum developed in the above medium in shake flasks (200 rpm) at 30°C for 48 h with spores from one-week-old slants. The following process conditions were maintained in the fermentor: working volume 50 L; temperature 30°C; initial pH 5.5; stirrer speed 300–400 rpm; air flow rate 300 (first 4 h)–375 L/min; fermentation period 24 h. After filtration to remove mycelia, the culture filtrate containing the enzyme was used.

For SSF, inoculum was developed as described above. Solid-state wheat bran medium (20 g wheat bran; 47 mg NaCl; 32 mg yeast extract; 1.25 mL 2 N HCl; 6.75 mL water) was mixed with 10% (v/w) of inoculum and incubated at 30°C for 60 h in a tray fermentor for PG production. The wheat bran used for SSF had an initial moisture content of 10.5% and the relative humidity of air was 54%. The mouldy bran was extracted with 0.1 M acetate buffer (pH 4.3; 1:10 ratio) in an orbital shaker (200 rpm) for 30 min, and used as enzyme source. Before downstream processing, the debris and spores were removed by filtration through Whatman No.1 filter paper (17).

To purify and concentrate the enzyme, IMP employed 450 nm microfiltration (MF) followed by 50 kDa ultrafiltration (UF) membranes. AAP was carried out using 0.5% (w/v) sodium alginate (pH 4.3) at 30°C as described by Nakkeeran et al. (15). The process flow diagrams followed for SmF-IMP, SmF-AAP and SSF-AAP (Figs. 1–3) were based on the studies done in our laboratory.

Polygalacturonase assay PG activity was determined using 0.5% (w/v) polygalacturonic acid (sodium salt) prepared in 0.1 M sodium acetate buffer (pH 4.3) as substrate. Assays were carried out for 10 min at 50°C (17) and the reducing sugars were quantified as galacturonic acid equivalents according to Nelson-Somogyi method (18). Activity corresponded to micromole galacturonic acid released per minute per milliliter.

Protein estimation Protein was determined by the dye binding method using Coomassie Brilliant Blue G 250 (19). Bovine serum albumin was used as a standard.

Economic analysis The base-case pectinase plant was designed to produce 30 kL purified PG concentrate (3000 U/mL) in 300 working days per year. Plant design and equipment sizing were worked out based on the laboratory process performance parameters keeping a high level utilization of process equipment. Specifications were drawn for major equipment and cost estimates obtained from a plant and equipment supplier. Six-tenths rule was applied wherever necessary for estimating the price of equipment (20). The prices of raw materials were obtained from chemical catalogues or directly from suppliers. Ceramic (MF) and polymeric membranes (UF and reverse osmosis, RO) were considered to have a life-time of 5 and 1 years, respectively (21,22). Accordingly, replacement costs were built in to the cost of consumables. The cost of chemicals needed for membrane cleaning was included in the raw material cost. The activity of the concentrate product was kept close to commercial pectinase concentrates (~3000 U/mL) and the conservative selling price applied in the revenue calculations was ~20% of the price of an equivalent commercial product (INR 22,000/L, net price without taxes). Total capital cost, total product cost and unitary product cost were worked out individually for upstream and downstream processing sections based on methodologies proposed by Peters and Timmerhaus (23).

All the experiments were carried out in duplicate and mean values are reported.

RESULTS AND DISCUSSION

Upstream and downstream process performance The performance of SmF and SSF processes for the production of *A. carbonarius* PG are presented in Table 1. The fermentation period by SmF was only 24 h as against 60 h by SSF while the PG activity (U/mL) was higher in SmF (230 U/mL) compared to SSF (89 U/mL). Generally, SSF provides a system with higher productivity due to larger enzyme yields and shorter fermentation periods (7). In the present study, the yield of PG (U/mL) obtained in SmF was 2.6 times higher than SSF while the yield was much higher to the extent of 10.3 fold when compared in terms of substrate quantity used. In absolute measure, 1 g of starch yielded 13,100 U by SmF as against 4940 U obtained in SSF process indicating that the utilization of starch for the production of enzyme was 2.7 times higher in SmF process. The results suggested that it is more appropriate to compare the enzyme yield as well as productivity in terms of the substrate used, more specifically the carbon source. Higher yield together with shorter fermentation period resulted in 6.6 times greater productivity favoring SmF for PG production over SSF. The higher PG productivity obtained by SmF could be attributed to the use of mutant *A. carbonarius* that was more effective in glucose regulation (Venkatesh, K.S., Ph.D. thesis, University of Mysore, Mysore, 2004).

The downstream processing using IMP and AAP were optimized for the purification of PG from *A. carbonarius* SmF culture broth and SSF extracts (14,17). IMP enhanced the specific activity of SmF-PG by

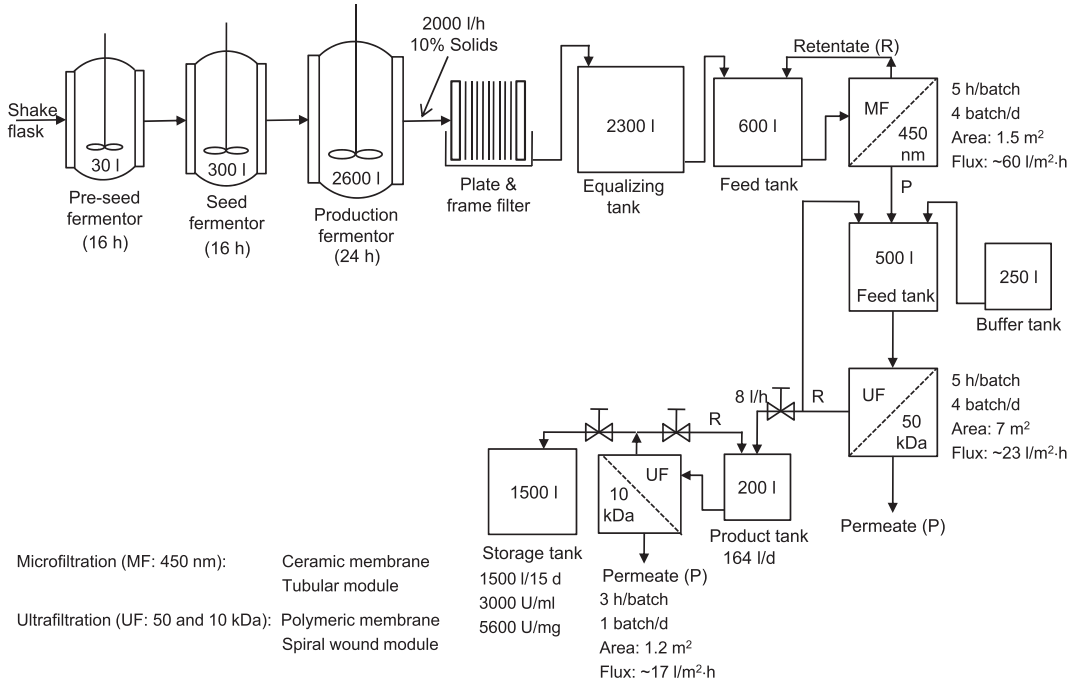

FIG. 1. Process flow diagram for upstream and downstream processing of *A. carbonarius* PG (SmF-IMP). PG, polygalacturonase; SmF, submerged fermentation; IMP, integrated membrane process.