

PRODUCTION OF ENZYMES BY SOLID SUBSTRATE FERMENTATION: RELATION SUBSTRATE/ENZYME AND INDUCTION/CATABOLIC REPRESSION

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Summary

Solid substrate cultures of moulds behave in quite a different way to conventional submerged cultures. Catabolic repression often observed in submerged fermentation can easily be overcome by using solid state fermentation. *A. niger* strains can be grown by SSF technique with the following advantages: enzyme titres and productivity are usually higher; there is a tendency to early enzyme excretion as compared to SmF technique.

Conventional enzyme production in a Stirred Tank Reactor (STR) by microbial organisms requires, an inexpensive and ready to use carbon source (sugar or starch derivative), a specific enzyme inducer, and a mixture of mineral salts and other organic compounds. Unfortunately, the presence of high levels of the carbon source often inhibit the production of many enzymes. For example, it has been reported that the addition of too much of sugar or even pectin to a STR inoculated with *Aspergillus niger* was detrimental to pectinase production [2, 3]. But continuous and controlled addition of small amounts of sugar (fedbatch reactor) increased four or five times pectinase productivity [2]. The explanation of this phenomenon was related to a dynamic balance between supply and demand of sugars in the SmF process leaving the sugar concentration at a low level, although the total supply of sugars was very high [2]. But, Ramesh and Lonsane [4] using *Bacillus subtilis* grown by SSF in fixed bed reactors and adding starch as inducer did not find the catabolic repression phenomenon commonly observed by the addition of high levels of carbohydrates.

Solis-Pereira *et al.* [5] confirmed that in shake flasks (SmF) inoculated with a strain of *Aspergillus niger*, called CH4, glucose levels of 30 g/L were inhibitory to pectinase production in the presence of 30 g/L of pectin (Table 1). But in SSF (packed bed reactors with bagasse) using the same strain and inducer, the addition of glucose enhanced pectinase production although the sugar level was 100 g/L in the absorbed broth (see Table 1).

TABLE 1. Effect of glucose addition to pectinase levels produced by SmF and SSF techniques [5].

Substrates	SmF (U/mL)	SSF (U/g)
30 g/L Pectin	0.75	5.00
A + 30 g/L glucose	0.00	11.0
A + 100 g/L glucose	N.D.	13.0

This observation has been confirmed in our laboratory (unpublished data) using different *A. niger* strains and inducers (sucrose, tannic acid) and assaying for the corresponding enzyme activities (invertase, tannase) as illustrated in figure 1.

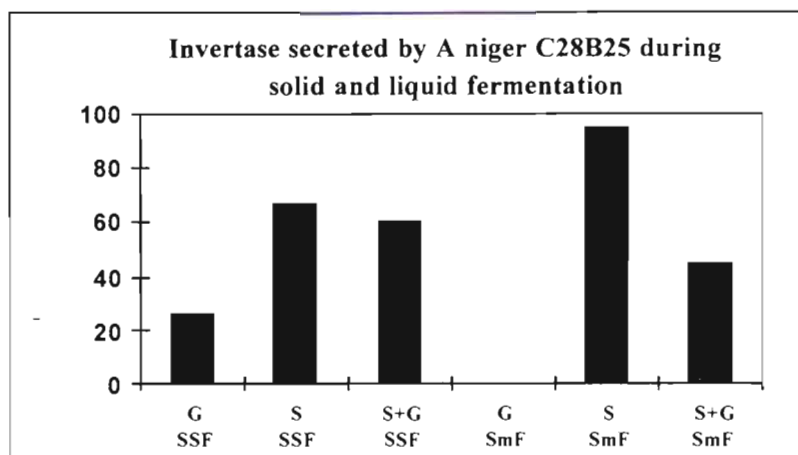


Figure 1. Invertase secreted by *A. niger* C28B25 during SSF and SmF. (G: Glucose, S: Saccharose).

Catabolic repression is observed in SmF at 40g/L of glucose (Fig. 1). The repression at the same concentration of glucose is much reduced in SSF. Glucose added to saccharose in SSF does not affect invertase production.

Production of other enzymes has also been tested in packed bed reactors with bagasse as solid support and in fixed bed reactors with polyurethane as solid matrix. In all those cases, enzyme production was increased using high levels of glucose or sucrose without much evidence of catabolic repression.

A consequence of such physiological behaviour is the increase of enzyme productivity by SSF. This was observed to a certain level. Garcia-Peña (11) studied the effect of adding increasing amounts of tannic acid as an inducer during an SSF process and observed that over and above 10% tannic acid, tannase production decreased dramatically, as shown in figure 2.

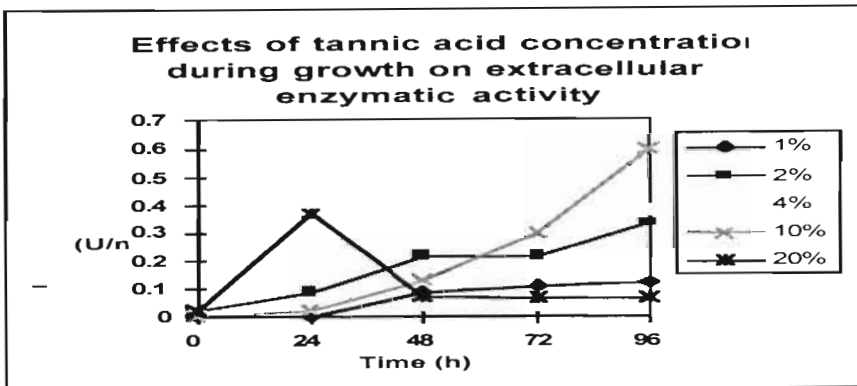


Figure 2. Effects of tannic acid concentration during growth on extracellular tannase activity

Another interesting observation is the fact that tannase has been reported to be bound to the hyphal biomass of *A. niger* when it is produced by SmF. Lekha and Lonsane (10) and Garcia-Peña (11) have found that this enzyme is mostly excreted to the culture medium of *A. niger* when produced by SSF (figure 3.). These intriguing differences between SSF and SmF techniques in the excretion of enzymes, seem to indicate that moulds can modulate the way to use enzymes, depending on the culture medium. Apparently, those organisms have "sensors" that pick up environmental signals and have also complex transducing systems that modulate their biochemical behaviour in order to adapt to a particular set of environmental variables (low or high a_w , good or bad mixing of substrates, low or high temperature, etc). This adaptation gives plasticity to those organisms in order to survive in changing culture conditions.

An unexplored solid state fermentation method using polyurethane foam (PUF) as inert carrier impregnated with a synthetic liquid medium was developed simulating the nutritional composition and culture conditions of solid state fermentation on sugar cane bagasse. with this system, biomass, the important parameter involved in SSF process, could be measured directly. Tannase production of various previously selected overproducing *A. niger* strains was tested (Figure 4.). The three strains produce maximum activity around 48 hours of incubation. The specific activities are similar to those obtained with bagasse [12]. However, maximum tannase production is observed much later (around 100 hrs) when sugar cane bagasse is used as solid support.

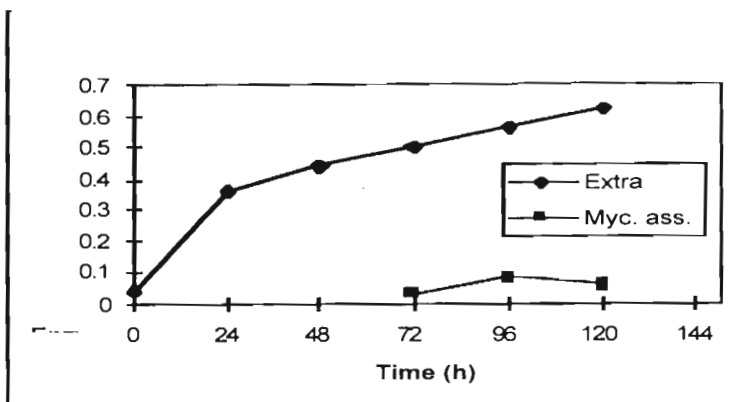


Figure 3. Comparison of tannase extracellular activity with activity associated with the mycelium.

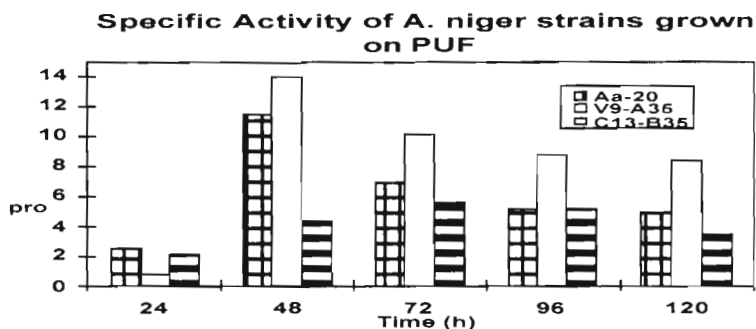


Figure 4. Specific activity of tannase from three *A. niger* strains during growth on PUF.

TABLE 2. Comparison of pectinase productivity by SmF and SSF cultures of *A. niger* CH4 [1].

Activity	Productivity ($U\ ml^{-1}\ h^{-1}$)		Ratio (SSF/SmF) (SSF/SmF)
	SSF	SmF	
Endo-pectinase	0.006	0.001	6.0
Exo-pectinase	0.14	0.002	51.5
Pectin-lyase	0.008	0.0002	29.2

Acuña-Argüelles *et al.* [1] found that *A. niger* CH4 had a much higher pectinase productivity when cultured by SSF as shown in Table 2.

Clearly, SSF cultures were more productive for all pectinase activities assayed, such as endo-pectinase by viscometry, exo-pectinase by the production of reducing compounds and pectin lyase by the changes in UV absorbing material [1].

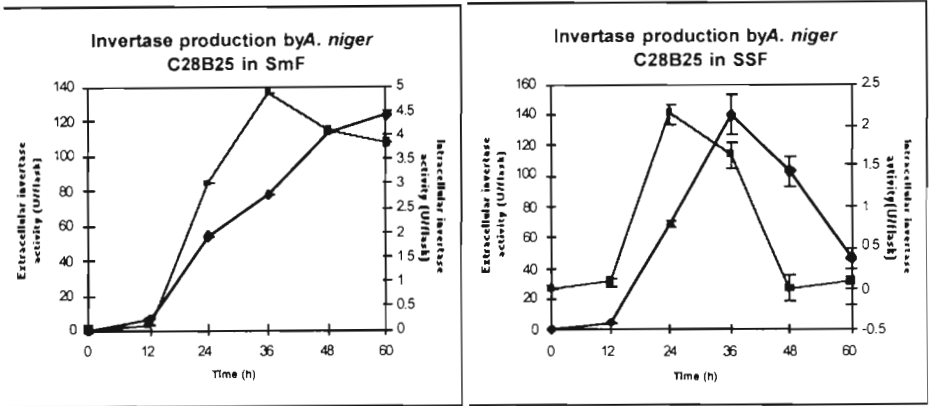


Figure 5. Invertase production of *A. niger* C28B25 in SmF and SSF. Squares represent extracellular invertase production and diamond represent intracellular invertase. [Note the difference in scales]

In SmF, intracellular accumulation is over twice that of SSF. Invertase is secreted earlier (maximum at 24 hrs) in SSF than in SmF (maximum at 36 hrs). Unlike tannase which is mycelium associated in SmF, invertase seems to be readily excreted in both SmF and SSF.

Such increases of productivity could have important economic consequences in the cost of enzyme production. Thus, Ghildyal *et al.* [6] have made an economic *pro forma* analysis of amylo-glucosidase production by SmF and SSF. Their calculations indicate that due to a higher yield of SSF using *A. niger* CFTRI 1105, which produced 10 times higher titres than by SmF, the overall economic picture was much better for SSF process. In fact there are reports of successful large scale production of pectolytic enzymes by SSF in India [7] and also of fungal amylase scale-up by SSF [8].

Pandey [9] has reviewed the reports of enzyme production by SSF including, cellulases, amylase, glucoamylase, beta glucosidase, pectinases, catalase and proteases with a list of 28 microbial species in which *Bacillus* and *Aspergillus* are the most frequently used genus.

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