EFFECT OF NITROGEN SOURCE ON LYSOZYME AND BIOMASS PRODUCTION BY *Aspergillus niger* ON SUBMERGED AND IMMOBILIZED CULTURE.

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Introduction. Filamentous fungi are a very attractive alternative for protein production, but while production level for native and homologous proteins are quite high, heterologous protein production level is normally very low (1). Protein production by Filamentous fungi is normally conducted by submerged fermentation (SmF) but it has been reported that immobilized Fermentation (ImF) can induce a higher protein production (2). The main problem with ImF culture is it normally is conducted on complex substrates that make culture analysis difficult, so we have been using polyurethane foam (PUF) as an inert substrate to study gene induction, kind and quantity of growth and protein secretion in order to get a higher understanding of the reasons that make ImF more productive than SmF.
The objective of this work is to study the effect of nitrogen source and carbon source level in a recombinant protein production.

Methods. Lysozyme producer *Aspergillus niger* B1 strain was cultivated by SmF and ImF on PUF; minimal culture media with $NaNO_3$ or NH_4SO_4 as nitrogen source was used; in each case 25 or 50 g/L of starch was included as only carbon source. For ImF 25 ml of inoculated media was added to 1 gr of 5 mm per side PUF cubes. Biomass production was determined as weight difference between paper filter or PUF before and after culture harvest. pH was determined from harvested supernatant. Lysozyme production was quantified according to Archer *et al*(3).

Results. When ammonium sulfate was used as nitrogen source lysozyme production was higher for ImF and biomass production was higher for SmF, while when sodium nitrate was used as nitrogen source higher biomass levels were obtained for ImF cultures but lysozyme production was higher for SmF. It is quite clear that a crossed effect between biomass and lysozyme production exist and it is conserved in both levels of carbon source used. The biomass obtained in NH_4SO_4 cultures is approximately 50% higher when carbon source is increased from 25 to 50 g/L, while biomass level remain unchanged if $NaNO_3$ is used despite of carbon source increase, and with NH_4SO_4 as nitrogen source the increase of carbon source has effect on biomass production that increase in a 60% approximately but there is not effect on lysozyme production. The use of NH4SO4 to get a higher production of recombinant proteins has been reported (4) but this is the first time the crossed relation of nitrogen source with recombinant protein production and biomass production is reported for ImF. Effect on lysozyme production is interesting specially as maximal secreted protein shows no effect despite carbon source level or culture system. NH_4SO_4 induces very low final pH values independently of the carbon source level or culture system used while $NaNO_3$ usage results in much higher final pH values again independent of carbon source levels or the culture system employed. Actually we are working in the determination if this pH values has an affect on lysozyme stability and protease production. This work shows that nitrogen source may be a very important choice for protein production using ImF cultures.

Culture Media			X_{MAX}	E_{MAX}	T_{Xmax}	Protein	pH_{MIN}
N source	C g/L	Culture System	g/L	U/L	h	mg/L	pH
NH_4SO_4	25	SmF	8.73	13100	72	128.11	2.18
		ImF	6.68	14160	72	165.89	2.28
	50	SmF	12.99	8600	72	164.78	2.07
		ImF	11.37	13240	72	156.89	2.32
$NaNO_3$	25	SmF	12.01	7412	60	105.67	4.32
		ImF	29.36	2602	48	136.68	4.12
	50	SmF	12.01	11122	60	169.51	4.25
		ImF	24.44	6374	60	182.54	4.94

Table 1. Maximal biomass (X_{max}), lysozyme (E_{max}) Time for maximal lysozyme production (T_{Xmax}), secreted protein (P) and minimal pH value obtained for Submerged and immovilized cultures of A, niger B1 strain.

Conclusions. Nitrogen source has a major effect to biomass and lysozyme production in both SmF and ImF cultures.

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