Maestría en Ingeniería en Diseño de Bioprocesos

Title

Hydrolytic enzymes production of industrial interest by agro-industrial wastes fermentation

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Hydrolytic enzymes production of industrial interest by agro-industrial wastes fermentation

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1. Introduction

Generally, around 30-40% of the production cost of industrial enzymes is accounted by the fermentation substrate (Hinnman, 1994). Therefore, the use of low cost substrate is one of the ways to greatly reduce costs (Díaz *et al.*, 2011). Throughout the world a large magnitude of various agricultural and agro based industrial wastes residues are being generated from current industrial processing practices (Saval, 2012). A wide range of agricultural/agro-industrial wastes and by-product residues such as orange peel, apple bagasse (Anwar *et al.*, 2014; Koser *et al.*, 2014) and tejocote between others, are potentially suitable feed-stock for their composition and disposition. Our research is focused in the revalorization of these residues, considering their composition, to use for the production of hydrolytic enzymes with industrial application.

2. Aim

- Isolate and select able microorganisms to produce hydrolytic enzymes.
- Characterize agro-industrial wastes of orange peel, apple bagasse and tejocote.
- Chose a substrate and microorganisms to produce hydrolytic enzymes.
- Produce hydrolytic enzymes in submerged fermentation and solid-state fermentation.

3. Method

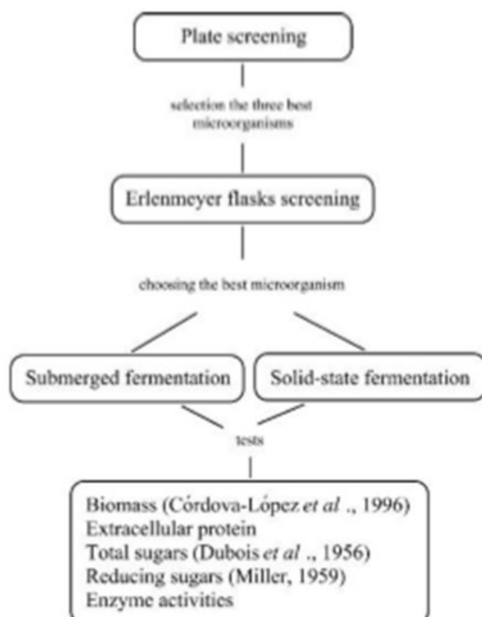

Figure 1. *Workflow general doing to obtained hydrolytic enzymes by agro-industrial wastes fermentation.*

4. Results

4.1. Plate screening

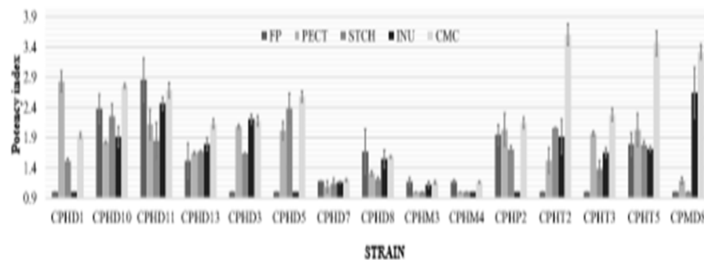

Figure 2. *Potency index values presented in strain fifteen, evaluated in carbon sources different.*

After to plate screening (Figure 2), the ANOVA analysis distinguished three strain like the best to excrete hydrolytic enzymes by halo formation. After the multiple comparative test, strains CPHD11, CPHD13 and CPHT5 were chosen. (Table 1).

Table 1. *ANOVA shows significantly differences between sources and strains (Software R was used for statistical analysis).*

Factor	DF	SS	MS	F-value	Pr(>F)
Source	4	13.4	3.4	12.8	7.7e-09
Strain	14	22.4	1.6	6.1	3.2e-09
Residual	131	34.2	0.3		

4.2. Erlenmeyer flasks screening

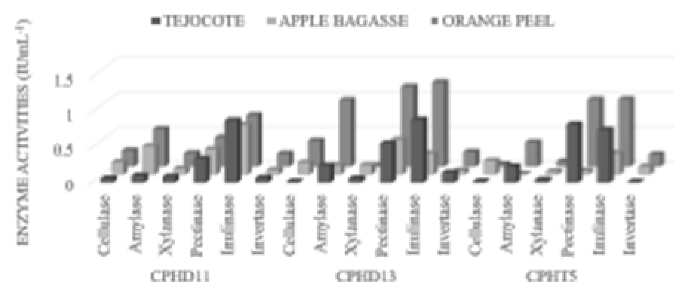

Figure 3. *Enzyme activities obtained by CPHD11, CPHD13 and CPHT5 strains growing on tejocote residues, apple bagasse and orange peel wastes at fermentation fifth day.*

The strains CPHD11, CPHD13 and CPHT5 were grown on Erlenmeyer flasks with tejocote, apple bagasse or orange peel; the flasks were incubated on an orbital shaker (150 rpm at 28° C) for ten days; samples were withdrawn at fifth (Figure 3) and tenth (Figure 4) day; and assayed for enzymatic activities: cellulase, amylase, xylanase, pectinase, inulinase and invertase.

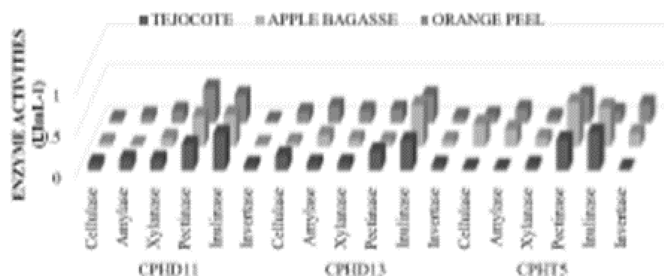

Figure 4. *Enzyme activities obtained by CPHD11, CPHD13 and CPHT5 strains growing on tejcocote residues, apple bagasse and orange peel wastes at fermentation tenth day.*

According to the Duncan comparative test made in software R (Table 2), the best strain CPHD13 was chosen to carry out submerged and solid-state fermentation.

Table 2. *Duncan comparative test to enzymes production considering strains, wastes and fermentation days.*

Enzyme activity	Strain		
	CPHD11	CPHD13	CPHT5
Cellulase	2.2 ± 0.5 **b**	3.7 ± 0.6 **a**	2.2 ± 0.5 **b**
Amylase	4.6 ± 1.2 **ab**	6.0 ± 1.5 **a**	3.4 ± 0.7 **c**
Xylanase	2.5 ± 0.2 **a**	1.9 ± 0.2 **ab**	1.5 ± 0.2 **c**
Invertase	1.4 ± 0.4 **b**	2.3 ± 0.5 **b**	4.9 ± 1.4 **a**
Inulinase	0.6± 0.2 *	0.6 ± 0.3 *	0.5 ± 0.3 *
Pectinase	0.4 ± 0.1 *	0.5 ± 0.3 *	0.5 ± 0.3 *

Equal letter: ANOVA with significantly difference (α=0.05); *: no significantly difference.

4.3. Enzymes production

4.3.1. Submerged fermentation

Strain CPHD13 was the best producer strain of hydrolytic enzymes. For this reason, submerged fermentation was carried out using 1L bioreactors with orange peel because, this waste was better than apple bagasse and tejocote. The solid-state fermentation was made in Erlenmeyer flasks.

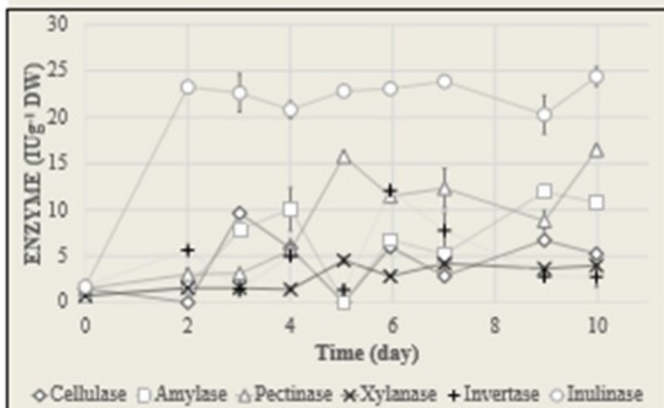

Figure 5. *Production profile submerged fermentation enzymes with orange peel.*

4.3.1. Solid-state fermentation

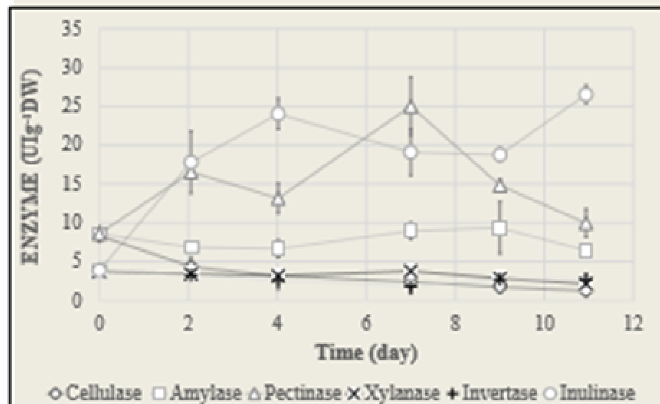

Figure 6. *Production profile solid-state fermentation enzymes with orange peel.*

5. Conclusion

The plate screening methodology can be a good tool to know the potential of a strain collection to enzyme production. In contrast, the screening flask directly with alternative carbon sources, indicate a direct correlation with the potential of each organism. On the other hand, the microorganisms presented production profile of pectinase and inulinase enzymes. In the same way, the production of hydrolytic enzymes in submerged fermentation, has a varied profile, with higher production of inulinase over other enzymes. However, in solid state fermentation protrudes inulinase and pectinase compared to the other enzymes that have a more uniform profile but much lower than the others.

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