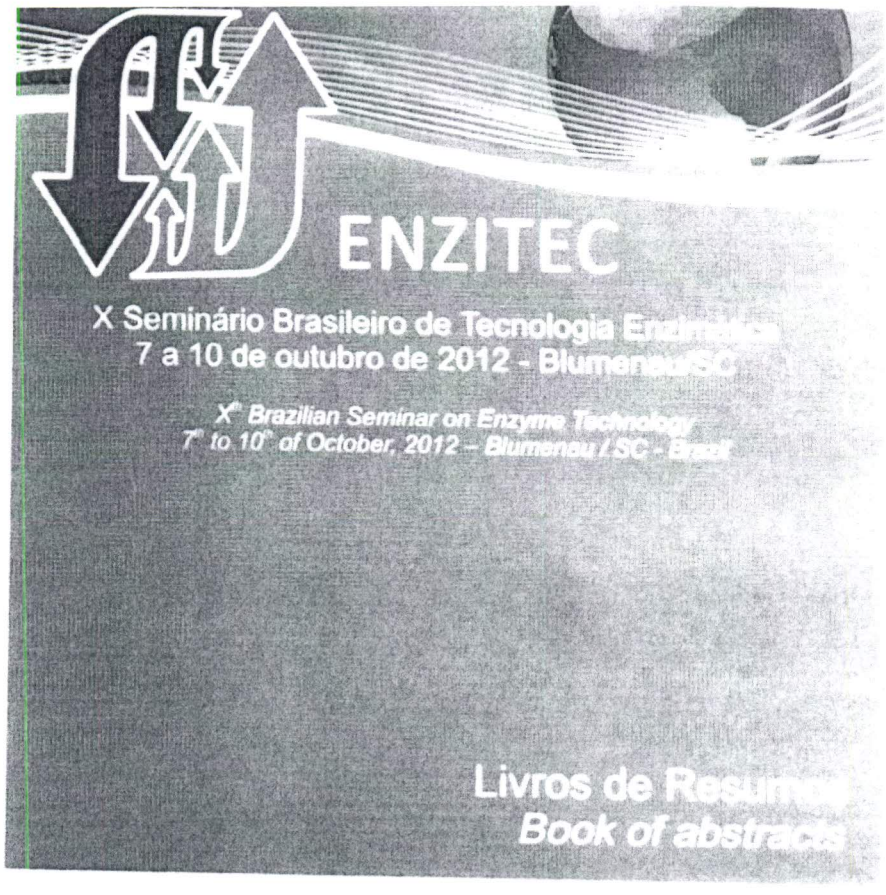
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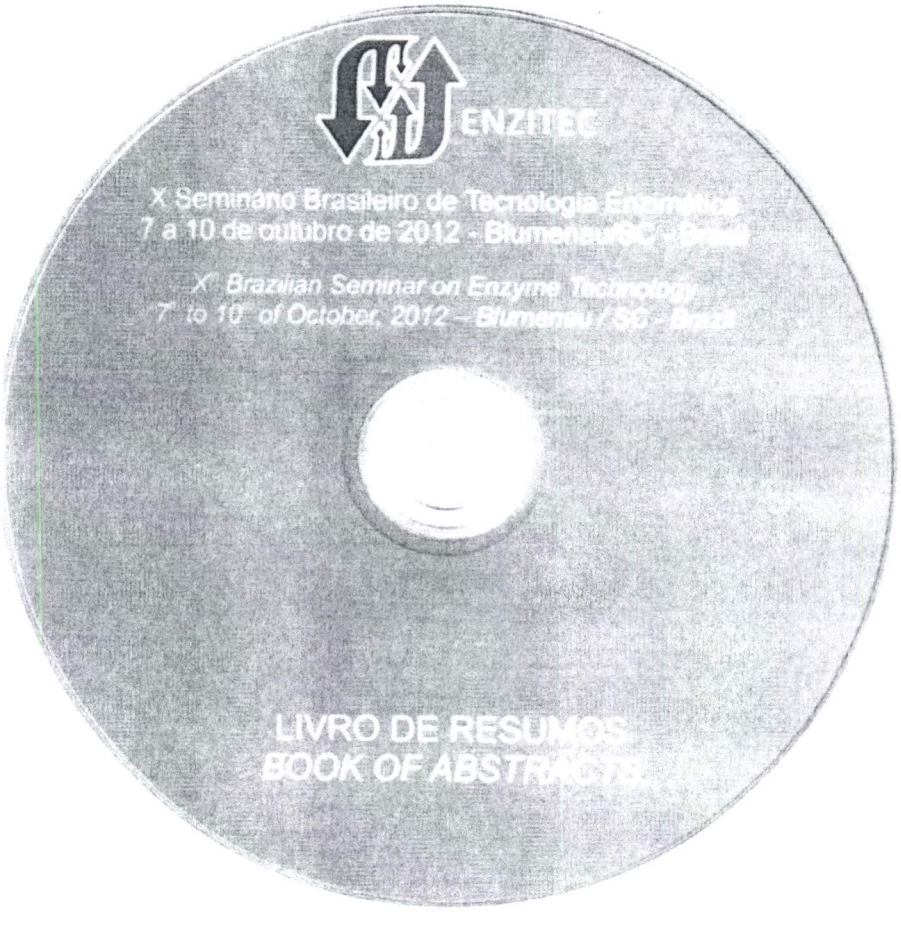
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Use of a new *Trichoderma harzianum* from the Amazon rainforest for on-site cellulase production

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INTRODUCTION

The on-site production of cellulases is an important strategy for the development of sustainable second generation ethanol production processes. This study concerns the use of a specific cellulolytic enzyme complex for hydrolysis of pretreated sugar cane bagasse. Glycosyl hydrolases (FPase, xylanase, and b-glucosidase) were produced using a new strain of *Trichoderma harzianum*, isolated from the Amazon rainforest[1] and cultivated under different conditions

RESULTS AND DISCUSSION

T. harzianum was initially grown in shake flasks in order to evaluate the influence of the carbon source on enzyme production. Steam-exploded bagasse (SB) and soluble carbon sources (fructooligosaccharide (FOS), sucrose, lactose, and glycerol) and their binary combinations were used at concentrations of 1% (w/v). Table 1 shows the maximum FPase, β-glucosidase, and xylanase activities in shake flasks for 120 h.

Table 1. Influence of different carbon sources and their combinations by *T. harzianum* during submerged fermentation in flasks.

Substrate	FPase (IU/mL)	Xylanase (IU/mL)	B-glucosidase (IU/mL)
SB	0.78	36.96	9.18
Sucrose	0.42	22.24	5.48
Glycerol	0.12	4.34	1.25
Lactose	0.22	23.47	1.73
FOS	0.43	23.27	4.11
SB+ sucrose	0.85	33.13	7.37
SB + glycerol	0.22	5.32	0.38
SB+lactose	0.35	17.92	4.84
SB + FOS	0.69	24.02	0.44

* SB: steam-pretreated bagasse; FOS: fructooligosaccharide

Sucrose was the soluble carbon source supplement that had the most significant positive effect on GH biosynthesis. A combination of sucrose with SB reduced the time required to achieve maximum FPase activity (ca. 0.85 FPU/mL) from 96 to 72 h. Therefore the addition of sucrose was studied using SB and DSB (bagasse pretreated using steam followed by delignification with NaOH) in a 3.0 L bioreactor at 29℃ in pH 5.0 (Table 2).

Table 2. Influence of sucrose on the FPase, xylanase, b-glucosidase production by *T. harzianum* in a bioreactor

Substrate	FPase (IU/mL)	Xylanase (IU/mL)	B-glucosidase (IU/mL)
SB	0.73	36.96	9.18
DSB	0.8	22.24	5.48
SB + sucrose	0.82	4.34	1.25
DSB + sucrose	1.21	23.47	1.73

CONCLUSION

This new strain of *T. harzianum* isolated from the Amazon rainforest appeared to be a potential candidate for enzyme production using sugar cane bagasse and sucrose as carbon sources. Both of these materials are in ready supply in a bioethanol production plant. This new strain showed an interesting ability of producing cellulase when grown on sucrose, suggesting that it might be less prone to carbohydrate repression than other Trichoderma strains.

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