EFFECT OF THE PH ON GROWTH AND ESTERASE ACTIVITY OF *FUSARIUM CULMORUM* GROWN ON MEDIA SUPPLEMENTED WITH DI (2-ETHYLHEXYL) PHTHALATE IN SUBMERGED FERMENTATION

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INTRODUCTION

Plasticizers are the chemical agents that provide flexibility to polymers such as polyvinyl chloride (PVC). The plasticizers most commonly used in the industry are phthalates. Di (2-ethylhexyl) phthalate (DEHP) is the phthalate with the highest production and a contaminant in the environment (Gao, 2015). This compound can affect gonadal development, being an endocrine disruptor. The filamentous fungus *Fusarium culmorum* is efficient in the degradation of DEHP, it is capable of using this compound as a source of carbon and energy (Ahuactzin-Pérez et al., 2016). In this work, it was observed how the initial pH of the fermentation modifies the growth and the enzymatic activity *of F. culmorum* when the DEHP is the only carbon source.

AIMS

General

To determine the optimal initial pH for growth and esterase activity of *F. culmorum* in medium supplemented with DEHP as the sole carbon source in submerged fermentation.

Specific

To evaluate the production of biomass (X) and the growth rate (μ) of *F. culmorum* in medium supplemented with DEHP at different pH values in submerged fermentation.

To determine the esterase activity (E) of *F. culmorum* with DEHP as a carbon source at different pH values in submerged fermentation.

To evaluate the yield parameters of esterase production: enzyme yield ($Y_{E/X}$), productivity and specific rate of enzymatic production (P_{RO} and q_p).

To carry out monodimensional electrophoresis to observe the enzymatic activity (zymography) of the supernatant of *F. culmorum* with DEHP as a carbon source at different pH values in submerged fermentation.

MATERIALS AND METHODS

Eight fermentations were performed at different pH values: 5.5, 6.0, 6.5, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0, taking samples every 12 h. Media contained (in g/L): K_2HPO_4, 1.0; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01 and $NaNO_3$, 3.0. 1000 mg of DEHP / L was used as a carbon source. Under sterile conditions, the media were inoculated and placed in an incubator with agitation at 25 °C and 120 rpm. Specific growth rate (μ) was obtained by dry weight of biomass (X) and calculated using logistic equation. The $Y_{E/X}$ is given by the enzymatic activity, obtained through a spectrophotometric determination using *p*-nitrophenyl butyrate as a substrate. The P_{RO} and q_p were determined as previously reported (Ferrer-Parra et al 2018). Zymographic analysis was performed using 12% gel electrophoresis of polyacrylamide with sodium dodecyl sulfate (SDS-PAGE). The pH profile of the fermentation was measured every 12 h using a potentiometer.

RESULTS

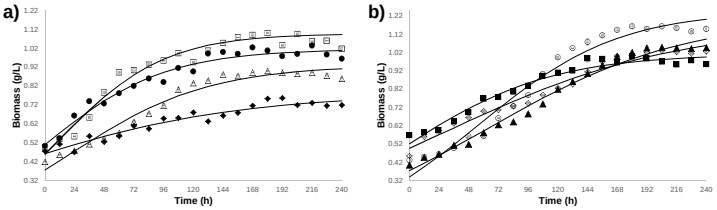

Fig. 1. Growth of *F. culmorum* in media supplemented with DEHP at different pH values in submerged fermentation. pH value **a)** ◖ 5.5, △ 6.0, □ 6.5, ◆ 7.0 , **b)** ■ 7.5, ○ 8.0, ◇ 8.5 ▲ ,9.0.

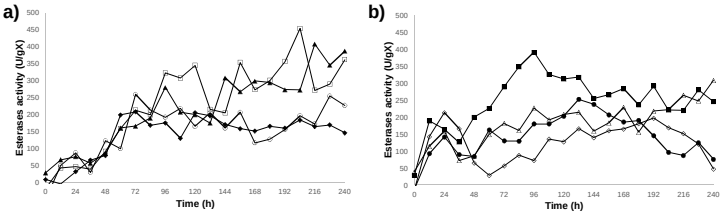

Fig. 2. Specific activity of esterase per gram of biomass of *F. culmorum* grown at different pH values in medium added with DEHP in liquid fermentation. pH value : **a)** 5.5, 6.0, 6.5, 7.0 y **b)** 7.5, 8.0, 8.5, 9.0.

Table 1. Growth and enzymatic yield parameters of *F. culmorum* in media supplemented with DEHP in submerged fermentation.

	pH of the culture media							
Parameter	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**
μ (h^{-1})	**0.02**[a] (±0.002)	**0.018**[a] (±0.001)	**0.023**[a] (±0.001)	0.01[b] (±0.001)	0.016[b] (±0.001)	**0.017**[a] (±0.001)	0.01[b] (±0.001)	0.012[b] (±0.001)
X_{max} (g/L)	1.014[b] (±0.002)	0.92[c] (±0.001)	**1.09**[a] (±0.002)	0.77[c] (± 0.001)	1.01[b] (±0.001)	**1.24**[a] (±0.002)	**1.16**[a] (±0.001)	**1.21**[a] (±0.001)
E_{max}(U/L)	**448.4**[a] (±49)	222.5[c] (±35)	**429.8**[a] (±45)	139.3[d] (±22)	293.5[b] (±37)	332.8[b] (±38)	216.2[c] (±25)	205.1[c] (±24)
$Y_{E/X}$(U Gx^{-1})	**444**[a] (±68)	241.8[b] (±32)	**394.3**[a] (±41)	181[c] (±21)	290.6[b] (±34)	268.4[b] (±33)	186.4[c] (±23)	169.5[c] (±18)
P_{RO}(U$L^{-1}h^{-1}$)	**2.2**[a] (±0.002)	1[c] (±0.001)	**1.9**[a] (±0.001)	1.2[c] (±0.002)	1.2[c] (±0.001)	1.7[b] (±0.002)	1.5[b] (±0.001)	1.1[c] (±0.001)
q_p(U h^{-1} gX^{-1})	**8.9**[a] (±0.006)	4.4[b] (±0.004)	**9.1**[a] (±0.006)	1.8[c] (±0.002)	4.6[b] (±0.003)	4.6[b] (±0.003)	1.9[c] (±0.001)	2[c] (±0.001)

*Values are expressed as mean ± SD (n=3); means within the same column not sharing common superscript letters (a-c) differ significantly at 5% level.

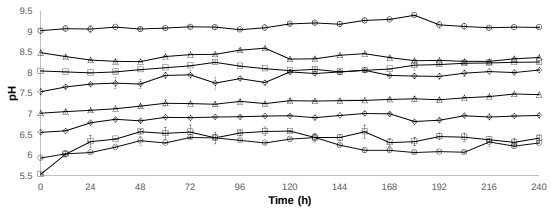

Fig. 3. pH of the cultures supplemented with DEHP during the fermentation process.

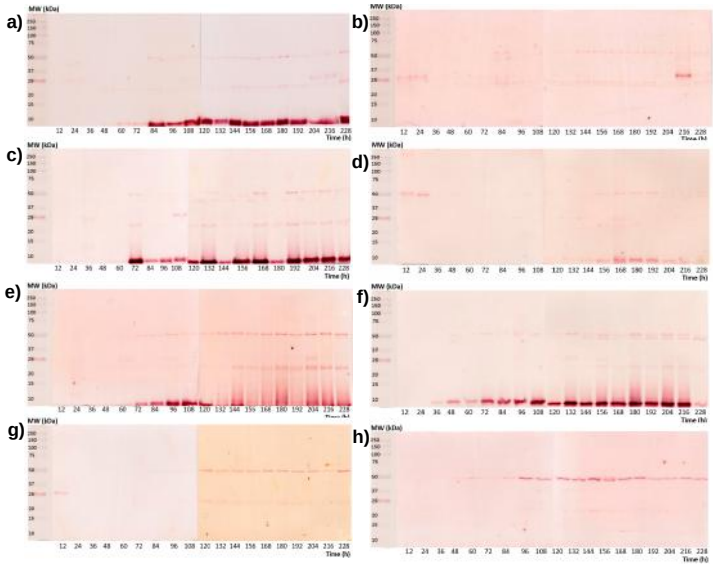

Fig. 4. Zymographic profiles obtained of the fermentation of *F. culmorum* in presence of DEHP at initial pH of: **a)** 5.5, **b)** 6.0, **c)** 6.5, **d)** 7.0, e) 7.5, f) 8.0, g) 8.5 and g) 9.0 in submerged fermentation.

CONCLUSION

Four esterase activity bands were observed in the DEHP-supplemented media, having a molecular weight of about 20 kDa, 25 kDa, 37 kDa and 50 kDa approximately. In general, the bands were observed between 72 and 228 h. These studies showed that 6.5 was the optimum pH for growth and esterase production of *F. culmorum*.

ACKNOWLEDGMENTS

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