Pichia stipitis is one of the most important host organisms used for high-level heterologous protein expression due to its capacity to promote proper protein folding and post-translational modification, especially for proteins from eukaryotic organisms. For this reason, as advancement of research in lignocellulosic ethanol occurs, Pichia stipitis has increasingly being used for expression of enzymes used in cellulose hydrolysis. In order to understand some of the factors that may affect the growth kinetics of Pichia, a 2 factor design experiment, with three replicates in the central point, was performed to study the effects of inoculum concentration, agitation and pH of medium on the growth kinetics of Pichia stipitis. The effect of those three factors was studied on the yields of biomass and ethanol productions, as well as glucose consumption. Results showed that after 24 h of fermentation, the initial concentration of inoculum was significant in determining the biomass and ethanol yield. According to results, an increase in the inoculum meant an increase in biomass yield, and a decrease in ethanol yield. Concerning the glucose consumption rate, all of the factors, along with its binary and tertiary interactions, showed to be significant at some point during fermentation, with inoculum and agitation being the most significant. Inoculum concentration was the only factor that affected all of the kinetics parameters studied, and it was the most significant of all the factors.

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