TITLE: *Lactobacillus* in the ethanolic fermentation: impact of the contamination by *L. fermentum* and *L. plantarum* and how to control the bacterial growth by adding ethanol to the acid treatment

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ABSTRACT:
Brazil is the greatest fuel ethanol producer from sugarcane and the fermentation is one of the most critical steps of this process. Some factors directly influence the fermentation efficiency, such as the fermentation system, the selected yeast and the bacterial contamination, especially from the genus *Lactobacillus*. The utilization of antibiotics and biocides can result in increased cost and environmental problems for the industry and the acid treatment (sulphuric acid solution, pH 2.0) of the cells between the cycles of fermentation is not always effective to combat the bacterial contamination. In the present study, the effect of the contamination by *L. fermentum* and *L. plantarum* was evaluated in a fermentation carried out by the industrial strain of *Saccharomyces cerevisiae* PE-2 in fed-batch system with cell recycle. Following, the effect of the addition of ethanol to the acid treatment of the cells was verified aiming the control of the growth of both bacteria and its effect on fermentation parameters. Initially, to evaluate the effect of the bacteria on the fermentation, six-cycle fed-batch fermentations with PE-2 in single and co-culture with *L. fermentum* or *L. plantarum* in sugarcane juice 16^{o}Brix for 9 hours were carried out. The minimum ethanol concentration to be added to the acidic treatment to control the bacterial growth was optimized in the range of 0 to 13% of ethanol in sulphuric acid solution, pH 2.0. Another set of fermentation trial was performed with PE-2 in co-culture with *L. fermentum* utilizing the acid treatment after each fermentative cycle, with and without the addition of 5% of ethanol. The bacterium *L. fermentum* was more harmful to the fermentation than *L. plantarum*, resulting in more sugar left in the fermented must and lower fermentative efficiency. *L. fermentum* lost completely the cell viability when 5% of ethanol was added to the acidic solution in pH 2.0. However, for *L. plantarum*, 9% of ethanol was demanded to cause similar effect. Only with the acid treatment, the population of *L. fermentum* reduced almost 3 log cycles at the end of the sixth fermentative cycle, however, when 5% of ethanol was added to the acid solution, the bacterium lost completely the cell viability right after the first fermentative cycle, as the initial experiment had revealed, and there was no impact to the fermentative efficiency, with low residual sugar concentration in the fermentative must at the end of the sixth nine-hour fermentative cycle.

Key-words: fermentation, ethanol, acid treatment, *Lactobacillu*