

Short Communication

DesinFix™ 135 in fermentation process for bioethanol production

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Abstract

Brazil has the world's largest ethanol production from sugarcane, but bacterial contamination decreases the ethanol yields. It was shown that the biocide DesinFix™ 135 can reduce the contamination without decreasing the yeasts' viability or negatively affecting the ethanol production.

Key words: ethanol production, fermentation process, sugar cane feedstock, biocides, bioethanol.

The dominant bioethanol feedstock in tropical regions is sugarcane. Brazil has the world's largest bioethanol production from sugarcane crop raw material. It was reported that the bioethanol production is expected to be about 23.96 billion liters in 2012 (Conab, 2012). Alcoholic fermentation is a biological process in which yeast (*Saccharomyces cerevisiae*) converts sugars, e.g., glucose, fructose, and sucrose into cellular energy with ethanol and carbon dioxide as metabolic products. Producing ethanol provides the yeast with a way to store energy but also to avoid competition, since the presence of ethanol creates a toxic environment to some microorganisms (Rosa *et al.*, 2006). However, several other microorganisms are able to share the same environment with the *S. cerevisiae* and consume sugar for their own metabolism. Therefore, the contamination in fermentation tanks by bacteria is one of the main problems in ethanol production mills.

Lactobacillus spp. bacteria are known as the most predominant contaminants in ethanol fermentation processes using tapioca and corn as feedstock (Chang *et al.*, 1995; Skinner and Leathers, 2004). According to a reviewed list (Oliva-Neto and Yokoya, 1998; Alcarde *et al.*, 2003; Meneghin, 2008; Saithong *et al.*, 2009) the five most common organisms present in the ethanol fermentation process with sugarcane juice and/or with molasses are: a) *Lactobacillus fermentum*; b) *Lactobacillus paracasei*; c)

Lactobacillus planetarium; d) *Leuconostoc mesenteroides*; e) *Bacillus subtilis*. These bacteria can cause inhibition of *S. cerevisiae* growth and limit the ethanol production. This is due to two major facts: [1] the production of acetic and lactic acids and [2] the competition for nutrients (Beckner *et al.*, 2011; Muthaiyan *et al.*, 2011).

Usually, fermentation mills combat the contamination problem by adding antibiotics, sulfuric acid, oxidants or other microbiocides in the fermentation tanks. The most common method for contamination control is a sulfuric acid treatment of the yeast cream. At the beginning of the regular fermentation process, sulfuric acid lowers the pH of the yeast cream to 2; then the yeast cells are incubated for 2 hours at that pH before returning to the fermenter. This common treatment is not completely satisfying, since it may have a negative influence on the yeast metabolism via low pH. The yeast metabolism is optimal for growth in an environment with a pH from 4 to 6. At lower external pH, the intracellular pH of the yeast cell may be affected, whereby enzyme functions may be impaired and as consequence, all yeast metabolism can be damaged. This has major influence on the fermentation time and ethanol yield (Narendranath and Power, 2005).

Kemira Oyj developed a new biocide for the control of bacteria in ethanol fermentation processes: DesinFix™ 135, a performic acid (PFA) solution produced on site. DesinFix™ 135 is a strong oxidizing, non-antibiotic agent

which kills microbes by destroying cell membrane functions. DesinFix™ 135 will decompose during the fermentation process into CO and H₂O, leaving no residues.

In order to evaluate the best conditions for the application of DesinFix™ 135, experiments were performed at laboratory and pilot plant at sugar mill. The main objective of this work was to test the efficiency and selectivity of DesinFix™ 135 in controlling the bacterial contamination while ensuring yeast viability and ethanol production.

The laboratory trials showed the efficiency of the product in a sucrose solution contaminated with a bacterial mix (Table 1). Sucrose solution was used as a model medium instead of molasses. After only 10 min of contact time with 10 mg L⁻¹ PFA, the microbial count of the sucrose solution decreased from 10⁷ to below 10³. The effect of PFA on the bacterial count was dose-dependent. This result shows that DesinFix™ 135 has a fast killing effect. The bacterial mix was composed of *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus planetarium*, *Leuconostoc mesenteroides*. These bacteria were provided by the VTT culture Collection in Finland.

Table 2 shows measurement data from pilot plant trials performed in a sugar cane mill in Brazil. These experiments were performed in different conditions on each day, each day representing one batch of fermentation. On days 1 and 5, 60 mg of PFA per liter was added to the yeast cream; on day 2, 20 mg of PFA per liter was added to the yeast cream and 20 mg of PFA per liter was added to the must tank; on days 4, 6 and 7, 20 mgL⁻¹ was added to the yeast cream and 5 mg PFAL⁻¹h⁻¹ applied to the must tank divided in 4 steps. Day 3 is the reference day without PFA addition. On the reference day only the mill's standard sulfuric acid treatment was carried out: sulfuric acid was added to the yeast cream until it reached a pH between 2.0 and 2.25. The wine was evaluated at the end of each fermentation. The level of bacterial contamination after PFA application did not differ significantly from that of the reference day.

Table 1 - Results from disinfection experiment with PFA in contaminated sucrose solution[§].

DesinFix™ 135 (mg PFAL ⁻¹)	THAB # ^{§§} (cfu mL ⁻¹ after 10 min from PFA addition ^{§§§})	THAB # ^{§§} (cfu mL ⁻¹ after 60 min from PFA addition)
0 (control)	6.5 x 10 ⁷	7.6 x 10 ⁷
5	3.9 x 10 ⁴	2.5 x 10 ⁴
10	< 1.0 x 10 ³	< 1.0 x 10 ³
> 20	< 1.0 x 10 ¹	< 1.0 x 10 ¹

[§]composition of solution: 200 gL⁻¹ of sucrose in distilled water with the addition of 0.5 gL⁻¹ urea ((NH₂)₂CO) and 0.01 mgL⁻¹ zinc chloride (ZnCl₂).

^{§§}THAB # = Total Heterotrophic Aerobic Bacteria, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus planetarium*, *Leuconostoc mesenteroides*.

^{§§§}CFU = Colony Forming Units; determined by plating samples on MRS-agar (Difco laboratories), incubating at 30 °C for 3 days and counting the number of colonies formed.

Table 2 - Parameters evaluated during the pilot scale experiments.

Days	Yeast Cream		pH	Must		Wine (at the end of fermentation)			
	Bacteria cfu*mL ⁻¹	°Brix		TRS g*L ⁻¹	Bacteria cfu*mL ⁻¹	Ethanol °GL	TRS g*L ⁻¹	Bacteria cfu*mL ⁻¹	Yield
1	3.3x10 ⁵	22.50	6.0	19.70	2.9x10 ⁶	8.10	0.07	4.6x10 ⁶	0.41
2	1.2x10 ⁴	18.16	5.9	14.48	1.1x10 ⁷	7.10	0.04	4.0x10 ⁷	0.48
Reference day	4.0x10 ⁴	21.41	5.6	16.56	2.2x10 ⁷	7.98	0.05	2.1x10 ⁶	0.48
4	1.0x10 ⁸	22.83	4.4	18.92	9.9x10 ⁸	8.68	0.05	2.7x10 ⁶	0.45
5	2.4x10 ⁶	20.32	5.8	15.46	1.1x10 ⁷	6.22	0.12	3.8x10 ⁶	0.40
6	9.3x10 ⁵	21.25	6.2	18.54	9.0x10 ⁵	8.26	0.08	8.7x10 ⁴	0.45
7	1.1x10 ⁸	20.35	n.m.	17.07	5.6x10 ⁵	7.24	0.10	1.8x10 ⁶	0.42

TRS = Total Reducing Sugars.

Yield = Ethanol °GL in the Must/TRS⁻¹ in the must.

Reference day: the plants usual sulfuric acid treatment of yeast cream was used.
n.m. = not measured.

These results show that Desinfix™ 135 is as effective in reducing bacterial contamination as sulfuric acid and can therefore be used as decontamination agent. Another point to be noted is that the pH is not negatively influenced by the use of Desinfix™ 135. A stable pH keeps the yeast metabolism functioning properly. The sulfuric acid treatment lowers the pH of the yeast cream to between 2.0 and 2.25, which is too low for the yeast cell to maintain its metabolic functions. When the must is fed to the yeast tank, the pH increases to approximately 5.0 and the yeast cell functions are recovered. Using Desinfix™ 135, this stress caused to the yeast cells by the sulfuric acid is avoided, since adding Desinfix™ 135 to treat the yeast cream during the tests from days 5 to 8 showed that the pH was kept between 4.6 and 5.3.

Several laboratory tests were performed in different kinds of media (containing sugar cane juice, molasses and water in different ratios) and the variability of the results seems to indicate that the efficiency of DesinFix™ 135 to control microbial contamination in sugar cane ethanol mills depends on the medium composition data not shown). Some oxidizing power of performic acid seems to be lost when the medium is very rich in color and organic matter (e.g. when the medium contained only molasses diluted with water, but no sugar cane juice). Therefore, the application procedure in industrial scale will depend on the practices of the particular mill. However, the results show that in suitable conditions, DesinFix™ 135 can reduce microbial contamination in significant amounts (at least 2 logs decrease with the amounts tested), maintain a stable pH, be at least as efficient as sulfuric acid but without the potential negative effects on yeast metabolism and fermentation performance associated with sulfuric acid treatment. The cost of DesinFix™ 135 treatment can be higher than sulfuric acid treatment alone. However, the effectiveness of Desinfix™ 135 makes additional use of antibiotics unnecessary and may even give slight increases in ethanol yields in the process, thus making the use of Desinfix™ 135 a cheaper op-

tion. The biocide Desinfix™ 135 can therefore be considered a viable non-antibiotic alternative to sulfuric acid treatment during industrial sugar cane/molasses fermentation for ethanol production.

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