

NCERC Summary Report:

LactoStab® is More Effective at Higher pH in Corn Mash Fermentation

Objective:

The objective of this study was to evaluate the efficacy of LactoStab® against lactic acid bacteria in corn mash fermentations at two different starting pH's:

- Phase 1: pH of ~5.80
- Phase 2: pH of ~5.50

The experimental design for Phase 1 and Phase 2 involved seven treatments and each treatment was tested in three replicates. The bench scale fermenter study was inoculated with a bacteria load of 1×10^6 CFU/ml and was performed in two separate weeks.

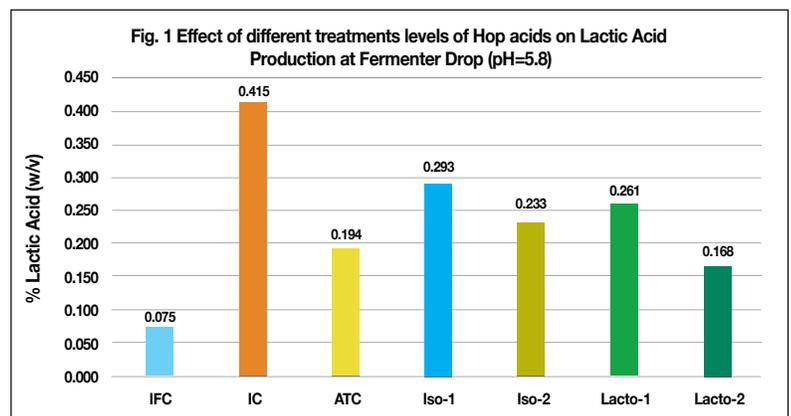
Table 1: Experimental Design for Phase 1 and Phase 2

| Treatment | Inoculation | IsoStab® (ppm) | LactoStab® (ppm) | Virginiamycin (ppm) | Corn Solids (%) |
|------------------------------|----------------------|----------------|------------------|---------------------|-----------------|
| IFC | Yeast | 0 | 0 | 0 | 33 |
| IC | Yeast + L. plantarum | 0 | 0 | 0 | 33 |
| Antibiotic Control (ATC-c) | Yeast + L. plantarum | 0 | 0 | 0 | 33 |
| IsoStab-dose 1 (IsoS-1) | Yeast + L. plantarum | 15 active | 0 | 0 | 33 |
| IsoStab-dose 2 (IsoS-2) | Yeast + L. plantarum | 30 active | 0 | 1 | 33 |
| LactoStab- dose 1 (LactoS-1) | Yeast + L. plantarum | 0 | 15 active | 0 | 33 |
| LactoStab- dose 2 (LactoS-2) | Yeast + L. plantarum | 0 | 30 active | 0 | 33 |

IFC=Infected free control IC=Infected control IsoS=IsoStab® LactoS=LactoStab® ATC=Antibiotic Control

Phase 1 Results:

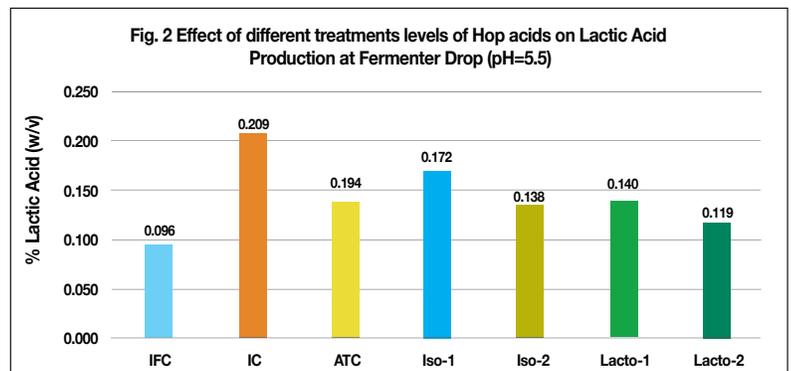
LactoStab-2 at the higher concentration level (30PPM active) performed the best with regards to controlling the amount of lactic acid formation, and the improvement was ~28% over IsoStab (2) or by a factor of 1.4 at the same treatment level. It also slightly outperformed the antibiotic treatment.



IFC=Infected free control IC=Infected control IsoS=IsoStab® LactoS=LactoStab® ATC=Antibiotic Control

Phase 2 Results:

LactoStab-2 at the higher concentration level (30PPM active) performed the best in terms of controlling the amount of lactic acid formation, and the improvement was slightly better than both IsoStab (2) and antibiotics.



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Phase 2a Test:

While the results of the Phase 2 test were positive in terms that it demonstrated the same trend as was observed in Phase 1, the fact that the infected control produced only **2.1 grams/L** at the end of fermentation indicated that the bacteria was not aggressive enough in Phase 2 as it was in Phase 1. There are a number of contributing factors, however we suspect that it was mainly due to the lower starting pH (more acidic media) in Phase 2 vs. Phase 1 that hindered or slowed the growth or development of the lactic acid bacteria during the course of fermentation.

To address this issue or concern, it was decided to challenge the system with a higher starting concentration of bacteria (**5 x 10⁷ CFU/ml**). The Phase 2a study was shorter but all other variables remained exactly the same as in the first two designs. Yeast concentration, urea and enzyme levels were all identical to the original setup of the experiment.

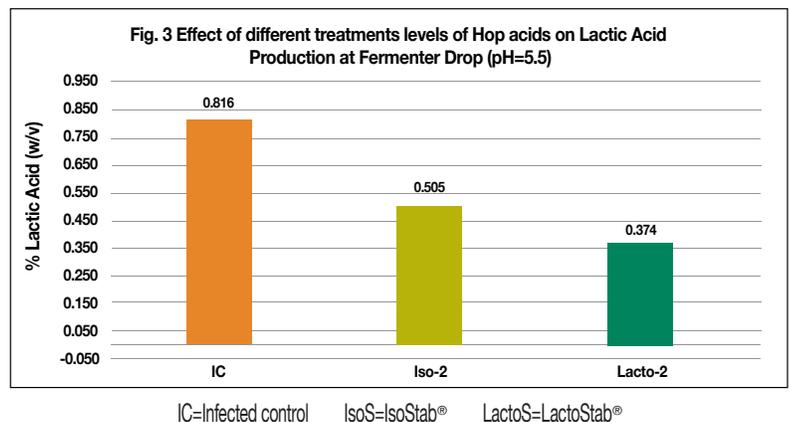
Table 2: Experimental Design for Phase 2a

| Treatment | Inoculation | IsoStab® (ppm) | LactoStab® (ppm) | Virginiamycin (ppm) | Corn Solids (%) |
|-----------------------------|----------------------|----------------|------------------|---------------------|-----------------|
| IC | Yeast + L. plantarum | 0 | 0 | 0 | 33 |
| IsoStab-dose 2 (IsoS-2) | Yeast + L. plantarum | 30 active | 0 | 1 | 33 |
| LactoStab-dose 2 (LactoS-2) | Yeast + L. plantarum | 0 | 30 active | 0 | 33 |

IC=Infected control IsoS=IsoStab® LactoS=LactoStab®

Phase 2a Results:

The challenged system clearly demonstrated that LactoStab-2 at the higher concentration level (30PPM active) performed the best with regards to controlling the amount of lactic acid formation, and the improvement was ~26% over IsoStab (2), or by a factor of **1.40** at the same treatment level. This improvement in performance was statistically different as well.



Conclusion

The bench scale lab study in corn mash fermentation demonstrated that LactoStab® is a more effective product in inhibiting lactic acid bacteria than IsoStab® at higher pH's. At pH levels of 5.5 and 5.8, LactoStab® outperformed IsoStab® in controlling bacterial contamination.

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