

## Fermentation Performance Case Study

### Introduction

One of the more important processes in fuel ethanol production is yeast fermentation of glucose into ethanol. When fermentation is running well, downstream operations run well. Unfortunately, if fermentation does not run well, downstream processes become fouled and low quality byproducts are produced. Also, fuel ethanol output is compromised, reducing facility profits.

Bacterial infections are one of the biggest contributing factors to poor fermentation. Infections inhibit yeast's ability to ferment sugar into ethanol. Ethanol facilities can spend hundreds of thousands of dollars annually on antibiotic use to stop the spread of infection. Often, antibiotics are overdosed to ensure overall bacterial control but also allowing bacterial strains to gain possible resistance to the antibiotics. One common example of the misuse of antibiotics can be seen in Figure 1. Over 20 bags of antibiotics were used per fermenter (ferm) at this facility. Yet only two ferms are problematic and influence the overuse/cost of antibiotics accelerating antibiotic resistance of bacterial strains. Furthermore, most commonly added antibiotics used are only effective against gram-positive bacteria (*Lactobacillus*) and not gram-negative bacteria (*Acetobacter* and *Psuedomonas*). Gram-negative bacteria consume valuable sugar and contribute to poor fermentation. The purpose of this case study is to evaluate the impact *Hydri-Maize™ HC 2759*, a liquid antibiotic-free antimicrobial, has on fermentation performance and its ability to inhibit gram-positive and gram-negative bacteria strains.

### Challenge

Twelve fuel ethanol plants' fermentation circuits were surveyed using Bacteria Profile Analysis technology and the following results were obtained:

- 8 out of 12...** Fermentation circuits had at least one fermenter that contained an excessive amount of *Lactobacillus* bacteria :  $1.0 \times 10^5$  cells/ml more than any other fermenter.
- 7 out of 12...** Fuel ethanol plants were adding 15 1/2-oz bags or more of antibiotics effective only against gram-positive bacteria to all the fermenters to address the high bacteria count from only one fermenter.
- 5 out of 12...** Fuel ethanol plants report gram-negative counts in one or more fermenters higher than the gram-positive counts.

The challenge is to introduce a fermentation antimicrobial that is effective against both gram-positive and gram-negative bacteria that:

- Is easy to apply (liquid form - simple feed pump on a timer)
- Will not allow bacteria to generate resistance
- Will have minimal to zero effect on yeast
- Will break down into environmentally friendly byproducts

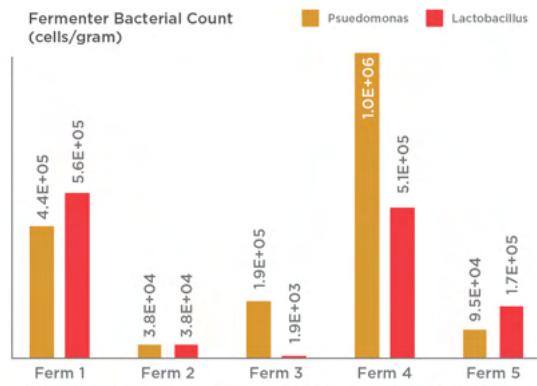


Figure 1: Ethanol facility with 5 fermenters, only two of which yield high bacteria counts

Figure 1

## Results

Hydri-Maize HC 2759 was introduced into a fuel ethanol facility (from the 12 surveyed) with high gram-negative and gram-positive bacterial counts ( $> 1.0 \times 10^5$  cells/ml). The same facility was also using greater than 15 1/2 bags of antibiotics. The simple test protocol was as follows (Figure 2). Adjustments were simple (reduction in antibiotics) and were made every fermentation cycle.

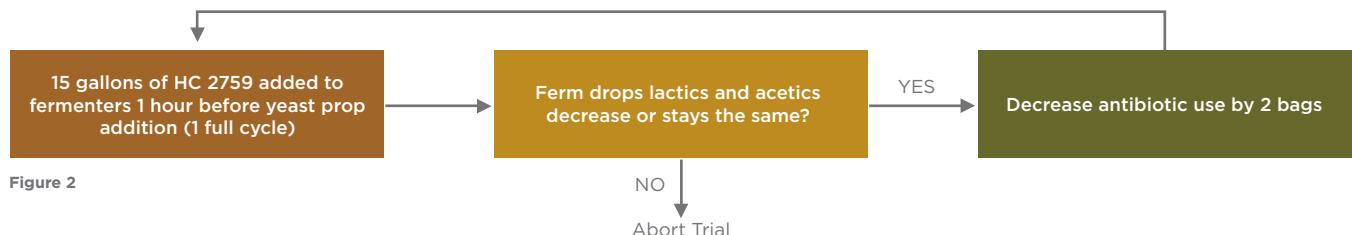


Figure 2

## Value Created

After applying the simple test protocol above, the test facility was able to:

- Decrease antibiotic usage by more than 60%
- Decrease acetic acid content by 28% when measured at the ferm drop (Figure 3), confirming HC 2759's effectiveness against gram-negative bacteria strains.
- Decrease lactic acid content by 41% (Figure 4), suggesting that HC 2759 is freeing up more sugar for fermentation

Overall, the value created for this ethanol was a decrease in antimicrobial costs by 40% and a dramatic reduction in both gram-negative and gram-positive bacteria content per fermenter.

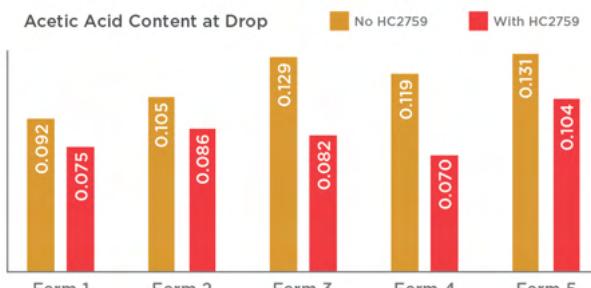


Figure 3: HPLC results for acetic acid content (%vol/wt) measured at the ferm drop. 41 fermenters with no HC2759, 40 fermenters with HC2759.

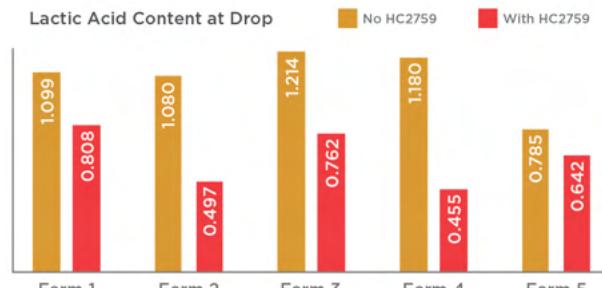


Figure 4: HPLC results for lactic acid content (%vol/wt) measured at the ferm drop. 41 fermenters with no HC2759, 40 fermenters with HC2759.

Figure 3

Figure 4



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HYDRITE  
17385 GOLF PARKWAY  
BROOKFIELD, WI 53045

WWW.HYDRITE.COM  
262-792-1450  
INFO@HYDRITE.COM