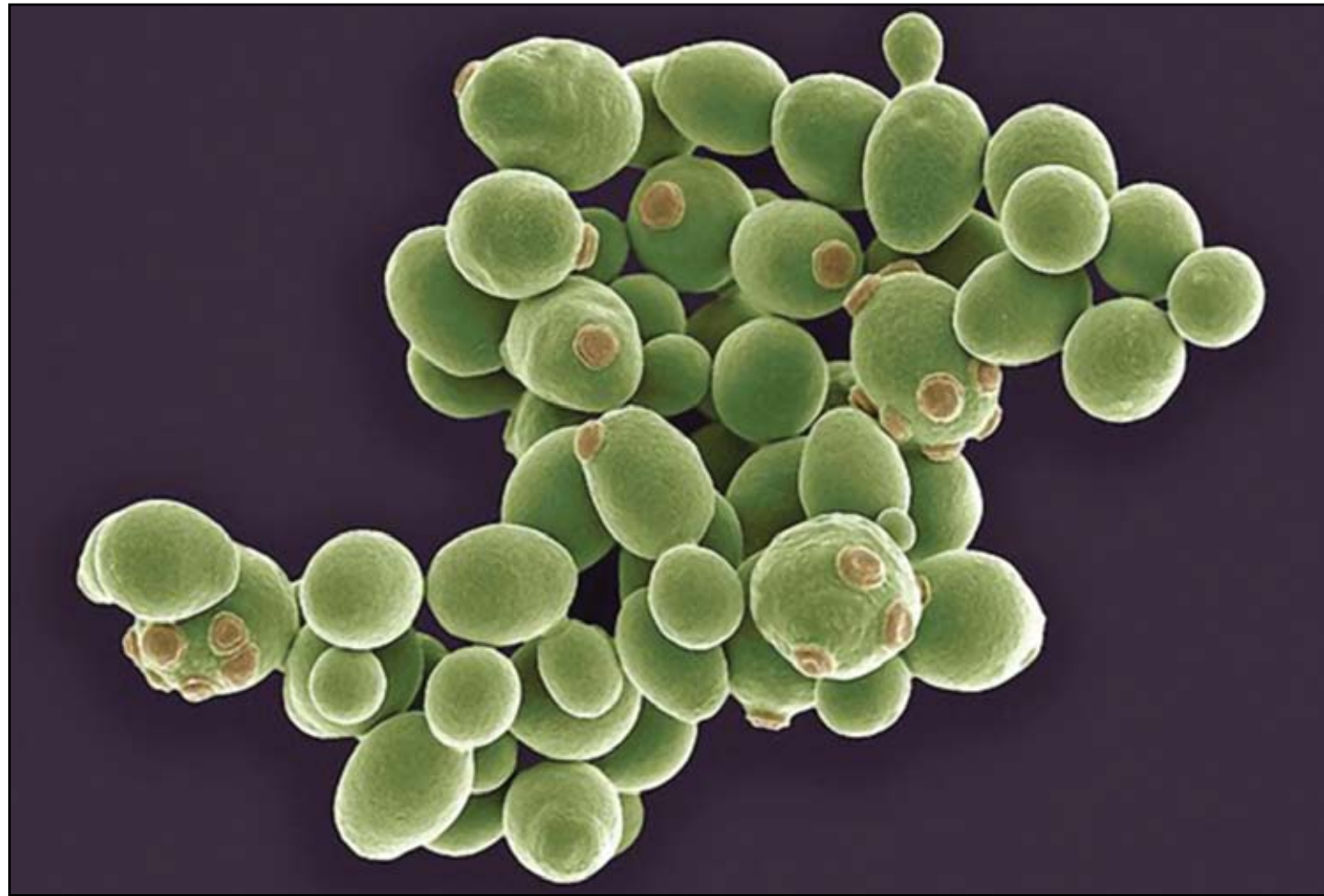


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Bacterial Inhibition Without Antibiotics

A method for controlling infection and maximizing ethanol yields

BY SAMI FAOUR

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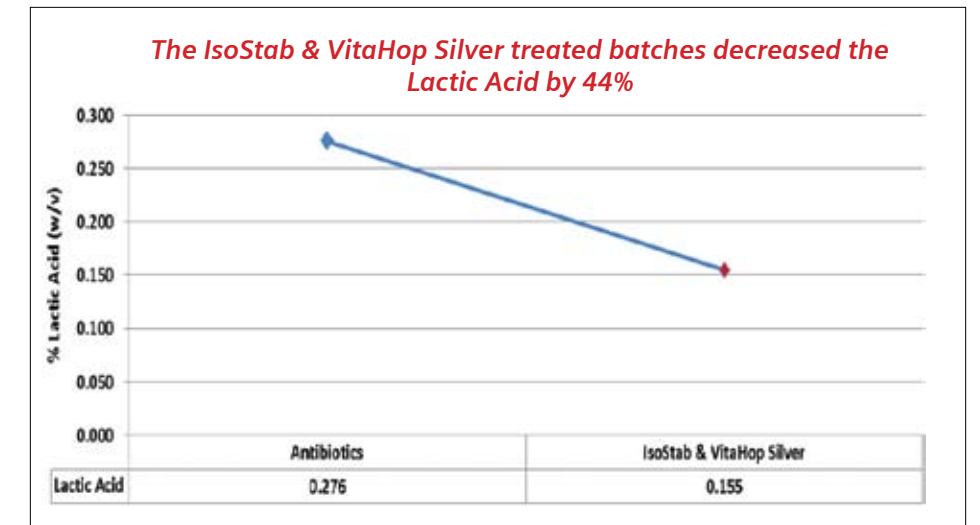
Since the beginning of the year, the ethanol industry has been facing an ever-changing market environment of higher input costs and increased competition. During periods of low-cost corn and high revenue, many operations overlook plant efficiency and focus on throughput. Now that the market dynamics have changed and the growth of the ethanol industry has slowed, and in order to have long-term success, producers have shifted their focus to increased ethanol yield and efficiency.

Increasing the ethanol concentration in a fermenter can be pursued by a number of strategies: purchasing high-quality corn that possesses high-starch and low-moisture content, trialing new enzymes with better starch-to-ethanol conversion or by trying a new yeast strain that is more robust than what's currently available in the market. But using any or all of these strategies does not necessarily maximize yield.

Operators must also address controlling the infection level and its organic acid metabolites with the proper antimicrobial, and maintaining healthier and more viable yeast during the course of fermentation with proper nutrition. BetaTec Hop Products has addressed both of these issues with two technologies which, used together, deliver bacterial inhibition, enhanced yeast health, higher ethanol yield and improved fermentation kinetics.

Infection Potential

Growth of contaminating lactic acid bacteria is a major problem in industrial alcohol fermentations. These bacteria can grow under the conditions of yeast fermentation and reduce alcohol yields by consuming glucose that could have been used by yeast for ethanol production. The end products of metabolism of lactobacilli, lactic acid and acetic acid are



SOURCE: BETATEC

detrimental to yeast growth and metabolism and, therefore, occur when propagations and/or fermentations are allowed to continue under less-than-sufficient microbial control. As bacterial numbers increase so do the acids formed. The metabolism of these organisms varies from that of *Saccharomyces* yeast with substantial production of lactic acid and lesser amounts of acetic acid made. In addition to operating the plant under as sanitary conditions as possible, the most common strategy to control *Lactobacillus* contamination is by the addition of antibiotics. Concerns are rising, however, about the overuse of antibiotics for industrial applications that may contribute to the emergence of resistant pathogenic bacteria strains harmful to animals and humans. Additionally, the presence of antibiotics in saleable coproducts (distillers grains) can negatively impact their marketability.

The preservative properties of hop acids that have been used in the brewing industry for more than a millennium constitute a legitimate naturally derived alternative. BetaTec's

trademarked IsoStab, an aqueous solution of isomerized alpha acids, has been successfully used in dry mill and wet mill ethanol plants since 2004. By adding the minimum inhibitory concentration of IsoStab to the yeast propagation tank and/or to the fermentation vessel, one can inhibit the growth of lactic acid bacteria during the production of ethanol and entirely avoid the need for antibiotics.

The mode of action for hop acids is based on the fact that the undissociated form of the hop acid is toxic to bacteria. Decreasing the mash pH increases the concentration of the undissociated form of hop acid, which is permeable to the cell membrane. The latter form is soluble in the phospholipid of the membrane and diffuses passively into the cell. Because of the higher pH inside the cell, the acid then dissociates and the liberated proton acidifies the cytoplasm, and as a result dissipates the pH gradient across the cytoplasmic membrane. This leads to the inhibition of nutrient uptake and, eventually, the bacteria starves to death.



More efficient fermentation is just a hop away.

Put BetaTec® natural hop extracts to work in your fermentation process to replace antibiotics and enhance yeast propagation. IsoStab® is the natural way to effectively control gram-positive bacteria while eliminating antibiotics and harsh chemicals. Plus, antibiotic-free DDGS adds value to your co-products. VitaHop® Silver yeast nutrient enhances yeast performance and vitality, inducing faster fermentations and larger yields. Combined with BetaTec® fermentation expertise and training, these technologies will significantly increase your plant's efficiency.



BetaTec® Plant Efficiency Formula

IsoStab® Technology

30% aqueous solution of isomerized hop acid extract

- Selective against gram positive bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bacillus*, and *Lactococcus*)
- Hop acid extracts are safe, GRAS & Kosher Certified
- Antibiotic-free DDGS

VitaHop® Silver Technology

A formulation of inactivated yeast, amino acids, vitamins and other ingredients

- Yeast Growth Enhancer
- Yeast Protectant
- Impact: Higher yeast cell count and viability, ethanol yield improvement & faster fermentation kinetics

Highlights of 20 Plant Trials

- ✓ Ethanol yield increase up to 3.2%
- ✓ Increase is statistically different from control
- ✓ More consistent fermentations
- ✓ Yeast cell counts increase up to 30%
- ✓ Yeast viability improvement 2-5%
- ✓ Faster fermentation kinetics 2-4%

BetaTec®...the natural hop to higher profits.
For more information specific to fuel ethanol producers, visit www.bthp.info.

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Enhancing Yeast Nutrition

The importance of yeast nutrition has been overlooked by the fuel ethanol industry. Its relevance to bacterial spoilage, yeast budding, fermentable ethanol ceiling and the rate of fermentation is poorly understood. Depending upon the feedstock used and yeast cell growth during propagation, the fermentation can be negatively affected by nutrient deficiency, causing yeast stress and consequently lower ethanol yield. Bacteria contamination can also deplete the availability of nutrients and, hence, undermine yeast efficiency by scavenging essential nutrients away from the yeast. In practice, just adding nitrogen to the propagators and to the fermenters is sufficient to promote successful fermentations. However, fermentation difficulties arise from a progressive reduction in yeast viability, which is caused by the combined effect of several factors:

- Loss of permeability in the cytoplasmic membrane of the yeast through insufficient synthesis of sterols.
- Adsorption of toxic fatty acids.
- An increase in the concentration of ethanol.
- Temperature fluctuation.

VitaHop Silver, a newly developed fermentation activator, is a proprietary formulation of dry cells of *Saccharomyces cerevisiae*, amino acids, vitamins and other ingredients designed to provide the yeast with the proper balance of nutrients for optimal growth and reproduction. In addition, VitaHop Silver increases yeast viability, particularly late in fermentation, resulting in ethanol yield enhancement.

Synergies in Combination

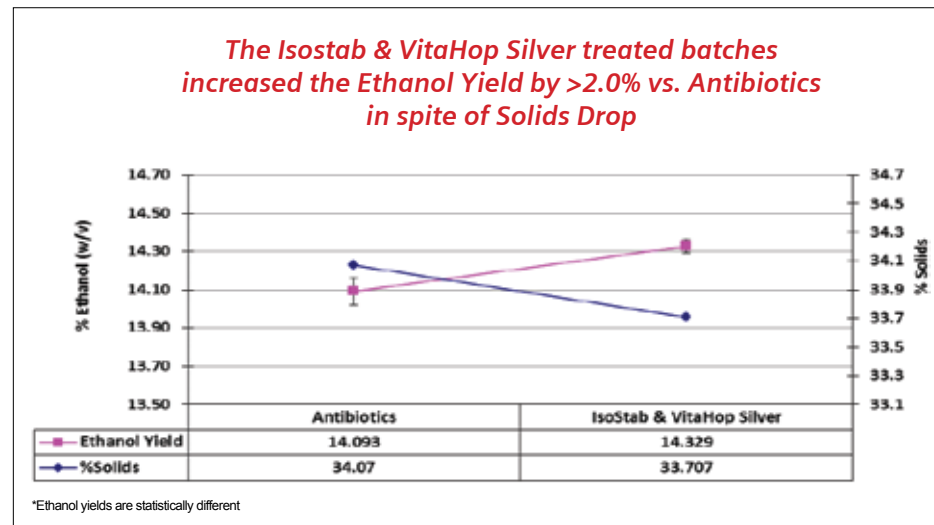
Laboratory research discovered that when both products, IsoStab and VitaHop Silver, are added simultaneously to a fermenta-

tion system, superior results in bacterial inhibition, ethanol yields, kinetics and yeast biomass and viability are achieved compared to when they are used individually or versus antibiotics. This suggested a complementary effect between the hop acid product and the proprietary yeast nutrient.

Those interesting findings prompted investigation of this combined performance at

MMgy plant utilized the BetaTec program and added both IsoStab and VitaHop Silver to the propagator. The plant continued to run at a steady state during the one-month trial.

Fermentation analysis of the plant data under this program showed positive results. HPLC data showed a reduction of 44 percent in lactic acid concentration and a relative increase of 1.7 percent in ethanol yield, despite



SOURCE: BETATEC

the commercial level. The key performance metrics examined in the case study were lactic and acetic acids, yeast cell count, yeast viability, ethanol yield and the kinetics (rate of ethanol production).

A Midwestern ethanol plant was used to trial the IsoStab and VitaHop Silver combination. The plant was experiencing slightly elevated lactic acids and there was interest in comparing their current antibiotic program with other options in order to minimize infection and maximize ethanol production. The 50

the drop in solids during the trial. Correcting it for solid loading, the relative increase in ethanol yield jumped to 2.7 percent (statistically significant as the data was processed at the 95 percent confidence level to ensure that the differences in the data are real). The rate of ethanol production (kinetics) followed a similar trend and the combination was able to increase the production rate by 5 percent at the height of fermentation.

A review of yeast data indicates that the IsoStab and VitaHop Silver combination had

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an 18 percent higher yeast cell count versus antibiotics. More substantial is the fact that viability in the propagator was increased by 5.5 percent, leading to healthier yeast in the fermenter. Although one might predict that excess yeast biomass will lead to reduced ethanol yield, accelerated trouble-free fermentations with higher ethanol concentration resulted. The nutrients and hop acids combined treatment lead to stronger yeast that diverted glucose destined for cell mass to ethanol production.

The magnitude of the ethanol-yield increase with the IsoStab and VitaHop Silver program is critical to the plant's bottom line. In this particular case, the average daily ethanol production was about 150,000 gallons of 200 proof. An increase of 2.7 percent in yield corresponds to 4,050 gallons of extra daily ethanol production. Assuming a price of \$2

per gallon of ethanol, this will add a profit of \$8,100 per day or \$243,000 per month to the plant's bottom line. The plant managers were so satisfied with the results that they continued on with the BetaTec program.

More than 20 plant trials have been conducted with the IsoStab and VitaHop combination technology with the following results:

- An ethanol yield increase, ranging from 1.50-3.2 percent (an increase that was statistically significant compared to the control).
- More consistent fermentations.
- Yeast cell counts increase, ranging from 15-30 percent.
- Yeast viability improvement, ranging from 2-5 percent.
- Faster fermentation kinetics, ranging from 2-5 percent.

Those improvements were observed

against any type of antibiotic that these plants employed and affirm the complementary nature of these technologies. The combined technologies will boost the operational flexibility of a plant by allowing the producer to maximize the quantity of ethanol produced. In addition, it constitutes the perfect alternative to antibiotics and would enable the industry to sell certified antibiotic-free DDGS to local and international markets where antibiotics are either banned or being discouraged.

Author: Sami Faour
 Technical Manager, North America, BetaTec Hop Products
 Sami.Faour@johnihaas.com
 (202) 777-4830



Susanne Retka Schill is the *Ethanol Producer Magazine* contributions editor. Reach her at sretkaschill@bbiinternational.com or (701) 738-4922.

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Sami Faour, Ethanol Technical Services Manager

Sami Faour graduated as a chemist in 1995. He finished his thesis on "Synthesis and Characterization of 3-methyl indole dimmers with alkyl and aryl sulfonic acids" in 1997 from George Mason University. Two years later he received his second Masters in Organic Chemistry from the University of Virginia. Later that year he joined BetaTec Hop Products a subsidiary of John I Hass, Inc as an associate researcher in hop chemistry. Sami has

been involved in synthesizing and formulating a library of hop products that comprised developmental and novel samples for antibacterial and therapeutic screening. Since 2006, he has been the Ethanol Technical Services Manager for Beta Tec in the fuel ethanol industry for North America addressing technical problems associated with field fermentation trials and miscellaneous technical activities like trouble-shooting. He is also actively involved in the development of a new line of products applicable for the ethanol industry.