

The Bactericidal Effect of Hop Derived β -acids

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Introduction

The detrimental effects of bacteria in the extraction process have been well documented throughout the industry. The negative aspects of a microbial infection are not limited to sugar loss alone. The by-products of microbial activity adversely affect purification, evaporation, and crystallization of the sugar syrups. The Amalgamated Sugar Company has evaluated many different biocides in recent years including formaldehyde, gluteraldehyde, carbamates, THPS, peroxyacetic acid, SO₂, ABS, essential oils, and others. During the 1999-2000 campaign a trial was conducted at one Amalgamated facility utilizing a hop extract containing a mixture of β -acids. The use of these β -acids in Europe has been described in several papers by Pollach, et. al.⁽¹⁾

Characteristics of microorganisms

Samples were taken from the diffusion loop and pure cultures obtained. The pure cultures were then characterized to better understand the organisms involved in sucrose destruction. Table 1 summarizes the results of some of these characterizations. Note that most

Table 1
Characteristics of Microorganisms

Location	O ₂	gram	nitrate	acid	spore	capsule	Comments
Press Water	fan	+	+	-	-	+	Bacillus species
	fan	var	+	+++	+	-	Clostridium species
	an	+	+	+	++	-	high % of cells with spores
Mid-tower	fan	+	+	-	-	+	Bacillus species
	ma	+	+	++	-	+	Lactobacillus species
	fan	+	+	+++	-	-	Enterobacter species
RJ @ mixer	fan	+	+	-	-	+	Bacillus species
	fan	+	+	+++	-	+	Erwina species-levan capsules
	fan	+	+	+++	-	-	
	ma	+	+	++	-	+	
	aer	+	-	++	-	-	
	aer	+	-	++	-	+	long thin rod: 5 μ ×1 μ

are gram positive facultative anaerobes. Most are acid producers and are able to reduce nitrate to nitrite. Spore formers are found in press water and diffusion juice. Capsule producers are in the tower and mixer. When applying biocides a knowledge of these characteristics can help in understanding the biocide's effectiveness.

Gram positive organisms are characterized by a peptidoglycan cell wall which composes up to 20% of the dry weight of the cell. By contrast, the gram negative cell wall is less than 2% of the cell weight but is covered by a lipopolysaccharide membrane which is much less chemically permeable. Two other components of a bacterial cell may also protect it from chemical and thermal decomposition. The capsule is composed of polysaccharide which results in higher thermal stability. Spores are formed when unfavorable growth conditions are encountered by the bacteria. Even though the spore does not consume sugar it allows an organism to pass through zones or time periods of chemical or heat treatments.

Biocides

Many different biocides have been evaluated over the years. Recently the most effective used by Amalgamated has been SO₂ either in liquid form or as ammonium bisulfite. The disadvantages of these products include:

- higher corrosion rates of process equipment
- higher ash content in sugar due to formation of imido-bis(sulfonic) acid as a result of reactions with microbial nitrite. The NH(SO₃H)₂ is formed by the reaction:
$$5\text{H} + 2\text{SO}_3^{2-} + 2\text{NO}_2^- \leftrightarrow \text{NH}(\text{SO}_3\text{H})_2 + \text{H}_2\text{O} + \text{NO}_3^-$$
- higher levels of ammonia in the juices
- H₂S production from the acidification of diffusion juice by SO₂ resulting in foam formation

A more environmentally desirable alternative was evaluated during a campaign-long trial during 1999-2000 in Mini-Cassia and 2000-2001 in both Mini-Cassia and Twin Falls.

β-acids

Humulus lupulus, or hops, is a member of the mulberry family. It is a perennial herbaceous climbing vine native to Europe, Eurasia, South America, and the United States. The female plants produce strobiles shaped like a scaly cone and covered with glandular hairs. The hairs contain resinous compounds which when extracted are responsible for the characteristic aromatic-bitter taste (the organoleptic properties) of beer. The α-acids of these resinous extracts are primarily used to flavor beer. However, the β-acid extracts have been shown to have bactericidal effects and have acted as preservatives in beer. Commercial preparations of these β-acids are now available for use in the sugar industry. Since they have been used in the production of beer for many generations the FDA has listed the acids as GRAS. There are three primary acids in the commercial preparation *BetaStab*. They are lupulinic, co-lupulenic, and ad-lupulenic acid. The acids are obtained from hop resins using supercritical carbon dioxide extraction.

The bactericidal mechanism of the acids are dependent on the hydrophobic properties of the bacterial cell. The immediate action is an alteration of the membrane permeability.⁽²⁾ Since gram positive cell walls are more permeable than gram negative the acids are most effective on gram positive organisms. Once inside the cell membrane the acids interfere with protein, DNA,

and RNA synthesis and lysis of the cell occurs rapidly. Of interest, *Clostridium* species are more susceptible than *Bacillus*.

Individual hop acid effectiveness varies with pH. At operating pH the most effective fraction is the lupulinic acid which has a minimum inhibitory concentration (MIC) of 1 ppm. The preparation of hop acids used in these trials was primarily lupulinic acid. The minimum bactericidal concentration (MBC) was shown to be 4 times greater than the MIC. Therefore the dosage used throughout most of the campaign was 4.2 ppm as acid (42 ppm as 10% solution).

Results

A side by side comparison of the effectiveness of ABS versus Betastab was possible in Mini-Cassia due to the two tower operation. Since the #2 (large) tower has been harder to control it was decided to use the Betastab there and use the #1 tower as a control with ABS. This direct comparison was done from the beginning of campaign (September 22) to November 23. The average thermophilic plate counts and lactic acids for this period are illustrated on Figures 1 and 2 below. The apparently good results using the Betastab resulted in a decision to use it on both towers. However, it was found that after a four or five day period the nitrite and lactic levels began to increase. Since the Betastab does not interfere with and may be synergistic with SO_2 ,⁽³⁾ ABS was added for two to three days before re-initializing the addition of Betastab. The thermophilic counts and lactic acid concentrations for the period between November 23 and the end of campaign (February 28) are shown in Figures 3 and 4. The synergistic effect of using alternating biocides is shown in the thermophilic count graph. The later period of campaign usually results in higher counts but using the ABS/Betastab combination lower counts than earlier in campaign were realized.

Figure 1

(Thermophilic count cfu/ml)

ABS / Betastab

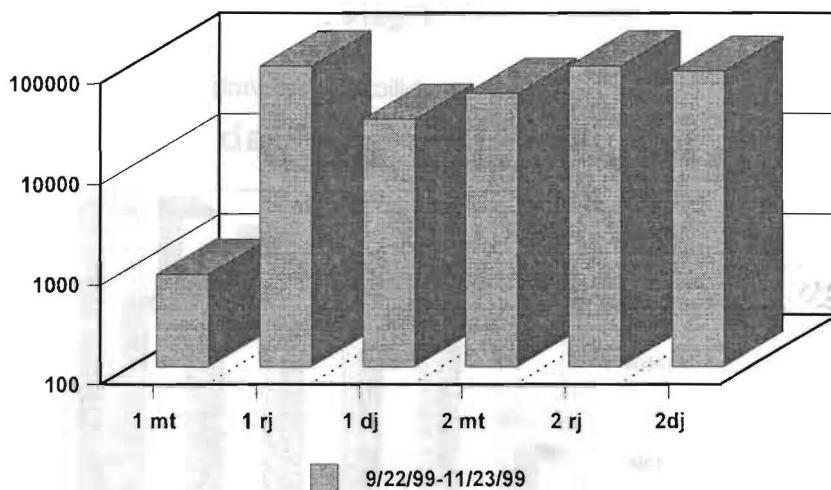


Figure 2

(Lactic acid ppm)

ABS vs. Betastab

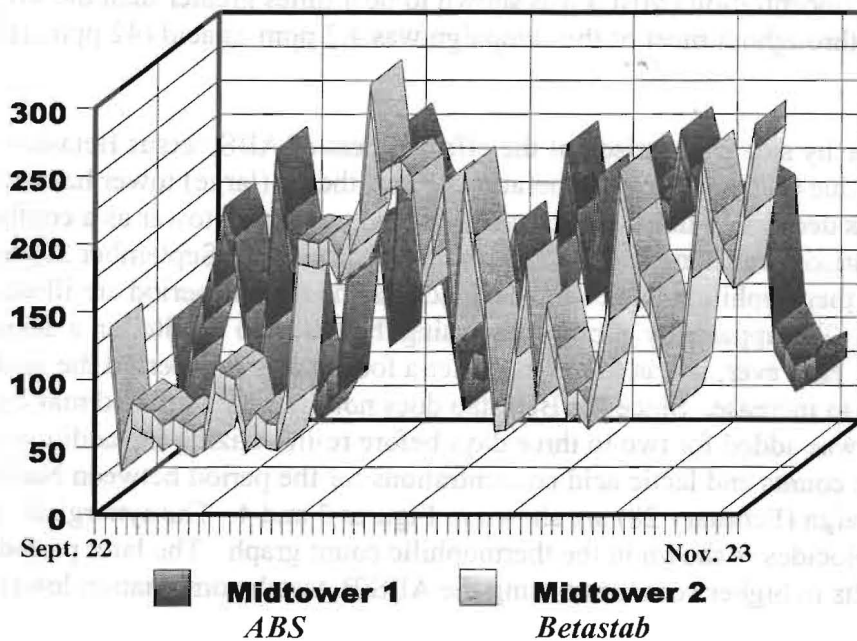


Figure 3

(Thermophilic count cfu/ml)

ABS / Betastab

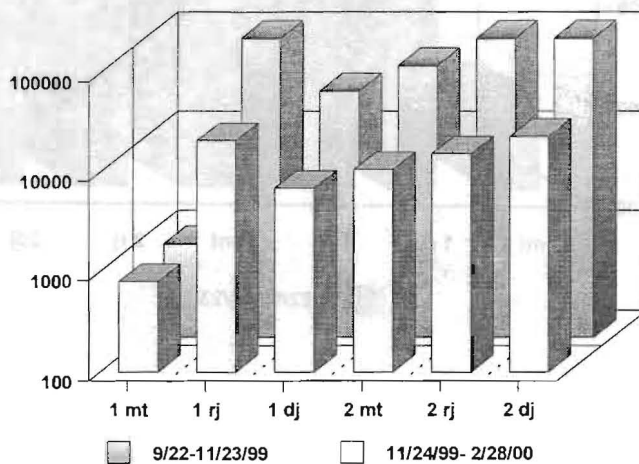
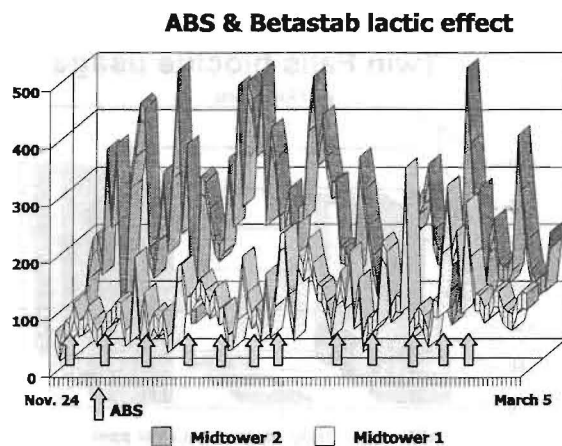


Figure 4



An additional benefit of the Betastab is a reduction in the amount of ammonia in the press water. The amount of ammonia in the press water directly affects both evaporation efficiencies and the amount of water which may be applied to waste water fields. During the Betastab addition periods a 52.8% reduction in the ammonia content of the press wash water was documented.

The success of the 1999-2000 trial in Mini-Cassia prompted the use of hop acids in both Mini-Cassia and Twin Falls this campaign. The application of hop acids allows substantial reduction of ABS usage as shown in graphs 5 and 6. The reduction of ABS has not had a detrimental effect on the lactic acid concentrations or the unaccountable losses.

Figure 5

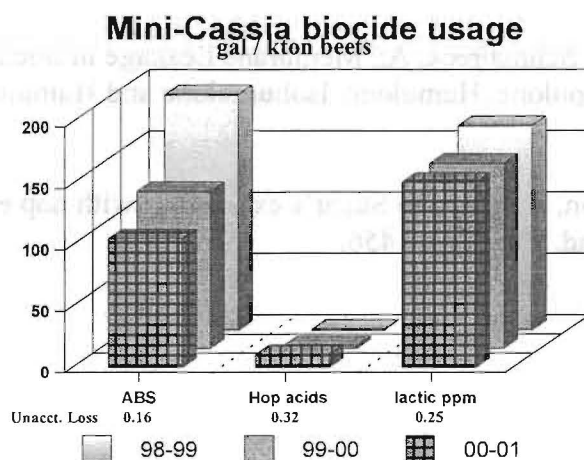
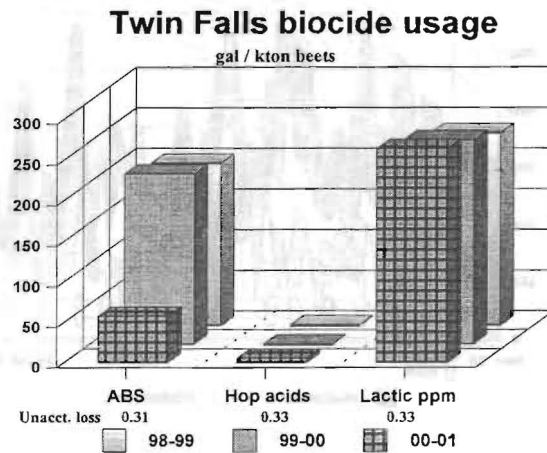


Figure 6



Conclusions:

Commercially available beta-acids from hops are a viable alternative to more corrosive or less effective biocides. The advantages of the use of Betastab include less corrosion, reduction of ammonia in condensates, reduction of SO₂ available for reactions affecting ash content, fewer employee contact concerns, reduction of H₂S production, and the potential for overall biocide cost reduction. These advantages encourage continued utilization and optimization of the hop derived beta-acids.

References

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