H_2S

HYDROGEN SULFIDE

& Beer

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INTRODUCTION

There's no mistaking the classic smell of rotten eggs, indicative of the presence of hydrogen sulfide (H_2S). This off-flavor can be controlled — and for some beer styles, H_2S is part of the normal flavor profile.

The presence of H_2S or other volatiles derived from H_2S may result in discarding an entire batch. Yet, this off-flavor and aroma can be controlled — once it is understood.

This white paper from Lallemand Brewing will help you to understand:

- **The composition of hydrogen sulfide,**
- **How it is produced during brewing,**
- **How failure to remove H_2S can result in formation of other, more stable, sulfur compounds,**
- **Detection methods,**
- **Prevention tips, and**
- **Techniques to remove H_2S**

Our experts gathered all the important literature and interviewed specialists to increase your understanding of H_2S in beer production so the natural flavor of malt and hops can shine through.

H_2S IS NOT ALWAYS AN OFF FLAVOR!

At low levels, hydrogen sulfide contributes to the typical character of lagers. Even at higher concentrations, H_2S is considered acceptable in certain traditional beer styles such as English pale ales from Burton-on-Trent.

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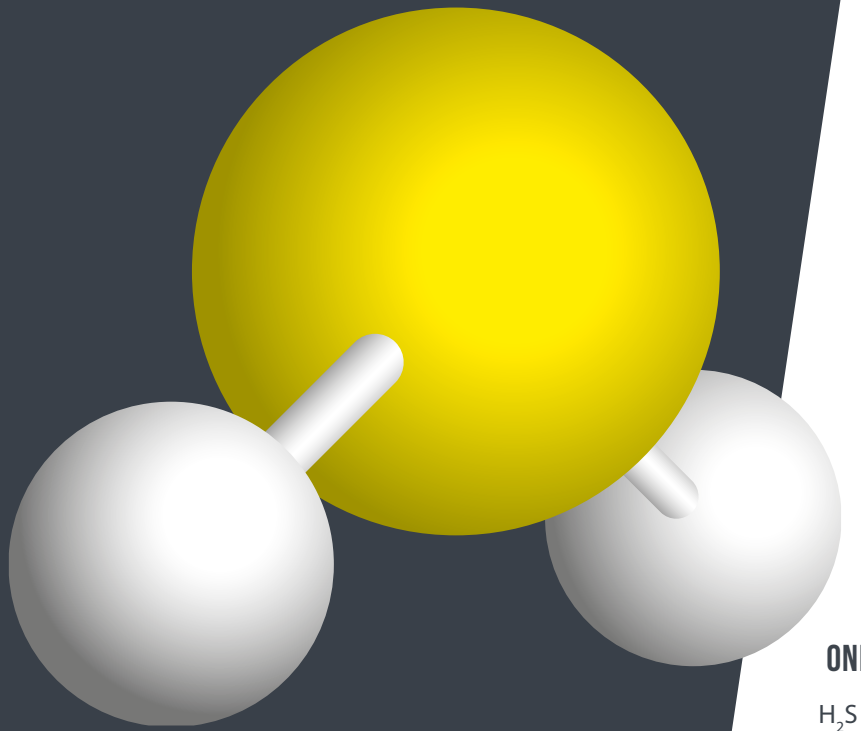
WHAT IS HYDROGEN SULFIDE?



THE MOLECULAR STRUCTURE OF H_2S

Yellow = sulfur, white = hydrogen.

Brewers have been aware of sulfur compounds in brewing since 1898. Today, we know that H_2S is a highly volatile compound with a low flavor threshold in beer, between 0.01 to 0.02 mg/L is detectable by most people.

H_2S is produced in different concentrations by brewing yeasts depending on a variety of factors, including wort composition, fermentation conditions and genetics of the yeast. H_2S can be produced:

- **By both brewing yeast as well as wild yeast contamination**
- **Bacteria contamination**
- **During fermentation or maturation**

ONE OF MANY VOLATILE SULFUR COMPOUNDS

H_2S is one of many sulfur compounds contained in beer, each with its own flavor profile. Sulfite (SO_2) has an aroma of burnt matches whereas dimethyl sulfide (DMS) has an aroma of corn or cooked vegetables. Mercaptans are characterized by a foul sewer-like, burnt rubber or rotting vegetable aroma.

H_2S IS CONVERTED TO OTHER COMPOUNDS

H_2S readily converts into other compounds in response to changes in pH, temperature and staling reactions, which makes it difficult to detect through analytical methods. For example, hydrogen sulfide may react with carbonyl compounds to produce pungent mercaptan off-flavors, which are very stable and difficult to remove from the beer after they are produced.

The amount of H_2S produced is determined by several factors, including:

- **Yeast strain**
- **Fermentation temperature**
- **Nitrogen composition of the wort**
- **Yeast handling practices**

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WHY IS H_2S PRODUCED? 1/3

H_2S is produced by the normal metabolism of the yeast *Saccharomyces cerevisiae* when sulfate ions are reduced for processing into amino acids. Due to the very reductive metabolism of alcoholic fermentation, there is an accumulation of a large amount of hydrogen ions (H^+) in the yeast cell. This creates an acidic environment in the cytoplasm, which is stressful to the yeast cell.

If the yeast does not eliminate these hydrogen ions, the survival of the yeast during fermentation would not be possible. H_2S is produced by the transformation of sulfur by enzymatic reduction of sulfates (sulfate reductase) in order to process and remove excess hydrogen ions from the yeast cell.

The production of abnormally high concentrations of H_2S can occur especially during alcoholic fermentation with nitrogen deficiency or at lower fermentation temperatures.

ROLE OF NITROGEN AND AMINO ACIDS

H_2S is produced as a bioproduct in the formation of the amino acids cysteine and methionine, which is facilitated by the sulfur reduction sequence (SRS) enzymes (See Figure 1). When enough nitrogen is present in the medium, the precursors O-acetyl serine and O-acetyl homoserine will sequester H_2S during the formation of methionine and cysteine respectively. *S. cerevisiae* yeast cultures can be induced to liberate H_2S by starvation of assimilable nitrogen.[1] If nitrogen is limiting, insufficient precursors are available, and free H_2S can accumulate in the cell and diffuse into the fermentation.

Other amino acids in the beer wort can influence H_2S production. Common amino acids in the wort that can favor the production of hydrogen sulfide include cysteine, homocysteine, aspartic acid, glutamic acid, glycine, histidine, homoserine, lysine, ornithine, threonine, and serine. Yeast can also produce other sulfur compounds from these amino acids.

Vitamin deficiency can also result in higher levels of H_2S. Vitamins act as cofactors for the SRS enzymes involved in amino acid metabolism. Low vitamin content in the fermentation can result in decreased levels of methionine, leading to increased levels of H_2S.

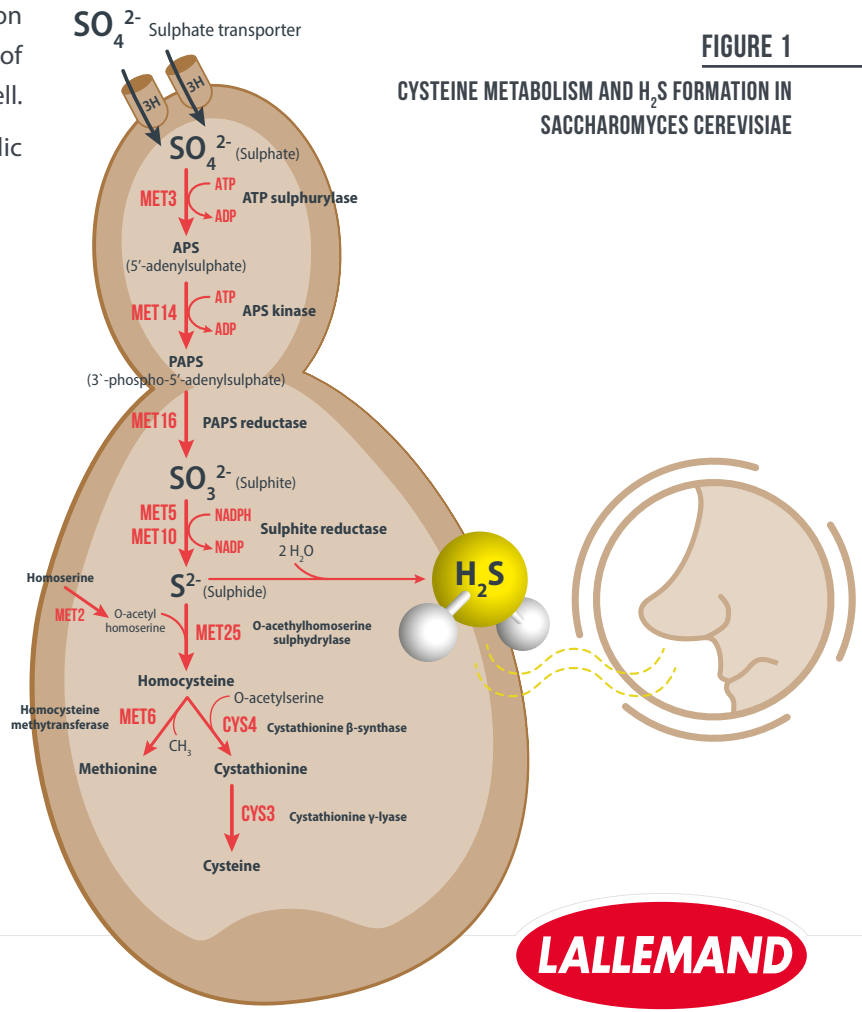


FIGURE 1

CYSTEINE METABOLISM AND H_2S FORMATION IN SACCHAROMYCES CEREVISIAE

WHY IS H_2S PRODUCED? 2/3

FIGURE 2

TYPICAL PATTERN OF H_2S BEHAVIOR AND YEAST IN SUSPENSION DURING FERMENTATION[2]

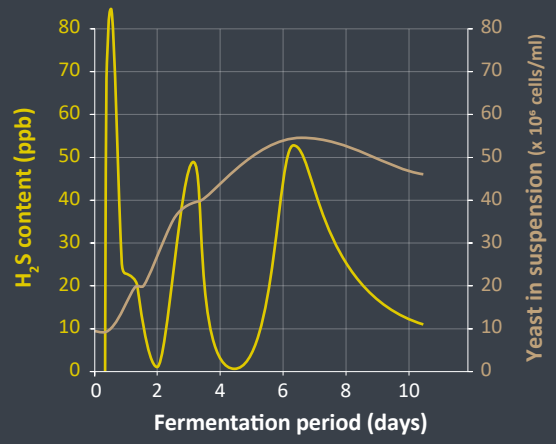
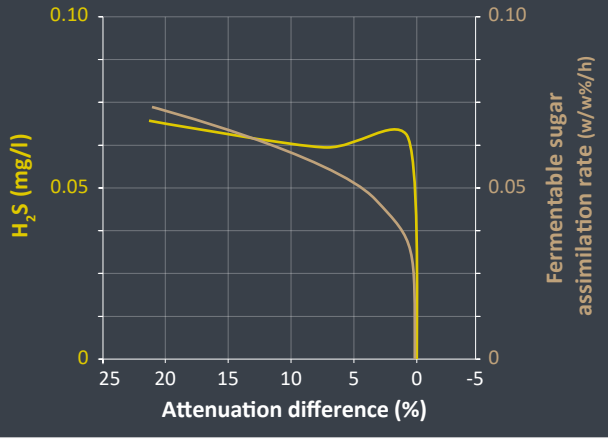


FIGURE 3

H_2S BEHAVIOR AND FERMENTABLE SUGAR ASSIMILATION RATE DURING LATE-STAGE FERMENTATION[3]



ENVIRONMENTAL FACTORS AFFECTING YEAST FORMATION OF H_2S

Sulfur metabolism is a dynamic process and H_2S levels rise and fall over the course of fermentation (Figure 2).[2]

During the first stage of fermentation, yeast take up sulfate and then reduce it to sulfite and then sulfide, which is used for sulfur-containing amino acid synthesis that supports yeast growth and cell division. When budding occurs, the H_2S in the fermenting wort decreases very rapidly, probably through yeast uptake. When metabolism slows, sulfite and sulfide are released from the yeast cells. In the mid and late stages of fermentation after yeast peak biomass, the decrease in H_2S content is said to be mainly attributed to CO_2 purging. As fermentation activity and CO_2 production slows towards the end of fermentation, other factors begin to contribute to decreasing the H_2S levels in the beer.[3]

There is a relationship between the assimilation rates of the fermentable sugars (glucose, maltose, and maltotriose) and the rapid decrease in H_2S at the end of fermentation. Once the fermentable-sugar assimilation rate drops below 0.05 w/w%/h, a rapid H_2S decrease is observed suggesting that the decrease in H_2S is triggered by the depletion of fermentable sugars (See Figure 3). Thus, the timing of rapid H_2S decrease is associated with the fermentation reaching its attenuation limit. [3]

At the end of fermentation, yeast will reabsorb H_2S resulting in a decrease in H_2S levels in the beer. The rate of H_2S reabsorption by yeast is related to the number of yeast cells still in suspension at the end of the fermentation. Extending the fermentation time prior to transferring the beer from the fermenter will provide more contact time with the yeast and a greater overall decrease in H_2S.[3]

CONDITIONS THAT INFLUENCE H_2S LEVELS IN BEER

Increased H_2S	Reduced H_2S
Low nitrogen levels	Adequate level of nitrogen
Yeast stress and autolysis	Vigorous fermentations
Lager yeasts	Higher copper concentrations

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WHY IS H_2S PRODUCED? 3/3

OXYGEN

The introduction of **oxygen** at the end of fermentation during packaging or beer transfer is associated with increased H_2S levels in the finished beer.[2] The introduction of oxygen after fermentation is complete may result in the yeast being stimulated to reactivate metabolism in a nutrient depleted environment, resulting in H_2S production.

STRESS RESPONSES

H_2S formation is also associated with **stress responses** in the yeast cell. Inadequate nutrition (low nitrogen or vitamins), insufficient pitching rate and too low or too high fermentation temperature can result in the overproduction or failure to eliminate H_2S from the fermenting beer. Yeast stress and autolysis will likely occur in parallel, resulting in a complex profile of off-flavors.

H_2S CAN BE FORMED:

- **During primary fermentation when yeast biomass is at its peak**
- **In the final stages of sugar consumption**

H_2S CAN BE REDUCED:

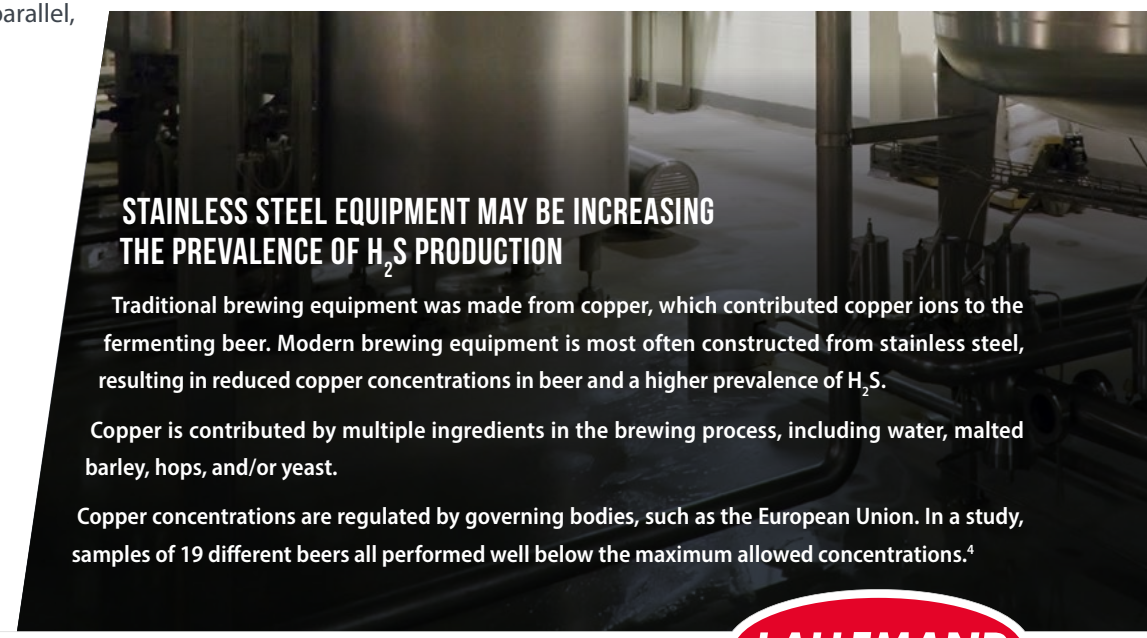
- **During yeast budding**
- **During active fermentation**
- **After achieving the attenuation limit**
- **During maturation while beer is in contact with the yeast**

YEAST STRAINS

Different **yeast strains** vary in their response to physiological and environmental factors in the production and reabsorption of reduced sulfide. It is important to know the potential of a specific yeast strain to produce and subsequently eliminate H_2S when selecting a strain for a particular beer style.

COPPER IONS

Copper ions in the beer can react with H_2S to form insoluble and non-volatile copper sulfide, which precipitates out of the beer. Low concentrations of copper can lead to more noticeable quantities of H_2S.[4]



STAINLESS STEEL EQUIPMENT MAY BE INCREASING THE PREVALENCE OF H_2S PRODUCTION

Traditional brewing equipment was made from copper, which contributed copper ions to the fermenting beer. Modern brewing equipment is most often constructed from stainless steel, resulting in reduced copper concentrations in beer and a higher prevalence of H_2S.

Copper is contributed by multiple ingredients in the brewing process, including water, malted barley, hops, and/or yeast.

Copper concentrations are regulated by governing bodies, such as the European Union. In a study, samples of 19 different beers all performed well below the maximum allowed concentrations.[4]

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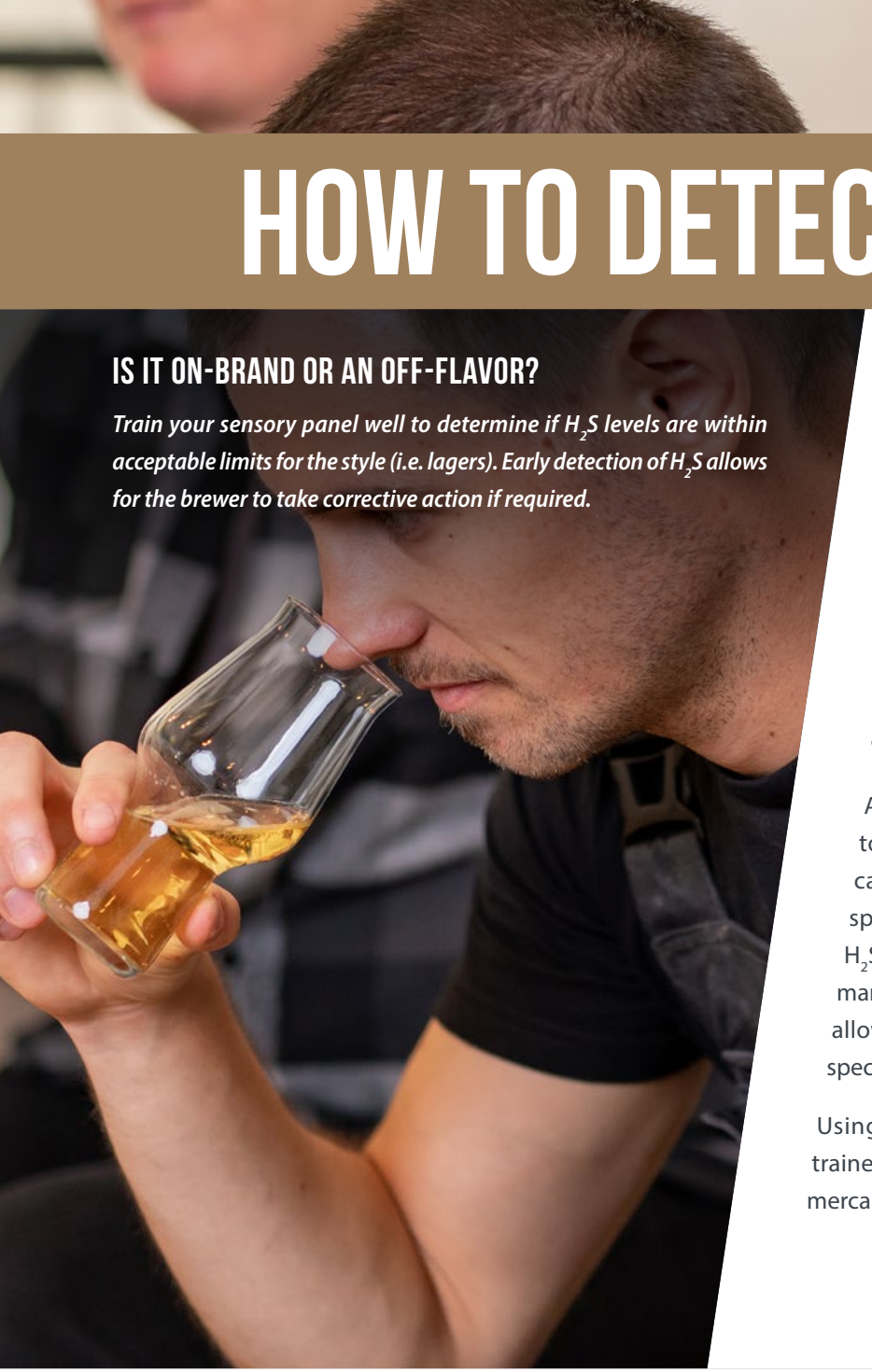
HOW TO DETECT H_2S?



IS IT ON-BRAND OR AN OFF-FLAVOR?

Train your sensory panel well to determine if H_2S levels are within acceptable limits for the style (i.e. lagers). Early detection of H_2S allows for the brewer to take corrective action if required.

Some methods exist for detecting H_2S in the lab by reaction with lead acetate, but these methods are non-quantitative and are typically not sensitive enough to detect trace quantities of H_2S in finished beer. For these reasons, these methods are not widely used in breweries.

Humans have developed a hypersensitivity to sulfur compounds, including H_2S and mercaptans. The presence of copper ions in the nasal mucus increases the binding affinity of sulfur compounds to receptors in the nose and increases the sensory threshold by up to 1000x. Sulfur compounds, in comparison to other common off flavors/defects in beer, are much easier to detect. For example, the average sensory thresholds for diacetyl (0.15 mg/L), acetic acid (90 mg/L) and lactic acid (140 mg/L) are much higher compared to H_2S (0.01 to 0.02 mg/L).

TRAIN YOUR NOSE AND FLAVOR PALETTE

A trained sensory panel is the most effective tool for detecting H_2S in the brewery. Brewers can train their palettes to be more sensitive to specific off-flavors and other aromas, including H_2S and mercaptans. Commercial sensory panel management courses and kits are available, which allow brewers to "spike" their beer sample with specific chemically pure flavor compounds.

Using this approach, a sensory panel can be trained to become increasingly sensitive to H_2S, mercaptans, other sulfur compounds, and more.

PREVENTING H_2S PRODUCTION

THERE ARE VARIOUS METHODS AVAILABLE TO AVOID H_2S PRODUCTION DURING FERMENTATION.

YEAST STRAIN SELECTION

Most ale strains produce lower levels of H_2S. Lager strains tend to produce more H_2S than other strains. Recently, specific yeast hybrids have been selected to produce lower levels of H_2S. These strains overexpress the MET10 gene, which encodes a sulfhydrylase (SHLase) capable of using either O-acetylserine or O-acetylhomoserine and have been shown to dramatically reduce H_2S production under both low and high nitrogen conditions.[6]

YEAST NUTRIENTS

All-malt wort usually contains everything a yeast cell needs for a healthy fermentation. Depending on your malt and water profile, your wort may be deficient in nitrogen, vitamins or minerals. Adding some nutrient might help to reduce H_2S formation. The type of nutrient and timing of addition is important.

Nutrients normally contain either a mineral nitrogen source (i.e. ammonium or urea), an organic nitrogen source (yeast derived), or a combination of both. Mineral nitrogen sources such as ammonium or urea result in very fast fermentations that rapidly deplete amino acids in the fermenting beer, which may trigger H_2S formation. Organic nitrogen sources are metabolized more slowly, resulting in more stable amino acid levels throughout fermentation and less H_2S formation. Nitrogen is not the only nutritional factor that influences H_2S production. A more complex nutrient including nitrogen, vitamins and minerals will reduce the chance of having H_2S in the finished beer.

Lallemand offers a range of yeast nutrient solutions, including YeastLife Extra™ - specifically formulated to provide a balanced blend of all essential elements required for a healthy fermentation - an insurance against H_2S production during fermentation.

LONG AGING/MATURATION PERIOD WITH MINIMUM OXYGEN INGRESS

Giving beer more time to age or mature in the presence of yeast can help reduce the H_2S concentration by allowing more time for reabsorption by the yeast. Longer maturation periods have other flavor consequences that can be positive (i.e. reduced diacetyl) or negative (i.e. reduced hop aroma). A longer maturation period is particularly important in lagers where yeast metabolism is slower at cooler temperatures.

> *"Choose the right yeast strain. Some lager strains are simply more sulfuric than others and require longer maturation times. It's much easier to avoid hydrogen sulfide production during yeast selection than after the beer is brewed."*

RICCIARDI GIANMARIA
TECHNICAL SERVICES MANAGER - ITALY & SLOVENIA

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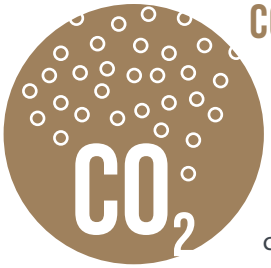
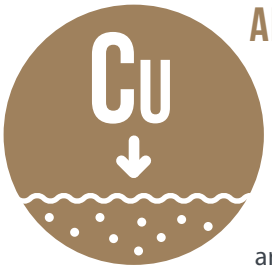
TECHNIQUES TO REMOVE H_2S



CO_2 PURGING

CO_2 purging refers to bubbling CO_2 slowly through the finished beer in the maturation tank. CO_2 purging is effective for removing sulfur in the form of H_2S, which has an aroma of rotten eggs. If H_2S has reacted to produce mercaptans with aromas such as garlic, onion, cooked cabbage, green beans, dishcloth, etc., then this technique will not be effective. If there is some oxygen ingress in addition to the CO_2, then this method may even be counterproductive and result in increased formation of sulphides and disulphides with very disagreeable aromas and very low sensory thresholds. CO_2 purging may also reduce other positive sensory characteristics such as hop aroma.



APPLICATION OF COPPER DERIVATIVES

The most effective techniques for removing H_2S from a beer after fermentation is complete are based on the application of copper derivatives, resulting in a redox reaction with the sulfur compounds.

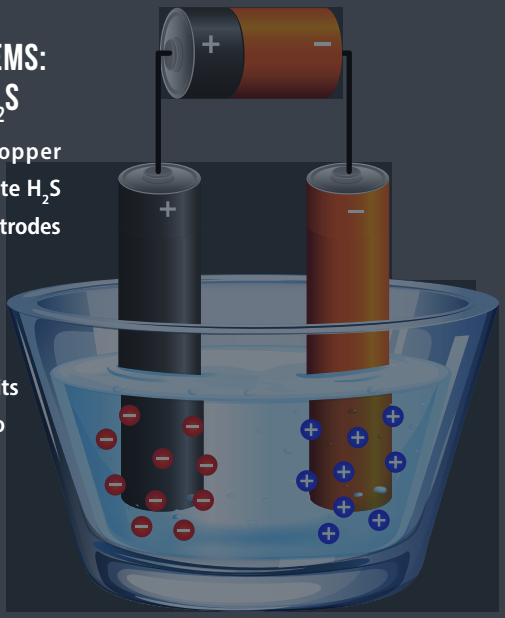
Copper sulfate added to beer quickly and efficiently removes all H_2S from the aroma through the formation of insoluble copper sulfide (CuS), which precipitates out of the beer. As an alternative, beer also can be run through copper tubing or treated with a copper electrolysis system to eliminate H_2S. Copper application may also remove mercaptan aromas from the beer.

Copper treatment is very effective and commonly used in cellars, but it does have some drawbacks. The most important among them is that it leaves many copper ions in solution in the media. The copper reacts rapidly with phenolic compounds causing sensory modifications in the texture of the tannins as well as dryness, metallic sensations and astringency. Copper is also a catalyst for oxidative processes, which may shorten the shelf life of the product.

COPPER ELECTROLYSIS SYSTEMS: ANOTHER WAY TO CONTROL H_2S

Researchers have designed a copper electrolysis system that can eliminate H_2S from beer. The unit consists of two electrodes in a stainless steel housing.

A power supply allows adjustment of the current by changing the voltage to precipitate H_2S as CuS. This system permits additions of minute amounts of copper to beer in the range of 30 µg/L or less.[4]



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CONCLUSION:

Keeping H_2S concentrations below the detectable threshold is an important goal for producing high-quality beers. The simplest way to remove H_2S from beers is to prevent its formation in the first place. This is accomplished by choosing an appropriate yeast strain, providing sufficient nutrition during fermentation and avoiding yeast stress. If H_2S is detected, there are corrective measures available to help rescue the beer by reducing or eliminating this distinct aroma before it reacts to form more stable sulfurous off-flavors.

FOR MORE INFORMATION

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In collaboration with Renaissance Yeast (Vancouver, BC, Canada), Lallemand Brewing recently launched their first 'low H_2S' yeast strain, **LalBrew Farmhouse™**. This novel and innovative Saison yeast, was bred using classical, natural breeding techniques to select low and non-H_2S producing strains.

From a yeast nutrition perspective, the AB Vickers nutrient, **YeastLife Extra™**, a blend of assimilable nitrogen, vitamins, minerals and specific amino acids is designed and formulated to reduce the level of sulfur compounds during fermentation.

www.lallemandbrewing.com/products/

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