DETERIORATION OF MOLASSES DURING STORAGE: POSSIBLE CAUSE AND MEANS TO PREVENT IT

By

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Abstract

MOLASSES has sugar content around 50% and, with a Brix above 85, it should not deteriorate during storage. However, it is not uncommon to observe reduction in sugars during storage. Sometimes, this deterioration is accelerated and instances of foaming with or without a rise in temperature are observed, which changes the colour and smell of molasses. There is a rapid reduction in sugars and a rise in acidity. Microscopic observation revealed that the microbial population is very high, and further microbial evaluation confirmed the growth of two bacteria growing in consortium as single bacteria which required a minimum sucrose content of 30% when grown under anaerobic conditions. When this consortium, was broken in aerobic conditions, none of the isolates could tolerate more than 5% sucrose. Their growth and thus molasses deterioration was controlled only with the use of Polmax ESR, a special biocide formulation used with continuous circulation of molasses with cooling. The possible reasons for such infection and efforts to control or avoid such deterioration in some factories will be presented.

Introduction

Molasses usually has more than 85^0 Brix i.e. only 15% moisture. It contains more than 45% fermentable sugars. If we apply theory, it is impossible to think of any microorganism which could survive in this environment for too long! We know that fungi, the best known living entities at low moisture, do not grow when moisture is lower than 18%, and there are very few microbes that can tolerate more than 30% sugar. It is believed that molasses is stable and does not deteriorate during storage.

All reports of degradation of molasses during storage indicate that it is due to the Millard reaction. This is the chemical reaction between reducing sugars and nitrogenous compounds / amino acids present in molasses. This reaction is exothermic, generates tremendous heat and usually is an uncontrollable reaction. Many molasses tanks are known to have exploded / burned where molasses mass is charred to black mass. The correct reason for the initiation of Millard reaction is yet to be known and nothing worth finding out is left behind when this reaction occurs!

For the first time, we got a chance to study degradation of molasses about 10 years ago in a 3500 TCD sugar factory in North India. Molasses foaming was seen and addition of an antifoaming agent did not have any effect. Molasses started flowing out; there was no rise in temperature. Molasses analysis showed an almost negligible quantity of reducing sugars and low purity, and pH was reduced to 4.3 from an initial pH about 5.2. Brix of molasses was reduced slightly to 89^0. Just due to curiosity, we conducted microscopic examination and, to everyone's surprise, it revealed that it contained huge amounts of short rod-shaped bacteria!

The factory was using quaternary ammonium compound (50% BKC) and dithiocarbamate (40%) based biocides alternatively for 15 days interval, and they did have some stock. Molasses circulation was increased immediately using an additional pump, cooling water was circulated

continuously for keeping temperature below room temperature, and 100 kg BKC was added while circulating molasses. This addition did not give any reduction in foaming or rise in acidity which continued at an alarming rate.

The factory then added the remaining stock of 200 kg dithiocarbamate biocide; this addition initially slowed the deterioration for a day. Deterioration picked up again and the situation looked alarming, the molasses colour turned darker, and its peculiar smell vanished! Luckily, a nearby factory had stock of 120 kg Polmax ESR biocide formulation known to have rapid killing action.

The addition of the 1st can reduced foaming significantly and, after adding the remaining 60 kg, foaming stopped. Several days later, stock of Polmax ESR arrived and, as a precaution, another 60 kg biocide was added. The reaction stopped completely; there was no rise in acidity and no reduction in purity either for almost a month till the molasses was sold. An untreated sample of molasses was studied for possible cause of the deterioration.

Such deteriorations occurred several times in many factories in North India and this experience helped us in controlling such degradation of molasses by treating it with 100 – 150 ppm dose of the biocide formulation Polmax ESR. In every case, foaming stopped immediately on addition of Polmax ESR, and the reaction did not reoccur even after storing molasses for more than a month.

Materials and method

Microscopy was conducted initially by staining a smear with crystal violet stain. Gram staining was also performed in the laboratory.

Various media were used for plate count: nutrient agar, nutrient agar supplemented with 10, 20 and 30% sucrose. Plates were incubated at room temperature: one set in an incubator and the other set in an aerobic jar. Standard microbiological methods were used for isolation of bacteria anaerobically.

Observations

Gram staining of molasses showed that there are two different bacteria in equal number in molasses. One was a Gram positive rod and the other was a Gram negative very short rod-shaped bacterium. Aerobically, none of the plates showed any growth, all plates looked like sterile plates, which prompted us to perform the test anaerobically, as we had seen plenty of bacteria microscopically!

Anaerobically kept plates of nutrient agar showed no growth, and none of the nutrient agar plates with addition of 5, 10, 15 and 20% sucrose showed growth of a single colony even in 1st dilution!

Nutrient agar with 30% added sucrose and kept in anaerobic jar showed mat growth on all dilutions and only the 10th dilution plates showed plentiful, tiny 1–2 mm white sticky amber shaped colonies. The plate looked like a plating of pure culture, as all colonies were identical, whereas microscopy showed the presence of two different bacteria! Gram staining of each colony revealed a similar picture! Each colony was not derived from one bacterium, but was derived from two different bacteria which differed in Gram staining!

Sub–culturing them in the same medium was futile, and third subculture slants showed no growth. Attempts were made to separate these bacteria anaerobically with no success.

However, plating of a single colony onto a nutrient agar plate aerobically gave separation of these bacteria. Two different colonies were observed; one 2 mm white buttery opaque convex colonies (Gram positive rods, possibly belonging to *Lactobacillus* family) and the other 3–4 mm flat translucent shining colony (Gram negative coco-bacilli possibly of *Pseudomonas* family). None of these purified bacteria grew anaerobically and none of them could grow above 5% sucrose concentration. We tried to grow them in the laboratory in combination using nutrient agar with 30% sucrose under anaerobic conditions, but they just did not grow!

Plating of molasses again revealed similar results with lower counts as nutrients were depleting faster. There was growth for several sub-cultures, but growth stopped suddenly after the third or at the most fourth sub-culture.

Subsequent incidences were treated with Polmax ESR. Only microscopy was conducted to see the involvement of similar microbes, but no microbial work was done, as the work was abandoned for the lack of funds and interest from sugar factories.

Conclusion/remarks

Foaming of molasses is caused by growth of microorganisms that have survived during process and have adapted to the conditions in the molasses tank. This growth is initially very slow and can't be noticed till visible foaming occurs; by this time, it is too late to control growth by low doses of an effective biocide.

Usually biocides are able to kill about 99.9% bacteria, in other words there are at least 0.1% survivors which could be alarming when they grow actively in billions!

Molasses contains a large amount of impurities that can protect microbes from the action of biocides; high viscosity also does not allow proper contact of biocide with microbes. Hence, for molasses preservation, the normal dose of biocide is 50 ppm and, in the case of high microbial count, it is advisable to treat molasses with a 100 – 150 ppm dose to ensure killing of almost all microbes to such a low count that they will not be able to grow further.

The best way to preserve molasses is to avoid such bacteria by an effective mill sanitation program. The key is to kill at least 90% microbes including thermophilic bacteria that have the capacity to grow at high temperature and high sucrose concentration at the mixed juice stage. The time available to achieve such killing is less than 20 minutes (many times about 10 minutes) and, when microbial load in harvested cane is known to be higher, i.e. stale / damaged / diseased cane, it is recommended to treat prepared cane with a biocide that has the ability to kill microbes in just 1 minute, as a continuous spray prior to shredder entry. This was also discussed at length at the time of negotiations.

Foaming of molasses studied in a few factories revealed that two types of bacteria are involved in the deterioration. These bacteria find their way to molasses from cane, form a consortium and initiate growth in combination under anaerobic conditions in molasses. One of them is a Gram +ve rod and the other one is a Gram – ve short rod. It was very difficult to separate them anaerobically, and they required minimum 30% sucrose concentration to grow. However, on separation, none of them could tolerate even 5% sucrose! They possibly belong to *Lactobacillus* and *Pseudomonas* respectively, and produce acid and gas by consuming sugars in molasses. This causes molasses to foam and the cell count is often more than 10^{10} per g!

We have controlled this foaming by treating it with continuous addition of Polmax ESR @ 100 ppm dose, while circulating molasses with cooling in more than 10 factories to date.

DÉTÉRIORATION DE LA MÉLASSE PENDANT LE STOCKAGE: LES CAUSES POSSIBLES ET LES MOYENS DE PRÉVENTION

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Mots-Clés: Détérioration de la Mélasse, Ensemble Microbien, *Pseudomonas, Lactobacillus*, Dégradation Anaérobie, Traitement Biocide.

Résumé

LA MÉLASSE qui a un taux de sucre d'environ 50%, et un brix de plus de 85, ne devrait pas se détériorer pendant le stockage. Cependant, on observe souvent une réduction du taux de sucre pendant cette période. Quelques fois cette détérioration est accélérée et des cas d'écumage avec ou sans hausse de température sont observés, ce qui provoque des changements au niveau de la couleur et de l'odeur de la mélasse. Il y a une réduction rapide dans le taux de sucre et une augmentation de l'acidité. Des observations microscopiques ont révélé une population microbienne très élevée, et de nouvelles évaluations ont confirmé le développement de deux bactéries vivant en symbiose et ayant besoin d'un seuil minimum de 30% de sucrose sous des conditions anaérobiques. Quand cet ensemble est dissocié dans des conditions aérobiques, aucun des deux organismes ne peut supporter plus de 5% de sucrose. Leur développement et aussi la détérioration de la mélasse n'ont été contrôlés que par l'utilisation de Polmax ESR, un biocide spécial utilisé avec la circulation continue de la mélasse pendant le refroidissement. Les raisons possibles de cette infection et les efforts pour contrôler ou prévenir une telle détérioration dans quelques usines seront présentés.

DETERIORO DE LAS MIELES DURANTE EL ALMACENAMIENTO: POSIBLES CAUSAS Y MEDIOS PARA PREVENIRLO.

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PALABRAS CLAVE: Deteriorode Mieles, Consorcio Microbial, *Pseudomonas, Lactobacillus*, Degradación Anaeróbica, Tratamiento con Biocidas.

Resumen

LAS MIELES tienen un contenido de azúcar alrededor del 50% y , con un Brix sobre 85, no debían deteriorarse durante el almacenamiento. Sin embargo, no es extraño observar reducciones de azúcares durante el almacanamiento. Algunas veces este deterioro se acelera y se observan espumación e incremento de temperatura, que cambian el color y el olor de las mieles. Hay una répida reducción de los azúcares y una elevación de la acidéz. La observación microscópica revela que la población microbial es muy alta y una evalución microbiológica posterior confirma el crecimiento de dos bacterias desarrollándose en cooperación como una sola bacteria que requiere un contenido mínimo de sacarosa de 30% cuando crece en condiciones anaeróbicas, ninguna de las dos en solitario es capáz de tolerar más de 5% de sacarosa. Su crecimiento y el consecuente deterioro de las mieles puede controlarse solamente con el uso de Polmax ESR, una formulación especial de biocida empleada con la recirculación contínua y el enfriamiento de las mieles. Se presentarán las posibles razones de estas infecciones y los esfuerzos para controlar ó evitar este deterioro en algunas fábricas.