State-of-the-Art Methods for Eradication of Pathogenic Microflora in Sugar Production

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Abstract. Producing sugar from sugar beet is a combination of physical and chemical processes not related to microbial life. Microorganisms in fact are pests for sugar production, as they negatively affect the production rates, lead to a loss of sucrose, and reduce the resulting sugar output while also worsening its quality; the products of microbial life reduce the pH value of sugar products, which leads to a faster corrosion of the equipment and piping. Microorganisms can come from sugar beet itself as well as from water, air, the staff, or the equipment. Non-compliance with the production standards may as well result in contamination. The entire sugar production process is negatively affected by microorganisms, as they cause a considerable loss of sucrose. Beet chips and juice are a good growth medium for pathogens that consume sucrose as a part of their metabolism.

1. Introduction

When sugar beet is exposed to pathogens, its root tissue starts rotting, leading to a catastrophic degradation of sucrose and a drastic increase in harmful nonsugars. The juice of rotten or tainted beet is rich in mucilage. Rotten mass has a profound impact on the process quality of sugar beet and effectively jeopardizes its processing. Diffusion leads to gassing, saturation leads to foaming, juice filtering is compromised, juice evaporation is slowed down, and massequite is crystallized and centrifuged. When the microbial processes progress too far, they make sugar beet unsuitable for further production [1,2,3]. Sugar production typically faces the following microbial groups: lactic acid bacteria Lactobacillus, Carnobacterium, Leuconostoc mesenteroides, Saccharococcus thermophilus, Enterococcus, Pediococcus, Lactococcus, Streptococcus, Vagococcus, Bacillus stereotheomorphillus, Clostridium thermohydrosulfuricum; yeast Saccharomyces sp. (esp. S. cerevisiae), Zygosaccharomyces, Candida; and fungi Aspergillus, Penicilliun, Geotrichum, Mucor, Rhizopus, Phoma Betae Botrytis. If the production conditions are kept optimal for sucrose extraction, especially the temperature, pathogens are not viable, and the unaccounted loss of sucrose in diffusion does not exceed 0.13% of the mass of sugar beet. However, if the production conditions are suboptimal, or the beet received for processing is of low quality e.g. it has too many fungi- or bacteria-infected tails and chippings, pathogen viability...
intensifies, and the unaccounted loss of sucrose rises to 0.5%. This negatively affected the diffusion unit’s and the entire facility’s performance, as each 0.1% of such loss reduces the sugar output by 0.20% to 0.25% of the mass of sugar beet. That is because sucrose degradation products (lactic and acetic acid, dyes, etc.) remain in the juice as salts and are strongly molassesogenic. L-lactic acid is the primary sucrose degradation product. Excessive microbial population may produce up to 600...700 mg/l of L-lactic acid in the diffusion juice, while its concentration normally does not exceed 50...100 mg/l [4,5,6].

2. Relevance and scientific significance
Sugar beet received by sugar factories has a diverse microbial flora consisting of mold fungi, yeast, and bacteria. Although the conditions in which sugar is made are not favorable for microorganisms: high temperatures and high concentrations of the dry matter in most of the intermediate products, some microbes adapt to these extremes and threaten to thwart the entire process.

Microorganisms are known to mainly come to the production facilities with the raw materials, air, and water. 1 g of healthy sugar beet contains 1 to 6 million microbes, whilst 1 g of freeze-damaged or long-stored beet has up to 25×10^6 CFU/cm^3 within, up to 9×10^7 CFU/cm^3 on the surface [4,7,8]. Besides, factories often receive sugar beet with excessive amounts of soil, which is also rich in microorganisms. Washing it does not help disinfect; rather, it may even boost the microbial counts because flume water is not sterile either. Water from rivers and ponds that factories use is also abundant in a whole diversity of microorganisms.

3. Statement of problem. Theory
Microorganisms are able to grow in any section of the production pipeline where the temperature is below 75ºC and the dry matter consists less than 70% of the product. However, it is the diffusion process that provides pathogens with the most favorable habitat. Diffusion juice is a good growth medium for a variety of microorganisms that enter the front of the diffusion unit together with chips and the rear with the pulp press water or barometric water. 1 ml of diffusion juice may contain hundreds of thousands to a few millions of microorganisms. In some cases, diffusion juice can get as rich as 40 million microorganisms per ml at 68...72ºC [5,9]. Their activity leads to the degradation of sucrose, generation of acids, gasification, steaming, production of mucilage, and corrosion of metals. The intensity of microbial growth mainly depends on the temperature. If the temperature is good, they grow quickly to 10^5–10^6 cells per ml. At optimal temperatures, microorganisms can be classified into mesophilic and thermophilic. Mesophiles metabolize and reproduce at 15...45ºC with 30...40ºC being the most optimal range. Thermophiles reproduce intensively at 50...70ºC, optimally at 55...60ºC. Temperatures above 70ºC suppress microbial life [6]. Leuconostok mesenteroides, Bacterium coli, Bacillus mesenteries, Bacillus subtilis, Bacillus stearothermophilus can all be found in diffusion juice. Mesophilic microorganisms enter the diffusion unit on poorly washed or damaged beetroots as well as in feedwater. Their metabolism consumes sucrose and produces organic acids and gases (CO_2, CH_4, H_2); it may even result in an explosion as the formation of gas pockets hinders the circulation of the juice-chip mixture. Microorganisms contained in the pulp press water represent the greatest hazard for the diffusion unit as they have already adapted to the extraction environment and to the disinfectant [1,11,12]. Keeping the diffusion unit at an optimal temperature is crucial for maintaining its sterility and reducing the loss of sucrose in extraction. Bacillus stearothermophilus can grow at extraction temperatures of 67...70ºC; at 72...75 ºC aerobic microorganisms grow no longer [7,13,14]. When feedwater is acidified with sulfur dioxide, sulfites react with formalin, which reduces the disinfectant effects of the latter, especially in the rear of the unit; this helps anaerobic bacteria grow. Occasionally, air is added to the juice-chip mixture at 1 m^3/h to suppress the growth of anaerobes in diffusion units. As the products of microbial digestion accumulate, they cause a loss of sucrose, ultimately producing a lower-quality diffusion juice. Microbial degradation of sucrose is the main contributor to the unaccounted loss of sucrose in diffusion. Besides, microbial metabolites are problematic for nearly any station of a sugar production facility [8,10,15]. Slime-producing and thermophilic bacteria
represent the greatest hazard. Slime-producing lactic acid bacteria (Leuconostok mesenteroides, Leuconostok dextranicum) that grow in the diffusion unit convert sucrose into dextrans, making juice viscous and nearly immobile as it becomes gel-like. This hinders juice filtering. The bacteria encapsulate themselves in a gelatinous shell that helps them survive even high temperatures of up to 87...88°C. Leuconostok colonies fill pipes and make colloids; a million of their germs is enough to degrade 0.001...0.006 mg of sucrose in an hour [9,16]. When processing freeze-damaged or tainted beet, where a variety of Leuconostoc species grow as early as in the pile, and the processing unit fails to suppress the bacterial reproduction, a substantial quantity of dextran, which is a bacterial metabolite, ends up in diffusion juice. Dextran is a highly branched polysaccharide. A dextran molecule consists of D-glucose residues dominated by α-1,6-glycosidic bonds. The molecular mass of dextran reaches 1 million or even more. Dextran forms a mucous layer on the bacterial cell surface that is referred to as the capsule. When dissolved in diffusion juice, dextran makes it far more viscous and less filterable, jeopardizing the cleaning process. The juice thus accumulates a higher concentration of reductants. As the filter fabric absorbs dextran, the filtration rate of diffusion juice goes down [17]. Diffusion juice is extremely favorable for thermophilic spore-forming bacteria that thrive at higher temperatures of 50...70°C. Most thermophiles live at temperatures that kill most mesophilic microorganisms. Some thermophiles can metabolize even at 78...85°C. The growth of thermophilic microorganisms alters the composition of diffusion juice and drastically increases the active acidity as they produce lactic acid and boost the concentration of invert sugar [18]. The degree to which the juice-ship mixture of the diffusion unit has been infected can be quickly measured by the change in the pH of juice sampled from 8 to 10 points on the vertical axis of the unit. When the diffusion unit is fully sterile, visualizing the pH of different points produces a straight line or a smooth upward curve from feedwater to diffusion juice. If there are downward outliers i.e. pH drops dramatically at some points, it is a sign of microbial activity [19]. At lower diffusion temperatures, mesophilic, putrefying, and butyric acid bacteria grow rapidly. Putrefying bacteria degrade the beet proteins and produce ammonia, acetone, organic acids, acetic aldehyde, and gases, which may even cause the diffusion unit to explode. Butyric acid bacteria hydrolyze pectins and starch; they ferment sugars and produce butyric and acetic acid, acetone, and a variety of alcohols and gases [20]. As diffusion juice cools down, yeast begins reproducing in the mixture as well; this process causes alcoholic fermentation of the juice that produces alcohol and gas and results in a loss of sucrose as well as in the accumulation of carbon dioxide within the diffusion unit. Food production facilities have disinfection routines in place to kill or suppress the growth of microorganisms. Depending on the agent type, disinfection methods are classified as physical or chemical. Physical methods include heat treatment (heating, steaming, or boiling), irradiation, ultrasonic and isotope exposure, etc.; chemical methods utilize a variety of antimicrobial chemicals. Death of bacteria is referred to as the bactericidal effect; death of fungi is referred to as fungicidal effect. Agents in use must be universal antiseptics as beet received by sugar factories tends to have a diverse microflora that is difficult to curb. Nearly any microorganism found in soil, water, or on the beetroot surface can be found in diffusion juice, pulp press water, and molasses. Physiologically, these are butyric acid bacteria, lactic acid bacteria, ammonifying bacteria, nitrifying bacteria, slime-producing bacteria, etc. Physically, they are anaerobes, aerobes, thermophiles, and cryophiles. Studies shows diffusion juice to have the following microfloral composition: 34.35% Pediococcus, 18.7% Micrococcus, 18.75% Lactobacillus, 3.2% Leuconostoc, 9.55% Streptococcus, 18.7% Corynebacterium, and 15.7% Bacillus [19].

4. Research findings. Experimental results

Russian Sugar Industry Research Institute has tested an electro-activated liquid system (EAS) for pre-storage treatment of sugar beet. The tests showed the rotting rate to drop by 50...55% over 60 days of storage, and the loss of sugar to drop by 30% against [13]. The effectiveness of Biopag as a disinfectant was compared to that of formalin by means of pre- and post-treatment microbiological analysis. The tests were designed to return the total counts of mesophiles, thermophiles, slime-producing mesophiles, and mold fungi. To obtain the total counts, mesophilic and thermophilic
microorganisms were inoculated in 1:10², 1:10³ and 1:10⁴ dilutions in Petri dishes onto meat peptone agar, slime-producing microorganisms were inoculated onto meat peptone agar with 10% sucrose, and fungi were cultivated in Czapek’s agar. Mesophilic, thermophilic, and slime-producing bacteria were then detected after 48 hours of growth in a thermostat-controlled chamber. Counts were made in 7 days. The research team counted colony-forming units (CFU) per 1 cm³ of diffusion juice or chip surface elution. The disinfecting action was thus evaluated by the number of grown colonies. Besides, the research team tested how the agent (1% solution, 8 liters per 100 tons of beet) and formalin (40% solution, 20 kg per 100 tons of beet) affected the bacterial activity of diffusion juice and beet chips, to which end they utilized spontaneous fermentation. To do this, 200 ml of diffusion juice or 50 g of chips + 150 ml of sterile water was placed in sterile flasks, subsequently adding formalin or Biopag for different experimental groups and keeping the flasks in a thermostat chamber for 24 hours or 6...8 hours in case of the mixture at 55 to 60°C. Juice pH was measured at the start of the experiment and then every 2 hours (every hour for the juice-chip mixture). These indicators were used to monitor the microbiological presence in diffusion juice or mixture to conclude on the microbial activity. Microbiological degradation of sucrose mainly produces lactic acid and acetic acid, both reducing pH. Some researchers have found the pH of diffusion juice to correlate with its microbial population, see Table 1. It means pH is an indirect indicator of the intensity of microbial growth.

### Table 1. Microbiological contamination of sugar beet diffusion juice.

| Contamination degree | Microbial population, CFU/cm³ | diffusion juice pH |
|----------------------|-------------------------------|--------------------|
| low                  | 10²…10⁴                       | 6.9…5.5            |
| medium               | 10⁴…10⁶                       | 5.5…4.5            |
| high                 | 10⁶                           | 4.5                |

With this in mind, the bactericidal effect of Biopag was measured by spontaneous fermentation. Early in the experiment, the research team selected beet chips as the strongest source of primary infection in the juice-chip mixture of the diffusion unit. Adding formalin to the original chip sample with its pH = 6.70 reduced pH of the juice slightly to 6.69 as a result of the agent’s acidic reaction (formalin has a pH of 3.5). On the contrary, Biopag has an alkaline reaction (pH = 9.92), so adding it to the chips increased pH to 6.72. Disinfecting laboratory-made diffusion juice showed Biopag capable of suppressing microbial growth as evidenced by a slight reduction of pH by 0.08 over 24 hours of temperature control. Formalin did inhibit the process, too, albeit to a lesser extent as it reduced pH by 0.43; thus, its effects were slightly less pronounced than those of Biopag. The diffusion juice used in the experiment has a high bacterial activity as shown by a reduction of 2.46 in pH. The bactericidal effects of Biopag were quantified by inoculating the elutions/dilutions of the tested objects onto appropriate growth media and then counting the grown microbial populations. The objects were beet chips and diffusion juice made in the laboratory. Table 2 shows the results of one of the microbiological experiment series. Control chips and juice had the highest microbial contamination for all groups of microorganisms.

### Table 2. Microbial contamination of beet chips and diffusion juice, CFU/cm³ after being treated with Biopag and formalin.

| Sample         | Microbial groups  | Beet chips | Diffusion juice |
|----------------|-------------------|------------|-----------------|
| Control sample | Mold fungi        | 10.5 × 10³ | 3.8 × 10²       |
|                | Mesophiles        | 9.8 × 10³  | 6.9 × 10³       |
|                | Slime-producing bacteria | 14.1 × 10³ | 4.8 × 10³       |
|                | Thermophiles      | 3.1 × 10³  | 2.2 × 10³       |
Treating chips and diffusion juice with formalin reduced the microbial population. It effectively cut the growth of mold fungi by $8.1 \times 10^3$ CFU/cm$^3$ or by a factor of 4.4 for the chips; it also suppressed the growth of mesophilic bacteria by $5.5 \times 10^3$ CFU/cm$^3$ or by a factor of 4.9 for diffusion juice; these were the greatest reductions observed in the experiment. Biopag showed similar patterns. Similarly, it was most effective against fungi on chips ($8.5 \times 10^3$ CFU/cm$^3$ or 5.3x) and mesophiles in diffusion juice ($5.7 \times 10^3$ CFU/cm$^3$ or 5.8x). Table 3 summarizes the effects of treating beet chips and diffusion juice with formalin and Biopag.

**Table 3.** Disinfecting effects of Biopag and formalin on beet chips and diffusion juice, %.

| Sample  | Microbial groups            | Beet chips | Diffusion juice |
|---------|----------------------------|------------|-----------------|
| Mold fungi | Mesophiles       | 77.1       | 55.3            |
|         | Slime-producing bacteria | 65.3       | 79.7            |
| Bacteria | Thermophiles       | 65.9       | 45.8            |
|         |                     | 41.9       | 50.0            |
| Average for all groups |                     | 62.6       | 57.7            |
| Mold fungi | Mesophiles       | 81.0       | 60.5            |
|         | Slime-producing bacteria | 64.3       | 82.6            |
| Bacteria | Thermophiles       | 66.7       | 50.0            |
|         |                     | 61.3       | 40.9            |
| Average for all groups |                     | 68.3       | 58.5            |

Apparentely, formalin had an average antimicrobial effect of 62.6% on beet chips, 57.7% on juice across all the microbial groups, peaking at 77.1% for mold fungi on chips and 79.7% for mesophiles in juice. Biopag had an average effect of 68.3% on chips and 58.5% in juice, peaking at 81.0% for mold fungi on chips and 82.6% for mesophiles in juice.

Thus, the preliminary finding is that Biopag has a pronounced antiseptic effect on the key groups of microorganisms infecting the juice-chip mixture and diffusion juice in diffusion units (mesophiles, thermophiles, and slime-producing mesophiles), and this effect is similar to, and in some cases even stronger than that of formalin. Biopag's total disinfecting effect was 58.5% for diffusion juice and 68.3% for chips compared to 57.7% and 62.6% for formalin. The agent was found to positively affect the process qualities of diffusion juice.

5. References

[1] Spichak V V, Belyaeva L I, Ananyeva P A 2009 Technological means in the production of sugar *Sugar* 9 pp 41–45

[2] Spichak V V, Sapronov N M, Saltyk I P 2008 Sugar beet is a raw material for sugar production (Kursk: RNIISP) 264 p

[3] Zharikova G P 2005 Microbiology of food products Sanitation and hygiene (M.: Academy) 304 p
[4] Dotsenko V A 2002 A practical guide to the sanitary supervision of processing industry enterprises (Spb.: GIORD) 148 p
[5] Spichak V V, Egorova M I, Buromsky V V 1995 Scalding of beet chips and the vital activity of microorganisms *Sugar industry* 6 pp 15–16
[6] 2001 Methodology for determining the chemical composition and quality indicators of sugar beet (Kursk: RNIISP) 47 p
[7] Nickisch Hartfiel A, Mauch W 1983 Untersuchungenvon Desinfektionsmittelfurderen Einsatzinz Extrakionsangel Zuckerindustrie 108 1 pp 24–35
[8] Sapronov N M, Kurasova L M, Rassolova G G, Smirnov K V, Shemyakin O N 2003 Antiseptic preparations for processing diffusion juice *Sugar* 3 pp 42 - 43
[9] Donchenko L V 1999 Safety of raw materials and food products (M.: Food industry) 352 p
[10] Dolgopolova N V, Shirokikh E V, Kosulin G S 2017 Technological indicators of the quality of sugar beet roots depending on their predecessors *Sugar beet* 3 pp 6-9
[11] Kaluzhskikh A G, Belyaev A G, Zaikina M A 2019 Spatial variability of the content of labile humus substances in black soils and their relationship with microbial biomass Conference on Innovations in Agricultural and Rural development (Kurgan)
[12] Podporinova G K 2010 On the question of the safety of root crops in kagats (Sugar beet) 7 37 p
[13] Apasov I V, Fomenko G K, Putilina L N 2011 The effectiveness of drugs to improve the safety of sugar beets during storage *Technology of high yields* 4 pp 37–39
[14] Kulneva N G, Shmatova A I 2012 Problems of sugar beet processing *Actual biotechnology* 2 pp 32-33
[15] Korneeva O S, Spivakova L V, Maltseva T V 2006 Fundamentals of microbiological and sanitary-hygienic control at the enterprises of the sugar beet industry: textbook (Voronezh) VGTA pp 5–22
[16] Shuvaeva G P, Korneeva O S, Grigorov V S, Ruadze I D 2003 Biology and Microbiology textbook allowance (Voronezh) VGTA pp 214-216
[17] Rabinovich G Yu, Sulman E M 2005 Study guide, first edition Sanitary and microbiological control of environmental objects and food products with the basics of microbiology 221 p
[18] Sapronov N M 2018 Storage of modern hybrids sugar beet with the use of multifunctional preservatives *Sugar production* 8 p 2628
[19] Kulneva N G 2014 Microflora of sugar beet production: problems and solutions *Vestnik VSUIT* 1 pp 193-196
[20] Spichak V V "Biopack" for processing diffusion juice *Sugar* 2 pp 38-40