ANALELE UNIVERSITATII DIN ORADEA
FASCICULA: ECOTOXICOLOGIE, ZOOTEHNIE SI TEHNOLOGII DE INDUSTRIE ALIMENTARA, VOL. VII, ANUL 7, 2008

ANALYSIS OF THE RISKS AND OF THE MAIN SOURCES OF MICROBIAL INFECTIONS IN A SUGAR FACTORY

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Abstract

In addition to these there are a multitude of other sources of microbial infections such as: the beet cutting machinery knives that accumulate beet fiber, beet roots fallen and left for a long time under the processing equipment, beet roots that remain for a long time in different narrow places of the equipment, overflowing of juices and syrups, collecting channels, the damp walls of the vacuum apparatus, the tanks and storage rooms where enters or where is produced sugar powder, water from the atmospheric pressure measuring tub, diluted syrups adhering to the interior surface of the storing reservoirs, etc..

Key words: microbiological, technological, sugar, temperature

INTRODUCTION

The most important sources of microbial infection from sugar factories are:

- load of beet;
- hydraulic water discharge - hydraulic transport - washing beet;
- the discharger itself;
- press water circulated in the diffuser

The microbiological loading of the beet affects its technological quality. Microorganisms can grow on soil surfaces that clinging to the beet and may occur as a result of diseases affecting sugar beet during the growing season. During the storage of the beet, even during short periods, existing microorganisms can grow or there can appear new species which can attack the beet root. Among microorganisms that have been identified on beet roots during storage there may be noted *Mucor hiemalis Wehm, Rhizopus nigricans Ehrenb, Botrytis cinerea Pers., Penicillium expansum Thjom., V. Tiegh Aspergillus niger, Aspergillus glaucus De Bary, Aspergillus ochraceus Wilh, Fusarium beta Desm Sacc, Fusarium oxysporum skiing., Phoma beta Frank., Alternaria tenuis Nees, Cladosporium heroarum Link.*

MATERIAL AND METHODS

1. Circumstances in which technological water helps infestation

Water is circulated in a closed circuit and it permanently contains sugar and active microorganisms. The number of bacteria found in the discharged water used in the hydraulic – transport and washing of the beet root are the following:

- 120 – 450 million of mezophilic bacteria / ml;
- 20 – 90 thousand of thermophilic bacteria / ml.

Fig. 1. Beet washing machine photographed in the sugar factory courtyard in Oradea

RESULTS AND DICUTION

2. The presence of microorganisms in the extraction diffuser

Sometimes, in the whole volume of the diffuser or only in certain areas can occur optimal conditions for the development of micro-organisms. These micro-organisms originate on the beet noodles, from the circulated press water and the fresh water. Beet noodles contain, in general, $10^5 – 10^7$ cells of microorganisms / g, derived from sugar beet, clinging soil, mostly found in the small absorbed strings from the longitudinal ridges, and from the washing water that adheres to the beet skins. Among these microorganisms we can find:

- *mezophilic microorganisms*, which develop between the temperatures of 5 ... 50° C, with the best field between 25 and 40° C;
- *thermophilic microorganisms*, which develop between the

temperatures of 25 ... 73° C, with the best field between 50 and 55° C.

The tilted gutter type diffuser has, at both ends, an area threatened by infection, where temperatures are below 60° C. These two areas are:

- *at the helm of the extractor*, on about 10% of its length, where the temperature is 15 ... 45° C, and for the the beet noodles to reach up to 73° C are needed 10-15 min. After heating at temperatures over 60° C, moisture and moderate temperatures loving microorganisms die, and in the juice remain their resilient forms, that can withstand heat and hot water, and moisture and high temperatures loving microorganisms;

- *at the other end of the diffuser*, microorganisms enter in the press along with water, which circulated inside the diffuser.

Fig. 2. Vertical diffuser

The press water is responsible for transporting the microorganisms that affect sugar and other beet components. Here are some of the microorganisms that get circulated along with the press water:

- *Bacillus subtilis*, which develops between 20 ... 25° C, with the best field between 28 ... 40° C. to develop *bacillus subtilis* requires high concentrations of oxygen and it's not destroyed in pre defecation or cold defecation. This bacillus is recognized because it turns azotații into nitrogen;

- *Baciullus stearothermophilus*, which develops between 37 ... 70° C, with the best field between 50 ... 65° C. It is resistant to high temperatures. It produces lactic acid and in smaller quantities citric acid;

- Lactobacillus, which develops between 28 ... 62° C, the optimum temperature being 35° C. it produces lactic acid;

- *Leuconostoc mesenteroides*, which develops between 11 ... 43° C, with the best field between 21 ... 25° C. Produces lactic acid and gelatinous capsules of dextran, which have the role coating an protecting, enabling the

microorganism to develop even in highly alkaline syrups and withstand the high temperatures inside the first body of the evaporation station;

- *Aerobacter aerogenes*, which do not cause loss of sugar, but determine the release of significant quantities of gases that cause foaming;
- *Pediculatum bacterium*, which produces gelatinous levulan, very similar to the dextran, but it rotates to the left the vibration angle of the polarized light.

2.1. The effects of the presence and activity of micro-organisms in the sugar factory

Analyzing the main species of microorganisms we find out that they are:

- yeasts, which transforms sugar into alcohol and acetic acid;
- lactic bacteria which convert sugar into lactic acid. Lactic bacteria find ideal conditions for development inside the diffuser and are the main cause for microbiological loss of sugar during the diffusion process.

In table 1 are listed species from the genus *Lactobacilus* producers of lactic acid, mentioning the optical isomer or isomers of lactic acid they produce.

- *Butyric bacteria*, which convert sugar into butyric acid, causing the smell typical of rancid butter;
- *Bacteria acetono butanol*, which converts sugar into a mixture of acetone and butyl alcohol;
- *Bacteria of the genus Leuconostoc*, which in active status, unprotected by coating of dextran, turn sucrose into dextran according to the reaction:

$$(\text{Glucose})_n + \text{Sucrose} \rightarrow (\text{Glucose})_{n+1} + \text{fructose}$$

The reaction is catalyzed by an enzyme called dextran sucrose. In addition to the losses of sugar, dextranul is likely to cause the warping of the filter canvas and to create abnormal crystallization of sucrose.

Table 1

Species from the genus *Lactobacillus* producers of lactic acid

The name of the species	**Optical isomer of lactic acid produced**
Lactobacillus animalis	Levo lactic acid
Lactobacillus acidophilus	Levo lactic acid, Dextrolactic acid
Lactobacillus helveticus	Levo lactic acid, Dextro lactic acid
Lactobacillus bulgaricus	Levo lactic acid
Lactobacillus lactis	Dextro lactic acid
Lactobacillus delbrueckii	Dextro lactic acid
Lactobacillus salivarius	Levo lactic acid, Dextro lactic acid

Lactobacillus casei	Levo lactic acid
Lactobacillus plantarum	Levo lactic acid, Dextro lactic acid
Lactobacillus xylosus	Levo lactic acid
Lactobacillus curvatus	Levo lactic acid, Dextro lactic acid
Lactobacillus coryniformis	Levo lactic acid, Dextro lactic acid
Lactobacillus farciminis	Levo lactic acid, Dextro lactic acid
Lactobacillus alimentarius	Levo lactic acid
Lactobacillus sharpae	Levo lactic acid
Lactobacillus amylophilus	Levo lactic acid
Lactobacillus brevis	Levo lactic acid, Dextro lactic acid
Lactobacillus buchneri	Levo lactic, acid Dextro lactic acid
Lactobacillus confusus	Levo lactic acid, Dextro lactic acid
Lactobacillus fructivorans	Llevo lactic acid, Dextro lactic acid

CONCLUSIONS

Following the practical and theoretical study it has been found that depending on the temperature of reproduction, in which activity is most intense, causing the biggest loss of sugar and the greatest difficulties in carrying out the technological process and the operation of machinery and plants, bacteria acting in the sugar factory can be categorized as follows:

- *Criophilic* bacteria, which reproduces at low temperatures and at temperatures in the area of 35 ... 40° C their activity is reduced;
- *Mezophilic* bacteria, which best reproduce at around 40° C. At temperatures of 60 ... 70° C, their activity becomes highly reduced;
- T*hermophilic* bacteria, which reach the peak of their reproduction and become extremely active at temperatures of 65 ... 70° C.

From the microbiological point of view, we can distinguish two different areas: before and after the pre heating of the beet noodles namely:

- before the preheating of the noodles the one that acts is the mezophiliac flora. The flora is naturally present in the roots of sugar beet and is likely to grow rapidly during the technological process of processing sugar beet. These micro-organisms cause loss by sugar fermentation;

- after preheating thermophilic germs develop, which, similarly, cause loss of sugar, but by the formation of lactic acid. These losses of sugar make obvious the necessity and an obligation of carrying out the disinfection process.

Many researchers have sought out to establish real values for microbiological losses of sugar trough diffusion. Thus:

- Sazavsky established that in the specific conditions from Poland, the microbiological losses resulted from diffusion ranged from the 0.2 - 0.3 kg sucrose / 100 kg beet;

- Schneider has established that in the specific conditions from Germany, microbiological losses during diffusion are about 0.2 kg sucrose / 100 kg beet;

- research carried out at the sugar factory in Oradea in the time frame 2003 - 2007 have established that the microbiological losses differ from caricature to caricature, as determined by several factors, including: outside temperature conditions, climate, the technological quality of the beet, technological discipline , managerial style, the level of training of employees etc.. The average loss of sucrose is situated between 0, 2-0, 7 kg sucrose /100 kg beet. Therefore, at the factory in Oradea, the problem of disinfection, prevention and combating of microbial infections is a priority and essential to ensure economic efficiency and profitability. Experimentally, it was determined that for the formation of one gravimetric part of lactic acid trough microbiological processes, there are metabolized two gravimetric parts of sucrose. These losses occur mainly in DDS type diffusers, in part where the noodles are heated which the heating at environmental temperature, with which they are introduced until they reach the temperature of plasmolize, which is 70 ° C.

Usually to determine the health status of a technological process of obtaining sugar, of a technological operations or phase, and to highlight the effect of treatment with a bactericidal produce there can be used several methods. These procedures are as follows:

- potentiometrical method with lipoical acid, which allows the measurement of the pH value;
- the microscopic examination of the Thomas cells, which allows the identification of viable bacteria, or of the Gram bacteria;
- testing with resazurine;
- culture in Petri dishes on agar medium;
- nitrite test, based on the identification of nitrites in the diffusion water;
- measuring the content of dextro acid and lactic levo.

Fig. 3. *Laboratory for determining and measuring microbiological components*

Measuring the pH**.** Measuring the pH value of a liquid is used often to identify the presence of microbiological infections. Falling pH value is determined by the presence of a microbiological infection and it is caused by the production of organic acids by bacteria. This method of identification of microbial infections should be examined with caution, and therefore it is necessary to combine it with other methods of diagnosis. Experimentally, it was proven and is known fact that the simple change the pH level cannot be regarded as the only indication of microbial infections, because certain microbiological germs do not produce acids.

The measuring of pH value of the sugar in Romanian factories takes place at a temperature of 20° C, which is considered to be the standard temperature for expressing the value of pH.

Microscopic examination of the Thomas cell. Examinating the diffusion juice from the cells Thomas allows accurate counting of bacteria present, such as cocoide bacteria, germs, amas bacteria that are superimposed with the increase in power in a bunch of cells, diplode type bacteria and chain bacteria, with chained growth. This method allows you to quickly determine the concentration from the microbiological germs identified. Although the method is fast, does not allow, however, establishing the activity of the identified germs. Also, the method does not allow determining the presence of certain germs like *Leuconostoc*.

Thomas cells are divided into squares. You determined the number of bacteria from 9 squares of the Thomas cell and you name that number "N".

Microbiological load is expressed in germs / ml and is calculated by the expression:

$$\frac{N}{9} \times 4 \times 10^6 \qquad \text{[germs/ml]}$$

Testing with Resazurine. It is a colorimetric method, accurate, which points out the concentration of the active germs. With this method it's measured the reducing activity of thermophilic germs using an indicator, which is the resazurine. But the method requires a heating temperature of 55° C and a time of revelation or identification, which is half an hour to an hour. Testing with Resazurine is accepted by the I.C.U.M.S.A. as a standard test to determine the concentration of active germs.

Culture in Petri dishes on agar medium**.** Culture in Petri dishes on agar medium is the only method that allows the determination of bacteria load from the genus *Leuconostoc*. It is a good method of identification is

reliable, but is limited in terms of accuracy. Incubation period is high, can reach 7-9 day and even more, depending on the temperature of culture.

The nitrite test**.** Beet in healthy condition does not contain or nitrates, but only nitrates or nitrogen. As a result of microbial infections that may occur during harvesting, primarily in the diffuser, but also in the press water, nitrites can be reduced to nitrogen. The cause is due to the presence and activity of reducing microorganisms, represented by Gram-negative bacteria that do not possess oxidase or cytochrome oxidase.

All species of Gram-negative bacteria metabolize glucose in the following two directions:

- To produce only organic acids, especially lactic acid;
- By producing organic acids, primarily lactic acid and visible emission of gases, especially hydrogen (H_2).

With the help of the nitrite test it is determined the value field of the contents of nitrate in diffusion juice, or press water, expressed in ppm. Depending on the content of nitrites, it is considers the degree of microbiological infection, it establishes the need for disinfection and will be assessed according the disinfectant or biocidal necessary.

Measuring the content of lactic acid**.** Experience has shown that the content of lactic acid is a fast and highly reliable method for measuring bacteriological activity in the sugar industry.
To measure the content of lactic acid using an electronic analyzer based on the ability of an enzyme to be used as the mineral electron acceptor. The reactions that are the foundation of this process are as follows:

$$\text{L – Lactate} + 2\ Fe^{3+} \Rightarrow \text{Piruvate} + 2\ Fe^{2+}$$

and

$$2\ Fe^{2+} \Rightarrow 2\ Fe^{3+} + 3\ e$$

Electricity generated by the electrons is proportional to the concentration of lactic acid.

The main method used is measuring the content of lactic acid, but it is always complemented by other methods as for example, measuring the pH value, counting the microscopic germs and microbiological cultures in Petri dishes with agar media. The parameter ensemble or information obtained with methods listed above enables the safe and rapid determination of the state of health of a technological process, a technological operations or technological phases to obtain sugar

The preparation of the water for the extraction of sugar

Modern technology involves the use of "unique water" for the following characteristics:

- pH value measured at 20° C = 5.2 - 5.5;
- temperature at the entrance of the diffuser = about 72° C;
- CaSO4 content below the solubility;

- the origin of "unique water" = mixing the following components: entire quantity of water resulting from grain milling; condensate;

- fresh water, cold, only if necessary;

- lime;

- sulfuric acid.

The role of $CaSO_4$ in the extraction water is to enhance the texture of the noodles after extraction and to allow thus pressing them to a dry matter content of about 28%.

For the dosage and the obtaining of the unique diffusion water it is necessary an automatic installation that allows:

- adjusting temperature, namely, platinum wells type PT 100 with temperature compensator, with 3-4 wires;

- maintain high standards, setting based on a type LT 1151 regulator which works on the membrane principle, with the signal of 4 - 20 mA on the supply wire of DC 24 V;

- Adjusting the pH, pH-meter with Ingold electrode;

- Determining and adjusting the flow of components that makes up “unique water”, fresh water, condensation, lime milk, sulfuric acid;

- Adjusting the flow of "unique water".

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