UDC 664.14:628.32:547.233.4
ISSN 1330-9862

*professional paper*

# Application of Quaternary Ammonium Compounds and Formalin as Disinfectants in Sugar Production

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Received: November 3, 1995
Accepted: April 12, 1996

*Summary*

*A combined disinfection with formalin and quaternary ammonium compounds was carried out in the Sugar Factory Virovitica. During the diffusion process microorganisms cause high losses of sugar. The main types of microbial flora entering the diffusers or growing during the diffusion process are as follows: aerobic sporeforming bacteria of the species* **Bacillus subtilis** *and* **Bacillus stearothermophilus**, *clostridia as* **Clostridium thermosaccharolyticum** *and* **Clostridium thermohydrosulphuricum, Leuconostoc mesenteroides** *and yeasts, principally of the genus* **Saccharomyces**.

*The metabolic products of these microorganisms are high molecular weight polysaccharides (dextrans and levans), organic acids (acetic, lactic, etc.), alcohols, carbon dioxide and hydrogen. The growth of these microorganisms can be controlled by addition of various disinfectants. Formalin is commonly used in our factories. Before the experiment with formalin and quaternary ammonium compounds, in the diffusers an optimum disinfection scheme was determined with formalin 0.03% of the mass of the beet (300 ppm) and the best results were obtained with 50% formalin consumption and by adding about 25 ppm of quaternary ammonium compounds.*

*The efficiency of quaternary ammonium compounds against thermophylic microorganisms has been confirmed. This kind of research should be continued to further reduce formalin consumption, or to substitute formalin completely with less toxic, biodegradable biocides.*

*Keywords:* quaternary ammonium compound, formalin, sugar production

## Introduction

The most important reason why the microbial analyses are being done during sugar production are sugar losses caused by microorganisms. These microorganisms need sucrose, glucose and fructose for their metabolism *(1)*. Beet pulp and raw juice are an excellent medium for microorganisms. Temperature and pH conditions are particularly important. Regarding these losses there are different values registered in the literature i.e. from 0.03 to 0.8% of the mass of the beet *(2–5)*. The growth and proliferation of these microorganisms can be controlled by addition of various disinfectants, as formaldehyde *(6, 7)*, quaternary ammonium compounds *(1,8)*, combination of formaldehyde and quaternary ammonium compounds *(9)*, iodophores *(10)*, iodoaceton *(11)*, bisulphites *(12)*, peracetic acid *(13)* and glutaraldehyde *(14)*. Formalin as disinfectant is commonly used in our factories. Consumption of formalin varies from 0.02 to 0.04% of the mass of the beet *(4)*. If the raw juice contains $10^6$ microorganisms/mL, sugar losses are 2.2 kg per t of t beet and per hour *(15)*. Using two fast chemical methods, resazurin method *(16–19)* and method for determination formaldehyde concentration *(20)*, an optimum disinfection scheme was achieved. Formalin consumption is approximately 0.03% of the beet mass daily with insignificant sugar losses (Fig. 1).

The aim of this work was to decrease formalin consumption during sugar production. A combined way of disinfection was carried out with formalin and Chimec 7365 (quaternary ammonium salts). Because of high toxic effects of formalin *(21)*, it will be useful to substitute it completely with less toxic and biodegradable biocides.

## Material and Methods

Sugar beet and juices were used in this experiment. Beet pulp was pressed and the obtained juice was analysed. Juices were sampled from the different places of the diffusion process and placed on sterile dishes. Testings were carried out on:

- sugar beet juice,
- juice after diffusion processing, rich with sucrose,

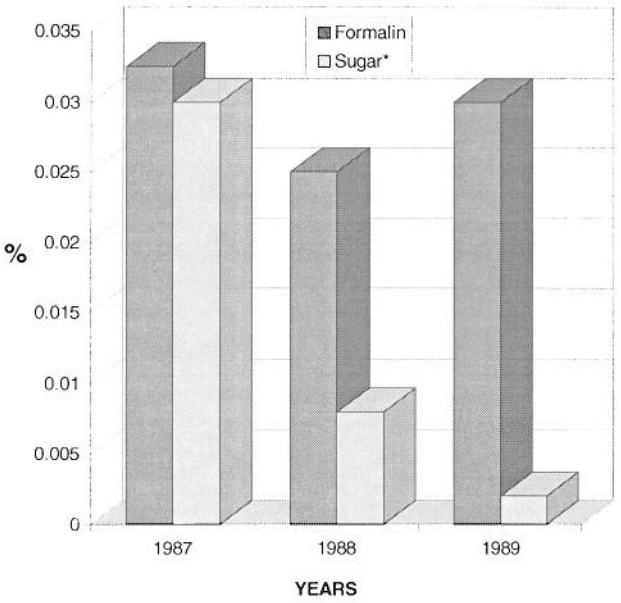


Fig. 1. Relation between sugar losses caused by microorganisms and formalin consumption in The Sugar Factory Virovitica
* Sugar = sugar losses counted as invert.

– beet pulp juice from the diffusers, contains sucrose and is returned in the diffuser, after sterilisation. It has to be sterilised because this juice shows significant bacterial activities.

. The following methods and tests were carried out on juices and beet pulp for the control of microbial activity and of the effects of disinfection:

a) Bacterial activity *(22)*,
b) Resazurin test *(16–19)*,
c) Determination of invert sugar *(23)*,
d) Formaldehyde test *(20)*,
e) Nitrite test *(24)*.

Samples were tested with the first two methods every four hours. In raw juice and beet pulp invert sugar was determined. Every two hours juices were tested on formaldehyde and nitrite content.

As disinfectants, formalin and Chimec 7365 were used. Formalin is the commercial name for about 40% water solution of formaldehyde. Chimec 7365 is a commercial name for a disinfectant based on quaternary ammonium salts, with specific antimicrobial effects for use in the sugar industry. It is efficacious in preventing and controlling the proliferation of bacterial infections in the beet. The product has a characteristic permanent action on the outer surface of the beet or slices. When utilized together with Chimec 7365, a synergism is obtained which allows formalin consumption to be reduced to a minimum. Chimec 7365 is biodegradable and thermolabile at usual processing temperatures and therefore does not cause undesired side effects. Chimec 7365 is water soluble in any proportion and can therefore be added as such or in solution form. A continuous use of the product is recommended in order to guarantee a check of bacterial growth. Typical data: amber-yellow liquid, density at 20 °C (0.98 ± 0.05) g/mL, viscosity at 20 °C (80–130) cPs, pH (1% water solution) 5.4 ± 0.5. Chimec 7365 is produced by the firm Chimec S.p.A. from Rome (Via delle Ande 19).

### *a) Bacterial activity (22)*

Samples were stored for incubation at the average temperature in the plant (55 °C) during 3 hours. pH value decreases depend on microbial activity of the samples. In each sample pH value was measured every hour. On the basis of pH decrease conclusions about intensity of bacterial activity can be drawn. If there is no bacterial activity, there is no pH decrease during the incubation time. But, if pH value decreases by 0.5 or more during the first incubation hour, that means very strong infection, and disinfecting is needed. Summarizing the pH decrease during three hours yields the points that indicate the presence or absence of microbial activity. Total decrease of pH value is in correlation with bacterial activity, as shown in Table 1.

Table 1. Correlation of bacterial activity with decreasing pH values

| pH decrease | Intensity of bacterial activity (points) |
|---|---|
| 0.2–0.4 | 1 |
| 0.5–0.7 | 2 |
| 0.8–1.1 | 3 |
| 1.2–1.5 | 4 |

### *b) Resazurin test (16–19)*

Resazurin is an indicator of microbial activity. This test helps to determine the place of infection in the plant and also provides information about the number of bacteria in the sample.

Juices and resazurin solution (50 ppm in water) are mixed together and incubated at average temperature of 55 °C. During 3 hours resazurin changes colour if there is bacterial activity in the juices. In case of infection, samples change colour from blue to violet, pink and finaly become colourless. If there is no bacteria in the sample, the colour stays blue during 3 hours. Resazurin colour changes depend on: pH values, temperature, reduced substances, number of bacteria and their enzymes. A correlation of colour change with the number of bacteria found is presented in Table 2.

Table 2. Correlation of colour change with the number of bacteria

| Colour stability/h | | | Number of bacteria per ml. |
|---|---|---|---|
| Violet | Pink | Colourless | |
| – | 1/2 | 3 | $10^8$–$10^9$ |
| 2 | 2 | 2 | $10^7$–$10^8$ |
| 1 | 3 | – | $10^5$–$10^8$ |
| 3 | – | – | $10^4$ |

### *c) Determination of invert sugar (23)*

A significant amount of sucrose is being inverted by hydrolysis to fructose and glucose. It is very important to do this testing. If the amount of invert sugar is higher in raw juice than in juice obtained from beet pulp, sugar losses are mainly caused by microorganisms.

*d) Formalin test (20)*

It has been observed that formaldehyde concentration in juices decreases steadily as a function of time. Standard curve for formaldehyde in juices was determined (see Table 3).

Table 3. Data for determination of standard curve for formaldehyde concentration in juices

| $w$ / ppm | $A$ (412 nm) | $\hat{w}$ | $w-\hat{w}$ |
|---|---|---|---|
| 2 | 0.2925 | 1.96 | 0.04 |
| 4 | 0.5675 | 4.01 | –0.01 |
| 6 | 0.8375 | 6.03 | –0.03 |
| 8 | 1.1075 | 8.05 | –0.05 |
| 10 | 1.3675 | 9.99 | 0.01 |
| 12 | 1.6275 | 11.93 | 0.07 |
| 14 | 1.9075 | 14.02 | –0.02 |

$w$ = mass fraction of formaldehyde
$A$ = absorbance

The relation of variables (absorbance and formaldehyde fraction) was assessed by the least square method, according to Doolittle *(25)* and is given by the equation $w/\text{ppm} = -0.2274 + 7.4722 \cdot A$.

*e) Nitrite test (24)*

Together with the resazurin test, the nitrite test makes microbial activity in juices clearer. Reduction of nitrates in juices proceeds in parallel with the production of lactic acid from sucrose, especially where thermophilic microorganisms are concerned. Standard curve for nitrite concentration in juices was determined (see Table 4).

Table 4. Data for determination of standard curve for nitrite concentration in juices

| $w$ / ppm | $A$ (530 nm) | $\hat{w}$ | $w-\hat{w}$ |
|---|---|---|---|
| 0.1 | 0.074 | 0.0967 | 0.0033 |
| 0.2 | 0.144 | 0.1998 | 0.0002 |
| 0.3 | 0.218 | 0.3088 | –0.0088 |
| 0.4 | 0.277 | 0.3958 | 0.0042 |
| 0.5 | 0.346 | 0.4974 | 0.0026 |
| 0.6 | 0.413 | 0.5961 | 0.0039 |
| 0.7 | 0.490 | 0.7096 | –0.0096 |
| 0.8 | 0.548 | 0.7950 | 0.0050 |
| 0.9 | 0.625 | 0.9084 | –0.0084 |
| 1.0 | 0.682 | 0.9924 | 0.0076 |

The model $w/\text{ppm} = -0.0123 + 1.4732 \cdot A$ was assessed by the least square method according to Doolittle *(25)*.

## Results

A combined disinfection was carried out with formalin and Chimec 7365. Formalin consumption was decreased significantly and sugar losses caused by microorganisms were reduced too. Before the combined disinfection, formalin consumption was 0.03% of the mass of the beet. During the experiment, the amount of formalin was decreased gradually, as shown in Table 5. Chimec 7365 was added constantly to beet slices and in the press water after sterilisation (Table 6). During the whole experiment, the absence of microbial activity was observed. There was no decrease of pH values during three hours of incubation. Only raw juice showed a decrease of pH value by 0.1 to 0.2, except when the diffusion processing was stopped for a few hours. In that case raw juice showed a decrease of pH value by 0.5. Resazurin test showed absence of microbial activity, as well. Juices were blue through the whole incubation time. It was found that without decreasing pH values during the incubation time, beet samples become coloured: blue to violet or violet. The reason is the presence of reducing sugars or other reduced compounds.

Middle of the diffuser and the press water after sterilisation maintained blue colour, which indicated the absence of microbial activity.

The results of formaldehyde and nitrite tests for the raw juice are shown in Fig. 2.

Table 5. Formalin adding*

| Days | Formalin consumption L | »Shock« doses L | Formalin / beet mass ratio ppm |
|---|---|---|---|
| 1 | 1200 | 3 × 400 | 300 |
| 2, 3 | 900 | 3 × 300 | 225 |
| 4, 5 | 810 | 3 × 270 | 202.5 |
| 6, 7 | 720 | 3 × 240 | 180 |
| 8, 9, 10 | 600 | 3 × 200 | 150 |

* Middle of the diffuser is the only place for adding formalin. Traditionally, the diffusers in sugar factories are disinfected by »shock« doses of formalin. For example: 1200 L are divided into three smaller amounts and added three times a day.

Table 6. Addition of Chimec 7365*

| Days | Chimec 7365/beet mass ratio ppm | |
|---|---|---|
| | Press water | Beet slices |
| 1 | – | – |
| 2–7 | 15 | 10 |
| 8–10 | 17 | 15 |

* On the first day the daily consumption of formalin was added. Chimec 7365 was added as 5–10% water solution constantly on beet slices.

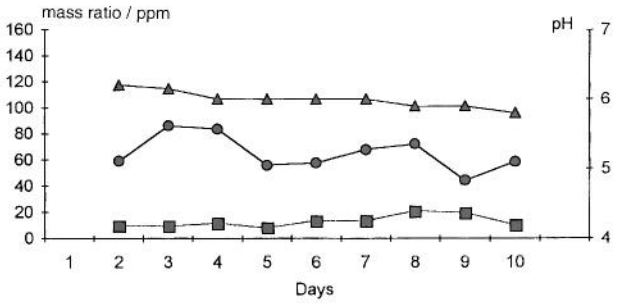


Fig. 2. Changes of nitrites, formalin and pH values in the raw juice (decreased formalin consumption by 50% with addition of ≈ 25 ppm Chimec 7365);(●) formalin, (■) nitrites, (▲)pH

As shown in Table 7, pH value of the raw juice was about 6. In comparison with beet, there was no increase of invert sugars and nitrites were kept at a low level.

Table 7. Average values of parameters in the raw juices (decreased formalin consumption 50% with addition of ≈ 25 ppm Chimec 7365)

| Parameter | Average value |
|---|---|
| pH | 6.000 |
| Invert sugar (% on 100 °Bx)–beet | 1.016 |
| Invert sugar (% on 100 °Bx)–raw juice | 0.900 |
| Mass ratio (nitrites)/ppm | 13.160 |
| Mass ratio (formalin)/ppm | 63.800 |

During the diffusion process, temperature and pH conditions, beet pulp and raw juice rich with sucrose are an excellent medium for growth and proliferation particularly of thermophilic bacteria. Quaternary ammonium compounds showed high effectiveness in the inhibition of thermophilic bacteria (Table 8).

Table 8. Efficiency of quaternary ammonium compounds in the inhbition of thermophilic microorganisms*

| | Number of bacteria per mL | | |
|---|---|---|---|
| | Aerobic bacteria | Thermophilic bacteria | Leuconostoc genus |
| Beet | $130 \times 10^6$ | 7 | $15 \times 10^3$ |
| Middle of the diffuser | $100 \times 10^3$ | 0 | $40 \times 10^1$ |
| Tail of the diffuser | $50 \times 10^2$ | 0 | $70 \times 10^2$ |
| Raw juice | $120 \times 10^4$ | 0 | $15 \times 10^2$ |
| Press water after sterilisation | 160 | 8 | 20 |
| Plasmolysator | $120 \times 10^3$ | 0 | $18 \times 10^1$ |

* Sulphitoreducing clostridia were not found

By further reducing formalin consumption with the same amounts of Chimec 7365 better results were not achieved. The second part of the experiment was an attempt to get better results concerning formalin consumption. But with reducing formalin under 150 ppm daily and with the same concentration of Chimec 7365, increase of invert sugars occurred. Because invert sugars in the raw juice got higher, formalin consumption was kept at 50% or 150 ppm.

## Discussion

Efficiency of quaternary ammonium compounds has been confirmed by many authors *(1, 26)*. Their effectiveness is higher than that of formalin *(26)*. The experiment was divided into two periods. During the first few days formalin consumption was reduced gradually by 50%, and later on under 50% of the daily consumption. In the Sugar Factory Virovitica, daily formalin consumption was about 300 ppm with insignificant sugar losses as shown in Fig. 1. Sugar losses vary depending on the quality of sugar beet and on conditions in the plant.

The method for estimating nitrites shows a correlation between nitrites concentration and bacterial activity. Formaldehyde reduces bacterial activity and also decreases the nitrite level *(24)*. Presence of nitrites during diffusion process confirms microbial activity, particularly of thermophilic microorganisms. The increase of nitrites usually occurs accompanied by lactic acid increase and they are both related to sugar losses *(24)*. Microorganisms which produce lactic acid can also reduce nitrates to nitrites.

## Conclusions

The results described in this paper about a combined dissinfection with formalin and quaternary ammonium compounds allow the following conclusions:

- the optimum disinfection scheme was achieved during the diffusion process;
- the addition of quaternary ammonium compounds resulted in the reduction of formalin consumption. With the addition of Chimec 7365 (about 25 ppm), formalin consumption was reduced from 300 to 150 ppm;
- the efficiency of quaternary ammonium compounds against thermophilic microorganisms was confirmed.

Finally, this kind of research should be continued to further reduce formalin consumption or to substitute formalin completely with less toxic, biodegradable biocides.

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# Primjena kvaternih amonijevih spojeva i formalina kao dezinfekcijskih sredstava u proizvodnji šećera

*Sažetak*

*U tvornici šećera Virovitica proveden je kombinirani način dezinfekcije s formalinom i kvaternim amonijevim spojevima.*

*Mikroorganizmi uzrokuju velike gubitke šećera tijekom procesa difuzije. Najvažniji predstavnici mikrobiološke flore koji s repom, zemljom i vodom dospijevaju u difuzere i razmnožavaju se za vrijeme difuzije jesu: aerobne sporotvorne bakterije vrste* ***Bacillus subtilis*** *i* ***Bacillus stearothermophilus****, klostridija kao* ***Clostridia thermosaccharolyticum*** *i* ***Clostridium thermohydrosulphuricum, Leuconostoc mesenteroides*** *i kvasci, osobito iz roda* ***Saccharomyces****.*

*Krajnji produkti metabolizma tih mikroorganizama su visokomolekularni polisaharidi (dekstrani i levani), organske kiseline (octena, mliječna itd.), alkoholi, ugljik(IV)-oksid i vodik. Razmnožavanje tih mikroorganizama može biti inhibirano doziranjem različitih dezinfekcijskih sredstava. U našim tvornicama najčešće se primjenjuje formalin. Prije postavljanja pokusa s formalinom i kvaternim amonijevim spojevima, postignuta je optimalna dezinfekcijska shema s formalinom (0,03% mase repe, tj. 300 ppm), a gubici šećera smanjeni su nekoliko puta. Najbolji rezultati postignuti su s 50% dnevne potrošnje formalina (150 ppm) i oko 25 ppm kvaternih amonijevih spojeva. Također je potvrđena učinkovitost kvaternih amonijevih spojeva prema termofilnim mikroorganizmima. Takva bi istraživanja trebalo nastaviti kako bi se još više smanjila količina formalina ili formalin potpuno zamijenio manje toksičnim, biorazgradljivim biocidima.*