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(54) Method for controlling bacterial infection in the sugar production porcess

(57) The invention concerns a method for controlling bacterial infection of raw sugar solutions obtained from sacchariferous material of vegetable origin, and in particular from sacchariferous plants such as sugar beet or sugar cane, based on the combined use of two different types of agents having antimicrobial and/or bacteriostatic activity, both suitable for use in the processing of products destined for human or animal consumption. The first of the two agents consists of one or more solutions based

on peracetic acid and hydrogen peroxide, at least one of which is continuously fed into the process, while the second agent, used as a secondary disinfectant fed into the process in a non-continuative manner, consists of a product based on natural vegetable extracts compatible with foodstuffs and having antibacterial activity, such as hop extract, vegetable resin extracts compatible with foodstuffs or palm kernel extracts.

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Description

[0001] The present invention concerns a method for controlling bacterial infection in the sugar production process. More specifically, the invention concerns a method for controlling bacterial infection of raw sugar solutions obtained from

- ⁵ sacchariferous material of vegetable origin, and particularly from sacchariferous plants such as sugar beet or sugar cane, based on the combined use - according to specific procedures - of two different kinds of agents having antimicrobial and/or bacteriostatic activity, both suitable for being employed in the processing of food products destined for human or animal consumption.
- [0002] As is known, in the production of sucrose or other sugar products based on raw vegetable material, primarily sugar beet and sugar cane, the vegetable material is first disintegrated mechanically and then undergoes extraction, or pressing, in order to obtain the raw sugar solutions from which, via subsequent operations, purified sugar in a solid state is obtained. Since they are agricultural raw materials, it is inevitable for the raw vegetable products to contain micro-organisms such as bacteria, yeasts or moulds, most of which are eliminated via initial washing. Those that remain, attached to soil particles which are not removed through washing, can easily develop within the processing system since the treated raw sugar solutions can act as putrients for these organisms.
- ¹⁵ the treated raw sugar solutions can act as nutrients for these organisms.
 [0003] Considering, for instance, the sugar beet production process (from Beta vulgaris), after the beet with leaves removed is washed and sliced, it is scalded in order to break down by denaturation the cells containing the dissolved sugar. In fact, slicing the beet only partly leads to breaking down the cell membranes, while the remaining cells remain intact. In order to obtain the sugary juice by extraction, the slices (cossettes) must undergo heat treatment in order to
- 20 denaturate the cell membranes. This treatment should take place at the highest temperature possible (such as 80°C) for a few minutes. Then, the cossettes undergo an extraction process by diffusion, normally carried out in counterflow continuous cycle devices (diffusers), in which the sucrose is transferred into an aqueous phase called raw juice or diffusion juice. The residual solid phase of this operation is composed of the so-called spent pulps or spent cossettes. The extraction by diffusion section is fed, in counterflow, by an aqueous extraction medium partly consisting of fresh
- ²⁵ water and partly recycled water coming from the pressing of the spent pulps. Further micro-organisms can thus be introduced into the system through the extraction water.

[0004] In view of the above, it is evident that the diffusers of a sugar beet extraction system are excellent incubators that can multiply the bacterial population, and that the contact time inside them influences bacterial growth, while the working temperature exerts a selective action on the species that prosper. In fact, the temperature inside the extraction

- 30 system influences the vital functions of the micro-organisms and, while low temperatures (< 40°C) tend to facilitate the growth of mesophilic bacteria, and particularly lactobacilli, high temperatures tend to promote thermophilic bacteria, such as *Bacillus stearotermophilus* and *Clostridium thermosaccharolacticum*, in particular. Both kinds of bacteria degrade the sugars contained in the treated raw sugar material, mainly giving rise to the forming of lactic acid and other products of their metabolism, such as lower fatty acids (acetic, propionic and butyric acids), ethanol, acetone and gaseous
- ³⁵ products such as NO₂. This obviously leads to sugar loss in the finished product and, in serious cases of infection can have economically serious consequences.
 [0005] Moreover, the micro-organisms that are present cause an enzymatic splitting of the sucrose molecule, with the production of the two constituent monosaccharides glucose and fructose (D(+)-glucose or dextrose, and D(-)-fructose or levulose). The hydrolised product thus obtained, which is made up of two reducing sugars whose aqueous solutions
- ⁴⁰ have an overall optic power opposite to that of the initial sucrose solutions (dextro-rotatory), is called "inverted sugar". The presence of inverted sugars in the diffusion juice, besides accompanying a loss in sugar, also gives the juice a darker colour and thus needs greater processing and further sugar losses for the final washing. [0006] The intensity of bacterial activity varies during the sugar production campaign, which is subject to considerable

(1000) The intensity of bacterial activity values during the sugar production campaigh, which is subject to considerable climatic changes that produce effects on the quality and on the state of cleanliness of the beet delivered to factories. The more deteriorated and dirty the beet, the greater the virulence of microbiological activity within the extraction systems. Slowdowns in production, due to a lack of beet supply or mechanical breakdowns, can have the same effect since they increase residence times and lower the temperature. Moreover, as noted, the direct economic damage caused by sucrose

destruction is accompanied by the indirect damage caused by the effects that the products of microbiological activity have on the process. The lactic acid destroys the natural alkalinity of the juices, while the reducing sugars increase their colouring.

[0007] The proliferation of mesophilic bacteria can be reduced by avoiding, as much as possible, long stays, temperatures lower that 40-50°C and production system stoppages, and is in any case avoided with the exposure to high temperatures. However, for the deactivaton of thermophilic species and for controlling infections due to these microorganisms is necessary to have regular treatment with suitable disinfectants. The sugar industry is aware of the

⁵⁵ potentiality of problems linked to bacterial proliferation and has long been using various bactericides. The most widely used of these is formaldehyde, followed by dithiocarbamate, glutaric aldehyde and quaternary ammonium salts.
 [0008] In particular, formaldehyde or formalin is used in 30-40% solutions, such as by adding it in considerable quantities for a very short time (an intermittent, shock, dosage) in one or more stages of the system, such as in quantities of

0.5-1.0% of the hourly flow rate of the raw juice (that is, 50-100 kg of formaldehyde per 100 m³ of treated raw juice per hour). The intervals between one shock dose and another can vary depending on the intensity of the infection, but on average there is one treatment every 8-24 hours (1-3 shocks per day). The effects of antibacterial treatment are normally monitored, by determining the lactic acid concentrations in the extraction system.

⁵ **[0009]** Although its antimicrobial effectiveness is well-known, the addition of formaldehyde to the raw sugar juices has the disadvantage of worsening the colouring of the juices, thereby increasing the sugar washing cycle - and thus the process time as a whole - as well as the sugar loss.

[0010] Moreover, and more critically, for formaldehyde there are normative restrictions for environmental reasons, given its evident carcinogenicity. Also the other aforesaid biocides are not devoid of possible negative effects when

- destined for uses linked to foodstuffs. It must be noted, in particular, that the spent beet pulps, which are traditionally destined for animal feeds, have recently been included, by the effects of legislation, in the category of products for human consumption. The greater attention that government and health organisations at European and world level are devoting to food safety places a serious possibility on the use of these biocides and justifies the current need for new systems for controlling bacterial infections that take these limitations into account.
- ¹⁵ [0011] One class of disinfectant compounds that were proposed and have been used for some time for controlling bacterial infections in the food industry and which do not involve the aforesaid safety risks is composed of peroxycarboxyl acids, and specifically of peracetic acid or peroxyacetic acid (PAA, with the formula CH₃-CO-OOH). Mixtures of peracetic acid and hydrogen peroxide (or oxygenated water, H₂O₂) in various molar ratios have been used as disinfectants in the sugar industry, in the implementation of methods involving additions of product at various dosages and different H₂O₂/PAA ratios, at different stages of the production process.
- **[0012]** Specifically, European patent EP 0678123 (Solvay Interox) describes a process for the disinfection of raw sugar juices in which a solution of peracetic acid with a H_2O_2/PAA molar ratio of at least 12:1 is fed into the pulp pressing water system and a solution of peracetic with a H_2O_2/PAA molar ratio lower than 10:1 is fed into an intermediate point of the diffusers. The bactericide solutions, which are marketed under the Proxitane[®] trademark, are preferably dosed: one in
- ²⁵ a continuous manner (such as at a concentration between 2 and 3% weight of PAA and 20-30% weight of hydrogen peroxide) in the press water system (in particular, so as to obtain PAA concentrations in the treated waters in the region of 5-50 ppm), and the other in the form of shocks in the diffusers (preferably in concentrations of 25-350 ppm of PAA, for example, with a shock concentration of 210 ppm, subsequently maintained at an average level of 130 ppm by a 10-minute dose every three hours).
- 30 [0013] Other technological proposals, still based on the use of disinfectant solutions based on peracetic acid and hydrogen peroxide, are described, in particular, in European patent EP 0943692 (S.E.P.P.I.C.) and in European patent EP 1138787 (S.E.P.P.I.C. and Bioxal). The former essentially proposes using a single formulation of peracetic acid solution with a H₂O₂/PAA molar ratio not above 4, introducing the solution in at least one stage of the system upstream of the diffusers and in at least one stage of the pulp press water system. The second document instead proposes a more
- 35 complex scheme, which involves dosing the peracetic acid/hydrogen peroxide solution, in various concentrations but still with the same ratio between the two components, onto the beets before slicing them, onto the already cut cossettes, in at least one stage of the system upstream of the diffusers and in at least one stage of the pulp press water system, as well as continuously in the recycled diffusion water system.
- [0014] It must be noted that peracetic acid, besides having a disinfectant action, presents some positive side effects on the sugar production process that no other type of bactericide can produce. Among these it is possible to cite the following.

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a) A positive effect on the pressability of the spent pulps. With equal diffusion operating conditions and equal doses of press coadjuvants (typically calcium sulphate and aluminium sulphate), the presses produce pressed pulps with a greater content of dry matter and less water content. This benefit can be exploited in order to reduce press coadjuvant consumption, increase the diffusion temperature or reduce fuel consumption in pulp drying.

b) An effect on the colour of the depurated juices: treating the diffusion juices with PAA leads to a reduction of the colour of the depurated juices, due both to direct action on chromophor groups, and indirectly because formalin use is avoided (formalin, as is known, has a colouring effect on the juices). Reducing juice colouring means greater value of the sugar produced or reducing the costs of refining white sugar.

- c) An effect, found during the studies carried out within the present invention, of reducing inverted sugar content. It was found that using PAA produces a drastic abatement of glucose and fructose in the raw diffusion juice, presumably because of inhibition of the enzymes dealing with converting the sucrose into the two simple sugars. This benefit, besides involving a reduction in sucrose loss, also reduces the negative effects of inverted sugars on juice colouring and on the content of the calcium salts soluble in the depurated juice.
 - d) A positive effect, as has been found within the studies connected to the present invention, on the content of calcium salts in the purified salts. The observed effect may be attributed both to the reduction in the sulphates introduced as press coadjuvants and to the reduced contribution to the forming of organic acids, in the depuration

phase, due to the presence of glucose and fructose.

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[0015] Despite the advantageous effects found in using peracetic acid as a disinfectant for the raw sugar juices produced in industrial sugar production (as is also demonstrated by the fact that different alternative methods of treatment have been proposed over the years in the sector, all consisting of application variations making use of the same biocide agent), any disinfectant method using only peracetic acid, however dosed and distributed inside the system, cannot guarantee total protection from bacterial infections that can arise during the sugar production process. The PAA and hydrogen peroxide mixtures, in fact, are in any case subject to gradual loss of efficacy due to the onset of resistance

phenomena. It is known that all micro-organisms, when subjected to repeated applications of the same antibacterial
 agent, can - in time - develop insensitivity to the specific agent, owing to the natural selection of strains resistant to the agent concerned.

[0016] Moreover, it has been found that PAA use poses further limitations when, in order to contrast bacterial resistance phenomena, it is decided to use higher doses of the same agent, or to suspend it in order to use another chemically different bacterial agent. In fact, the increase in the presses efficiency in terms of dry matter content, and thus of energy

- ¹⁵ consumption for the subsequent drying stage (which is one of the effects of using peracetic acid as a disinfectant for raw sugar juices), subjects the presses to greater torsional stress which, if not controlled, may seriously damage them. [0017] Both lactic acid, produced by bacterial activity, and PAA act as pressing coadjuvants. In a system under control, the combined effect of lactic acid and PAA used as a moderator of bacterial activity, and thus of the lactic acid level, enables controlling the presses stress simply by reducing the use of calcium sulphate and aluminium sulphate (which,
- as is known, are commonly used as coadjuvants to increase press yields). When infections go out of control and the lactic acid concentration sharply rises, causing an increase in the presses stress, it may become impossible to use PAA as an antibacterial agent so as not to accentuate the risk of damaging the presses themselves. In this stalemate situation, it is necessary to suspend the PAA treatment and use an alternative antibacterial agent. However, suspending PAA and using an alternative method causes a decompensation in carrying out the process, due to the fluctuation of those
- 25 parameters influenced by PAA (pressability, colour, glucose and calcium salts in the juices). [0018] In view of the above, the present invention thus aims at providing a method for controlling bacterial infection in processes for the production of sugar from vegetable materials, and particularly sugar beet and sugar cane - the method making use of disinfectant products compatible with the food industry and devoid of any negative effects for their use in both human and animal foodstuffs, but at the same time able to effectively combat the bacterial infections
- that can arise during the sugar production process, reducing as much as possible the loss of product and all the other inconveniences that result from the said bacterial infections. The process studied according to the present invention is based on improving the performance of the peracetic acid/hydrogen peroxide system and overcoming its limitations of use, in order to exploit, without any fluctuations disturbing the conductance of the extraction process, the technological benefits brought by this system and avoiding the onset of critical conditions to the presses, due to the loss of control over lactic acid production.

[0019] To this end, the present invention proposes the joint, but not alternative, use of a second type of bactericide - also having compatible characteristics for use in foodstuffs - consisting of natural plant extracts such as those obtained from hops, wood and the exudates of conifers, or from palm kernels.

- [0020] The antimicrobial properties, or in any case bacteriostatic ones, of certain natural plant extracts such as hop extract or resin extracts like rosin or other natural resins, have been known since ancient times, but only recently have these products been proposed as antimicrobial agents in the sugar industry. Specifically, European patent EP 0681029 (Zuckerforschung Tulln) describes the use of hop extracts as inhibitors of bacteria proliferation (thermophilus bacteria) in sugar extraction processes using an aqueous medium, wherein these extracts, or their components, are dosed - either continuously or as shocks - in raw sugar juices, to work at temperatures ranging between 50°C and 80°C. More specifically,
- 45 the description illustrates the use of hop extracts as they are (whose bacteriostatic properties are already well known and used in beer production) or their active constituents, the so-called beta-acids or "lupulons" and the so-called alpha-acids or "humulons", contained in hops, either in a dissolved or emulsion form in the aqueous medium. These agents, of which a commercial version is available under the BetaStab[®] (BetaTec) trademark, are considered very active against the types of bacteria found in raw sugar juices during sugar production processes. However, also in this case their sometime the second to the production processes.
- 50 continual use for a certain period of time may lead to the onset of resistant bacteria strains. [0021] Similarly, and in order to broaden the range of natural products that can be used as antimicrobials in the sugar industry, US patent no. 6770147 (Zuckerforschung Tulln) proposes formulations based on natural resins compatible with foodstuffs, and preferably resin acid extracts obtained from conifers, either substituting or in addition to hop extracts, to be used when treatment with the former gives rise to resistance. As is known, conifer resin extracts (rosin or colophony)
- ⁵⁵ are composed of various diterpenes, amongst which the most important are abietic acid, neoabietic acid, palustric acid and levopimaric acid, and they have a natural antibacterial activity.

[0022] Also within the same range of natural antibacterial products of vegetable origin, the extracts of the palm kernel (or palm seed oil) of an African palm from which palm oil is traditionally obtained have recently been proposed. The fatty

acids of palm kernel, amongst which there is firstly myristic acid (linear aliphatic carboxy acid of 14 carbon atoms), but also, for example, lauric acid (dodecanoic) and palmitic acid (hexadecanoic), also have a marked antibacterial activity and can be used as natural agents for controlling bacterial infections in raw sugar juices of the sugar industry.

- [0023] According to the present invention, it has been found that the continuous application regimen of the PAA-based disinfectant system may be advantageously integrated, in order to eliminate episodes of resistance to this antibacterial treatment, with a simultaneous treatment for short periods with an antibacterial agent made up of natural plant extracts of the kind just mentioned, which will be cumulatively indicated as NVE (natural vegetable extract), regardless of the specific chemical constitution.
- [0024] The efficacy of the coordinated use of NVE was experimented, according to the present invention, along with the constant use of a powerful oxidant as peracetic acid/hydrogen peroxide, from which there was expected a marked inhibiting action on organic matrix agents (as is, indeed, a natural vegetable extract). On the contrary, according to the present invention, it was found that the second agent, operating in an environment made absolutely devoid of species resistant to this second agent - thanks to the continuative use of PAA - has a considerable effect able to rapidly eliminate PAA-resistant species. In practice, the combined use of the two agents, instead of giving rise to interferences, leads to a synergic effect, enhancing the efficacy of both agents concerned.
- a synergic effect, enhancing the efficacy of both agents concerned.
 [0025] Thus, the present invention specifically provides a method for controlling bacterial infection in the sugar production process starting from sacchariferous vegetable materials, wherein to sugary material to be treated there is added one or more solutions based on peracetic acid and hydrogen peroxide, as a primary disinfectant, at least one of which is fed to the process in a continuative way, characterised by the fact that a further product is also added as a secondary
- 20 disinfectant, and namely a product based on natural vegetable extracts compatible with foodstuffs and having antibacterial activity.

[0026] For the purposes of the present description, the term "continuative" is generally taken to mean the use of a disinfectant agent that may consist of the continuous feeding of the said agent, without interruptions, into one or more stages of the sugar production process, or of discontinuous feeding, at intervals, but which is nonetheless on a regular

- ²⁵ basis over time and with no interruptions. The "continuative" use of the PAA/H₂O₂-based disinfectant may advantageously consist of, for example, a continuous feed in one stage of the extraction system, accompanied by "shock"-based feeding at regular intervals in another stage of the system, on condition that at least one of these two feeds does not incur interruptions for the entire duration of the treatment session considered.
- [0027] Still for the purposes of the present description, the term "non-continuative" is taken to mean the use of the disinfectant agent in a sporadic or episodic manner for a limited and circumscribed period of time with respect to the overall duration of the treatment session considered (and specifically, a sugar production campaign). Within the periods of use, the disinfectant used in a "non-continuative" manner may be fed into the process both with a continuous flow or with shocks at regular intervals.

[0028] Furthermore, for the purposes of the present description, by "antibacterial activity" is meant to be an actual bactericide activity or also a merely bacteriostatic activity, that is, such to inhibit the growth and proliferation of bacterial micro-organisms without necessarily causing their death.

[0029] Preferably, according to the present invention, the NVE-based product is selected from the group consisting of: hop extract or one or more of its constituents having antibacterial activity; vegetable resins compatible with food consumption or one or more of their constituents having antibacterial activity; palm kernel extracts or one or more of

- 40 their constituents having antibacterial activity; or a mixture of two or more of the said products. In particular, the NVEbased product is preferably composed of hop extract, or of a mixture of hop beta-acids, or of a mixture of hop alpha-acids. [0030] According to some embodiments of the proposed procedure, the said NVE-based product is a moderately alkaline aqueous emulsion containing 5-20% by weight of acids or other natural compounds having antibacterial activity. In the case that the NVE-based product is based on hop extract, it will contain, preferably, 80-90% by weight of water,
- ⁴⁵ 1-3% by weight of potassium hydroxide, 8-12% by weight of beta-acid, and 1-5% by weight of other hop compounds. [0031] The method for controlling bacterial infection proposed according to the present invention, when applied to sugar beet production processes, preferably involves an application scheme in which, of the said one or more PAA-based solutions, a first solution is continuously added in the press water system (that is, in the juice obtained from pressing the pulp and which is wholly recycled in the diffusers) and a second solution is added in the form of shocks, in
- ⁵⁰ one or more of the following stages of the production process: inside the diffusers, in the extraction juice system, in the juice-cossette mixer, in the juice-cossette line, according to where infection areas are located, monitored by analysing parameters indicating the intensity of microbiological activity.

[0032] These solutions based on peracetic acid and hydrogen peroxide preferably contain - similarly to the commercially available solutions of the prior art - 2-15% by weight of peroxyacetic acid and 15-50% by weight of hydrogen peroxide, and may also contain 5-25% by weight of residual non-reacted acetic acid as well as 0.5-1.5% by weight of other acids used as stabilisers in the peracetic acid manufacturing process.

[0033] The first peracetic acid-based solution continuously added in the press water system is preferably fed at a dosage ranging between 0.1 and 30 ppm of peracetic acid, preferably in the dosage range of 0.3-10 ppm of peracetic

acid, while the second peracetic acid-based solution is preferably added in the form of shocks with an average dosage ranging between 3 and 120 ppm of peracetic acid, preferably between 3 and 60 ppm and with a frequency ranging between 3 and 12 times a day.

[0034] During continuative use of PAA, the disinfectant process provided by the present invention envisages the timely and/or preventive check for the onset of PAA-resistant species. At the same time, to avoid the secondary bactericide

5 agent - and namely NVE - from causing the development of resistant species, this must be used for short periods and at intervals not too close to one another.

[0035] According to certain specific embodiments of the proposed method, the NVE-based product compatible with foodstuffs and having antibacterial activity used in a non-continuative manner is fed into the process on the onset of

- 10 species resistant to the peracetic acid-based disinfectant. In this case the NVE-based product is preferably fed in the form of shocks with a frequency ranging between 1 and 6 times a day and in dosages between 5 and 50 kg of product per shock, until such time as a pre-established level of reduction in bacterial activity is ascertained. In these conditions, which correspond to the most critical situations, it is worth having recourse to a timely check, that is calibrated in dosage and frequency of the shocks, according to the bacterial activity ascertained.
- 15 [0036] According to other specific embodiments of the proposed method, which are generally applicable to a fully operational production plant and with beet of good quality, the NVE-based product compatible with foodstuffs and having an antibacterial activity is fed into the process - for preventive purposes - at pre-established time intervals. In this case the NVE-based product is preferably fed in as shocks with a frequency of between 1 and 3 times a day and in doses ranging between 5 and 20 kg of product per shock, one day a week.
- 20 [0037] In each of the two cases mentioned, however, the frequency of the shocks and the dosages depend on the operating conditions and on the state of the beet concerned.

[0038] In intermediate conditions in terms of type of control, the dosage and frequency of shocks must be determined according to the parameters indicating the intensity of the microbiological activity.

[0039] Also the secondary disinfectant based on NVE can be fed into the process at one or more (such as by rotation) 25 of the following stages of the production process: inside the diffusers, in the extraction juice system, in the juice-cossette mixer, and in the juice-cossette line.

[0040] The specific characteristics of the present invention, as well as its advantages and relative operative procedures, will be more evident with reference to the detailed description presented merely for exemplificative purposes below, along with the results of the experimentation carried out on it and the comparative data with respect to the prior art. The

30 present invention is also illustrated in the attached drawings, wherein:

> Figure 1 shows a table graphically summarising the field trials carried out in order to assess the method according to the present invention during a sugar production campaign, with the indication of the various agents used and the treatment duration.

35 Figure 2 shows the trend of the L-lactate values, in g/l, assessed in the beet cossettes fed to the process and in the average raw juice, determined during the field trials summarised in Figure 1.

Figure 3 shows the trend of the glucose values, in g/l, assessed in the beet cossettes fed to the process and in the average raw juice, determined during the field trials summarised in Figure 1.

Figure 4 shows the trend of the concentration of calcium salts, expressed as hardness in French degrees, in the 40 clear liquid of 2nd saturation and in the thin juice, determined during the field trials summarised in Figure 1.

EXAMPLE 1

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[0041] The experimentation had an overall duration of about 51 days and took place in three separate periods preceded 45 by a conventional formalin treatment and alternating with a trial with vegetable extracts having disinfectant activity (NVE - hops). The diagram shown in Figure 1, herewith attached, graphically summarises the periods concerned and the operation trend.

[0042] Period using formalin: from the beginning of the campaign until 25 August, carried out at an average dosage of 390 ppm. Moreover, about 75 t of aluminium sulphate and a little under 276 t of CaSO₄ was also used. The lactic acid stayed at an average level of 270 ppm.

[0043] Period using PAA no. 1: this started on 26 August and lasted 17 days. Not having yet acquired the handling competence necessary to manage the lactic acid levels, on the one hand, and the absorptions of the presses, on the other, it became necessary to use formalin often at higher doses than necessary in order to maintain control of the press absorptions. That is why there was an accentuated variability in the lactic acid values, with repercussions on the colour and calcium salt values.

[0044] The use of aluminium sulphate was completely suspended throughout the period and the use of chalk was reduced.

[0045] Period using NVE-hops: this started on 12 September and lasted about 10 days. In the first part, thanks to

the quick pace and absence of resistant species, the efficacy appeared very satisfactory. In the second part, instead, also following a slowing down in pace, it became necessary to use formalin.

[0046] In this period, the disappearance of the technological benefits linked to colour, to glucose in the juices and to the pulp pressability was observed, so much so that it became necessary to resume use of aluminium sulphate and to go back to the habitual dosages of calcium sulphate.

[0047] Period using PAA no. 2: this started on 22 September and lasted for 17 days until there was a halt on 8 October for lack of beet supply. The experience gained, during most of the period in which the operating pace was characterised by a constant quick pace, enabled maintaining both the control of the lactic acid levels and the press absorptions, without the need to resort to the second biocide.

10 [0048] The second half of the period saw the employment, by implementing the procedure according to the present invention, of targeted shocks of the second bactericide, and namely consisting of NVE-hops (natural vegetable extracts from hops).

[0049] In none of the occasions above was use made of aluminium sulphate, while calcium sulphate was reduced to about 1/3.

15 [0050] This period was selected as significant for comparison with the formalin period, thanks to the acquired management regularity.

[0051] Period using PAA no. 3: this started on 13 October, straight after the pause for lack of beet, and ended on 24 October in order to run down formalin stocks.

[0052] The period was characterised by very low and inconsistent operating paces which made it necessary, from time to time, to make use of a second biocide, and namely formalin (as noted, in order to run down stocks)

20 [0053] Table 1 below reports the dosages of the disinfectants and press coadjuvants used in the various periods.

| 25 | | Sugar production campaign - Periods | | | | | | | | | | |
|----|-----------------------------------|-------------------------------------|--------------|---------|--------|---------|--|--------|--|--|--|--|
| 25 | | | Formaldehyde | PAA 1 | Hops | PPA 2 | | PAA 3 | | | | |
| | | | | | | | | | | | | |
| | Cut beet | t | 264,014 | 184,340 | 95,119 | 156,518 | | 89,341 | | | | |
| 30 | Formaldehyde | g/t | 348 | 74 | 73 | 4 | | 34 | | | | |
| | Hops | g/t | - | - | 21 | 6 | | 0 | | | | |
| | PAA 3% | g/t | - | 39 | - | 56 | | 50 | | | | |
| 35 | PAA 12% | g/t | - | 76 | - | 63 | | 63 | | | | |
| 00 | AI(SO ₄) ₂ | g/t | 457 | - | - | - | | - | | | | |
| | Ca(SO ₄) ₂ | g/t | 1,292 | 413 | - | - | | - | | | | |

TABLE 1

Operating conditions 40

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[0054] The PAA/H₂O₂ application model was adopted, which refers to patent EP 0943692, based on the following operating procedures:

■ PAA in continuous dosage on the press water; namely, a 3% solution was used. 45

■ PAA dosed in shocks inside the diffusers and in the mixers/scalders; namely, a 12% solution was used.

[0055] In sum, an average dosage of about 50 ppm of 3% solution on the press water and of about 70 ppm, on average, of 12% solution in the diffusers was recorded.

[0056] The handling, stockage and distribution of solutions containing PAA is a critical factor. That is why particular 50 care must be taken in adopting measures and provisions geared to assuring the maximum safety standard possible. [0057] As regards the 3% solution, the dosage was carried out in continuous mode by means of a dosage pump, directly on the press water tank upstream of the depulping filters.

[0058] The 12% solution was dosed by means of electrovalves activated from the control room, mainly at 1/3 of the

Conclusions

[0059] It was possible to see that the second bactericide, applied as a support to PAA with primary bactericide functions, had a greater than expected efficacy, finding itself operating in an environment devoid of resistant species. This behaviour

turned out to be extremely useful for controlling the critical situations that arise, for example, during operation at a slower pace.

[0060] For its part, the PAA applied regularly as a primary bactericide confirmed the expected technological benefits relative to:

- 10 Pulp pressability
 - Juice colour
 - but also had a significant impact on the following parameters:
 - Reduction of the inverted sugars
 - Reduction of calcium salts
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[0061] It may thus be concluded that the continuative use of PAA, besides having the known disinfectant action, produces a series of technological benefits of high economic return, while the use of a second bactericide, applied in an environment characterised by the development of PAA-resistant species, but extremely sensitive to different molecules, appears extremely effective in supporting the basic control function entrusted to PAA.

20 **[0062]** The combination of PAA with NVE, and namely hop extracts, but also resin extracts of conifers or palm kernel extracts, or one or more of the respective constituents having antibacterial activity, meet the food safety standards required for all those products to be applied in direct contact with human foodstuffs or assimilated as such (spent pulps).

ANALYSIS OF THE RESULTS

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Control of infections

[0063] The control of the infections was conditioned by the pace of the diffusions and by the press absorptions.

[0064] The low temperature conditions and high retention times that come about during reduced operating paces make it difficult to control the infections. Moreover, during the experimentation with PAA, it became necessary to face an additional critical factor: press absorptions.

[0065] As is known, the PAA used in order to control lactic acid levels and the pH of the juices also exerts a strong influence on the solid matter of the pulps and on the absorption of the presses: this condition, especially in the first trial period, led to using occasional formalin shock treatments for fear of damaging the presses.

³⁵ **[0066]** In the second period of PAA use, half characterised by a regular quick operating pace, it was not necessary to use the second disinfectant proposed according to the present invention. With a reduced operating pace - a situation that arose in the second half of the period concerned - use was made of 1-2 shocks a day of NVE from hops as a support biocide.

[0067] The attached Figure 2 reports the lactic acid levels in the average raw juice throughout the period under study, with respect to the one contributed by the beet cossettes.

[0068] In sum, it was possible to observe that the second biocide, used in the absence of resistant species, turned out to be very effective.

Impact on inverted sugars

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[0069] During the experimentation it was possible to observe an interesting behaviour of glucose in the diffusion juices, and this is well illustrated in the graph of the herewith attached Figure 3.

[0070] In the first month of the sugar production campaign, coinciding with the use of formalin, there was an increase of glucose, equal to 380 ppm, with respect to the one coming from beet. A similar behaviour was found during the 10 days of hop treatment. For the rest of the campaign characterised by PAA use, except for the week run with hop NVE, the trend decreased until it finally annulled itself completely. In other words, with PAA treatment the excess glucose disappears, with modest increases of lactic acid levels (from 0 to a maximum of 80 ppm).

[0071] Finally, it was observed that the drastic reduction in inverted sugar in the diffusion juice was accompanied by:

- A reduction of the colour of the thin and dense juices by about 1000 UI.
 - A reduction of calcium salts in the clear juice of 2nd saturation and in the thin juice by about 10°F.
 - A reduction of glucose in the dense juice.

[0072] Since the above-described phenomenon appears interesting both from a technological and economic standpoint (a reduction of losses), an attempt was made to explain the reason for this.

[0073] During the extraction process there is the formation of inverted sugar thanks to the enzymes (invertase) that accompany the beet cossettes. Moreover, some bactericides, which owe their action to the destruction of the lipoprotein

⁵ membrane of the micro-organisms, give rise to the immediate release of the invertase present inside the cells. This phenomenon is widely described by Günther Pollach in his recent work on the antibacterial effects of fatty acids on diffusion juices (G. Pollach et al., The concept of different natural antibacterials for the sugar industry, Zuckerindustrie 129, (2004), No. 8, 555-564).

[0074] The above-described phenomena, either singly or in combination, could explain the presence of newly formed glucose during the use of formalin and hops.

[0075] Vice-versa, to explain its disappearance when PAA is used, the most likely hypothesis is that no additional inverted sugar is formed, thanks to a possible inhibiting action by PAA on the invertase and/or to the lack of destructive action on bacterial membranes on the part of the PAA, to which would correspond the lack of release of new enzymes in the diffusion juice.

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Impact on the calcium salts in the depurated juices

[0076] The graph of herewith attached Figure 4 shows the trend of the calcium salts in the clear liquid of 2nd saturation and in the thin juice. There is a significant decrease in calcium salts from the time PAA starts to be used and up to the end of the campaign, with the exception of the hop period, which resumes the trend found with formalin.

[0077] The variation is seen with a "step" right at the point where there is the shift from formalin to PAA on 26 August, despite some formalin shock due to inexperience.

[0078] Table 2 below reports the averages in hardness, relative to the two juices, for each of the periods the experimentation was divided into.

TABLE 2

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| | Hardness °F | | | | | | | | |
|----------------------------|-------------|-------|-------|-------|--|-------|-------|--|--|
| | Formalin | PAA 1 | Нор | PAA 2 | | PAA 3 | Δ | | |
| Thin | 19.26 | 11.19 | 12.02 | 11.05 | | 11.35 | 8.20 | | |
| 2 nd carb. | 44.42 | 38.80 | 36.87 | 32.79 | | 27.47 | 11.63 | | |
| $\Delta 2^{nd}$ carb./Thin | 25.17 | 27.62 | 28.85 | 21.73 | | 16.12 | 3.43 | | |

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[0079] Comparing the first period (using formalin) of the campaign with the 2nd period (with PAA) it is possible to see a reduction of about 10°F both on the clear juice of 2nd saturation and in the thin juice after the resins, in which the hardness value is halved.

[0080] It is plausible to attribute the reduction of calcium salts to a lower presence of organic acids on the depurated juice, justifiable with a lower inverted sugar content in the diffusion juice - something that would also rule out the hypothesis

of biological destruction of the inverted sugar with the forming of acids.

EXAMPLE 2

- [0081] For the sugar production campaign of the year after the one of Example 1, a similar experimentation was carried out this too of a duration equal to that of the whole campaign which saw the use, for practically the whole period, of the PAA system (3%+15%) and Natural Vegetable Extracts (NVE) based on hop, palm kernel and pine resin extracts.
 [0082] For the whole campaign, no particular difficulties were found in controlling the infections in all operating conditions encountered throughout the process, thanks to the acquired familiarity in handling the PAA/NVE system.
- [0083] From the point of view of controlling the infections, the vegetable extracts used alone, in an intermediate period of the campaign of the duration of 15 days, performed well, save for confirming the progressive loss of efficacy over time.
 [0084] The PAA/NVE combination turned out to be an optimal technological choice both as regards the control of infections, thanks to the contrasting effect with respect to the establishment of resistant species, and also as regards the possibility of avoiding the undesirable effect on press absorptions caused by the massive use of PAA concomitantly with peaks of lactic acid in the juices.
 - [0085] The result was that it became possible to maintain the control parameters within the ranges considered optimal, equivalent to 5.8-6.0 for the pH and 250-400 ppm (0.25-0.40 g/l, see Figure 2 for comparison) for the L-lactic acid formed.
 [0086] The dry matter of the pressed spent pulps was virtually constant throughout the campaign at values comparable

to those of Example 1 when not, indeed, better than them, with sampling points being equal. The best performances of the pressing station were confirmed by higher electric absorption values than the ones recorded during the previous campaign - indicating a better use of the machines that produced a quantity of pressed pulp that was considerably higher than in the previous year.

5 [0087] The use of aluminium sulphate was totally avoided during the campaign period except for the 15 days of trial with only vegetable extracts.

[0088] As regards chalk, the average dosage was around a value slightly lower than the average value of the previous campaigns.

- [0089] As also demonstrated by the experimental results shown for exemplificative purposes above, the method for controlling bacterial infections based on the combined use of PAA as the main antibacterial, fed into the system in a continuative manner, and of NVE as a secondary antibacterial, to be used in a non-continuative manner, allows having a complete advantage of the technological benefits linked to using peracetic acid while, at the same time, controlling the development of PAA-resistant species that can be effectively eliminated via sporadic targeted treatment with natural antibacterial agents without interrupting the primary treatment.
- ¹⁵ **[0090]** The present invention has been disclosed with particular reference to some specific embodiments thereof, but it should be understood that modifications and changes may be made by the persons skilled in the art without departing from the scope of the invention as defined in the appended claims.

20 Claims

- A method for controlling bacterial infection in sugar production processes starting from sacchariferous vegetable material, wherein to the sugary material to be treated there is added one or more solutions based on peracetic acid and hydrogen peroxide, as a primary disinfectant, at least one of which is fed in a continuative way to the process, characterised by the fact that a further product, based on natural vegetable extracts compatible with foodstuffs
- 25 characterised by the fact that a further product, based on natural vegetable extracts c and having antibacterial activity, is added as a secondary disinfectant.
 - 2. A method according to claim 1, wherein the said product based on natural vegetable extracts is selected from the group consisting of: hop extract or one or more of its constituents having antibacterial activity; vegetable resins compatible with foodstuffs or one or more of their constituents having antibacterial activity; palm kernel extracts or one or more of their constituents having antibacterial activity; or a mixture of two or more of the said products.
 - **3.** A method according to claim 2, wherein the said product based on natural vegetable extracts is hop extract, or a mixture of hop beta-acids or a mixture of hop alpha-acids.
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- 4. A method according to claims 2 or 3, wherein the said product based on natural vegetable extracts is a moderately alkaline aqueous emulsion containing 5-20% by weight of acids or other natural compounds having antibacterial activity.
- 40 5. A method according to claim 4, wherein the said product based on natural vegetable extracts is based on hop extract and contains 80-90% by weight of water, 1-3% by weight of potassium hydroxide, 8-12% by weight of beta-acids, and 1-5% by weight of other hop compounds.
- 6. A method according to any one of claims 1-5 for sugar beet production processes, wherein of the said one or more solutions based on peracetic acid and hydrogen peroxide, a first solution is added continuously in the press water system and a second solution is added in the form of shocks in one or more of the following stages of the production system: inside the diffusers, in the extraction juice system, in the juice-cossettes mixer, in the juice-cossettes line.
 - **7.** A method according to claim 6, wherein the said solutions based on peracetic acid and hydrogen peroxide contain 2-15% by weight of peroxyacetic acid and 15-50% by weight of hydrogen peroxide.
 - 8. A method according to claim 7, wherein the said solutions based on peracetic acid and hydrogen peroxide also contain 5-25% weight of unreacted residual acetic acid as well as 0.5-1.5% by weight of other acids used as stabilisers in the peracetic acid manufacturing process.
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9. A method according to any one of claims 6-8, wherein the said first solution based on peracetic acid added continuously in the press water system is fed into the process at a dosage ranging between 0.1 and 30 ppm of peracetic acid.

- **10.** A method according to any one of claims 6-9, wherein the said second solution based on peracetic acid is added as shocks with an average dosage ranging between 3 and 120 ppm of peracetic acid, and with a frequency ranging from 3 to 12 times a day.
- 5 11. A method according to any one of claims 6-10, wherein the said product based on natural vegetable extracts compatible with foodstuffs and having antibacterial activity, used in a non-continuative manner, is fed to the process on the onset of species resistant to the peracetic acid-based disinfectant.
- A method according to claim 11, wherein the said product based on natural vegetable extracts is fed to the process as shocks with a frequency of between 1 and 6 times a day and in dosages ranging between 5 and 50 kg of product per shock, until such time as a pre-established decreased level of bacterial activity is detected.
 - **13.** A method according to any one of claims 6-10, wherein the said product based on natural vegetable extracts compatible with foodstuffs and having antibacterial activity used in a non-continuative manner is fed to the process for preventive purposes and at pre-established time intervals.
 - **14.** A method according to claim 13, wherein the said product based on natural vegetable extracts is fed to the process as shocks with a frequency ranging between 1 and 3 times a day and at dosages ranging between 5 and 20 kg of product per shock, one day a week.

15. A method according to any one of claims 11-14, wherein the said product based on natural vegetable extracts is fed to one or more of the following stages of the production process: inside the diffusers, inside the extraction juice system, in the juice-cossettes mixer, in the juice-cossettes line.



FIG. 1

SUGAR CAMPAIGN - PERIODS

lactate in cossettes g/l



Raw Juice Vs. from Cossettes

L-LACTATE

FIG. 2

glucose in cossettes g/l



FIG. 3

GLUCOSE Raw Juice Vs. from Cossettes

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FIG. 4

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EUROPEAN SEARCH REPORT

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