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PREPARATION OF NOURISHING SOLUTIONS FOR THE PRODUCTION OF CITRIC ACID BY THE FERMENTATION METHOD

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It is known that certain kinds of moulds of the species Aspergillus, Citromyces, Mucor and Penicillium are capable of converting sugar from its solutions into citric acid. Further it is known, that it is possible to use as the fermentation solution diluted sugar making molasses (German Patent No. 461356) whose content of nourishing materials may be enhanced, if necessary, by the addition of mineral nourishing substances, particularly phosphates, which usually are present in the molasses in an insufficient proportion. The fermentation is effected by inoculating a sterile nourishing solution of molasses with spores of suitably selected and cultivated moulds of the above mentioned species. These spores germinate rapidly on the surface of the nourishing solution at a suitable temperature, e. g. 30 degrees Centigrade, and form there a continuous mould mycelium in which the biochemical process of conversion of the sugar into citric acid takes place. During such a biochemical process a part only of the sugar is converted into citric acid while a part of the same is spent for building the organic substance of the mould mycelium and another part of the sugar is burnt to carbon dioxide by respiration whereby the energy necessary for the process is provided. For the commercial success of the whole process it is necessary to have the largest possible proportion of the sugar converted into citric acid as rapidly as possible. Therefore, the amount of sugar which is converted into mould substance and that amount which is lost as carbon dioxide by respiration should be as small as possible. For this reason it has been proposed to reduce the respiration of the moulds by the addition of poisons in order to increase the yield of citric acid (German Patent No. 544.589), or to restrict the development of the mould mycelium beyond the necessary limit by destruction of the growth-producing substances. Since, however, the region where the process of producing citric acid from sugar takes place is the mould mycelium on the surface of the fermentation solution, it is necessary for the mould mycelium to be formed rapidly, in a sufficient amount and with a correct and intense functioning capacity. Sound mould mycelium of high efficiency is not too thick, forms a continuous crack-free resistant film having a dry surface which is strongly undulated, and shows a highly articulated growth in depth and an otherwise smooth inner surface which is in contact with the nourishing solution.

Upon inoculation of molasses solutions, or also of other nourishing solutions, with spores of the before mentioned moulds it may be observed in many cases that although the spores germinate well a very thin mould film is formed which becomes wetted through and then drops below the level of the solution, or no continuous mould film is formed at all but only separate spots of moulds. If such an "unhealthy" mould mycelium becomes wetted through or broken, or drops below the level, it is necessary to effect a new inoculation whereupon generally a continuous new mould mycelium is formed. However, the duration of the fermentation period is thus increased due, on the one hand, to the increase of the time necessary for the mycelium to be formed and, on the other hand, to the fact that the wetted mycelium, which has sunk deep, impedes the free diffusion of the nourishing solution into the new sound mycelium. Such wetting through of the mould mycelium also increases the danger of infection, since the moist surface of the mould film may be infected much more easily by bacteria and other micro-organisms. Further it has been found that a mould film which shows an inclination for becoming wetted through, or forms a continuous film at a slow speed only, is rough on its inner surface, instead of being smooth, and shows a bad permeability for the nourishing solution into the cells.

Now it has been found that this "unhealthy" formation of mould mycelium on molasses solutions is the result of superficially active substances, which accumulate on the surface of the nourishing solution without separating from the same as a new surface phase. These substances modify the surface tension and impart to the solution a tendency to foaming. It has been ascertained that this unfavourable phenomenon may be eliminated by removing the said substances influencing the surface tension of the solution, i. e. the strongly superficially active substances. The provisions permitting to remove these substances and to eliminate the above mentioned unfavourable effects of the same may be of various character. Sometimes it is sufficient merely to strip off the surface. However, far more efficient are such steps which permit an adjustable removal of the superficially active substances. Among such steps belongs first of all the production of foam in the nourishing solution by blowing in finely divided air or other gases. The active substances are discharged from the surface of the nourishing solution with the foam produced, and it is possible to regulate such removal by altering the amount and pressure of the air blown, or the duration of the foaming period. By varying these factors the most favourable conditions for the growth and fermentation may be ascertained by the aid of a series of experiments on laboratory scale before undertaking the actual fermentation process on commercial scale.

Another intervention permitting to remove completely or partially the superficially active substances consists in the application of adsorption during the preparation of the nourishing solution. In such a case the working method may be carried out by admixing with the nourishing solution preformed insoluble adsorbing bodies which after a predetermined period of action are removed from the solution by decantation or filtration. As such bodies, charcoal or activated coal, clays (bleaching clays), silica gel or alumina gel may be conveniently employed.

According to another method precipitation is effected, in the nourishing solution, of bodies which have a considerable adsorption effect. This method covers for instance the production of precipitates of barium sulphate, insoluble sulphides, hydroxides or carbonates or of other insoluble compounds of the heavy metals or of the alkaline earths, the precipitant being added to the nourishing solution. By modifying the amount of the adsorbing bodies added, or of the kind and amount of the precipitant, of the cource and speed of the formation of the precipitate, it is possible to ascertain by preliminary experiments the most favourable conditions and extent of the removal of the superficially active substances and thus the best working conditions for the preparation of nourishing solutions.

It is obvious that the described methods for preparing the nourishing solution may be combined one with another to obtain the most favourable effect.

The following description refers to several examples, which facilitate the understanding of the working process and explain the influence of the described provisions.

To carry out the process according to this invention on laboratory scale enamelled vessels are used having a diameter of 20 cm. (fermentation surface 315 sq. cm) and a depth of 10 cm. These vessels are filled with two liters of a nourishing solution which has been prepared by diluting 600 grams of sugar molasses. The nourishing solution is acidified with about 20 cubic cm. of binormal (2n) sulphuric acid and about 4 cubic cm. of a 10% phosphoric acid solution are added. The solution is sterilized by boiling and introduced into the experimental vessels in a sterile space. The solution cooled down to about 35 degrees Centigrade is inoculated in a manner known per se with specially cultivated spores of appropriate moulds. After a twelve-day fermentation at a temperature of about 30 degrees Centigrade the well fermented solutions contain only about 2 to 5% of unfermented sugar, calculated on the original amount. The fermentation is then interrupted, and the mycelium is removed, pressed and extracted. The liquid extract is added to the fermented solution, and by the addition of lime under boiling conditions calcium citrate is precipitated. The analytically determined citric acid in the same, calculated on 100 parts of the sugar present, represents the fermentation yield.

In each of the following examples five similar experiments are carried out under uniform conditions in order to obtain a good average. Each vessel has been charged with 600 grams of molasses, that is 300 grams of sugar.

Comparative example

In the first group of experiments the above mentioned fermentation solution of molasses was treated without any further provision. After inoculation a very moist mould mycelium was obtained upon expiration of 24 hours, and in three amongst the five vessels the mycelium dropped into the solution so that it was necessary to inoculate these vessels anew. In two other vessels the mould mycelium recovered after 24 hours. In two vessels a rather strong infection was ascertained; these two vessels had a soft uneasily pressable mould film. After a twelve-day fermentation 993 grams of dry lime citrate containing 705 grams of citric acid were obtained from the 1500 grams of sugar, so that the yield amounted to 47 grams of citric acid per 100 grams of sugar.

Example 1

The same molasses as in the comparative example were used and a similar fermentation solution was prepared. However, after sterilization the total amount of 10.5 liters of nourishing solution was caused to foam by blowing in air which was distributed by means of a porous ceramic slab under a pressure of 100 millimeters of mercury during a period of 10 minutes. From the 10.5 liters of nourishing solution half a liter was removed by the foaming operation. The remaining 10 liters were distributed to five fermentation vessels and subjected to fermentation. The growth of the mould mycelium and the entire fermentation were perfect. A strong and sound mould film free from infection was obtained. After a twelve-day period of fermentation 1345 grams of dried lime citrate containing 976 grams of citric acid were obtained from the 1500 grams of sugar, so that the fermentation was effected with a yield of 65 grams of citric acid per 100 grams of the sugar present.

Example 2

The same molasses as in the comparative example were used and the fermentation solution was obtained in a similar manner. The difference consisted in that, for the total charge of 10 liters, 25 grams of activated coal were added during the sterilization. After agitation for half an hour of the boiling solution the latter was filtered and after cooling the inoculation was effected as in the preceding example. A sound mould mycelium was obtained and the entire fermentation was effected without infection. After a twelve-day fermentation 1280 grams of dried lime citrate were obtained in total, with a content of 952 grams of citric acid, from the 1500 grams of sugar, so that the yield amounted to 63.5 grams of citric acid per 100 grams of the sugar present.

Example 3

With the molasses of the preceding examples a similar nourishing solution was obtained. 5 grams of zinc sulphate were added to the solution and during sterilisation of the same an addition of a sodium sulphide solution in small excess was made to form a strongly adsorbing precipitate of zinc sulphide which was separated by filtration. After inoculation a strong and sound mould mycelium was obtained. The fermentation process was very uniform. After twelve days 1408 grams of lime citrate having a content of 1038 grams of citric acid were obtained from the 1500 grams of sugar. Thus the yield in this case was 69.2 parts of citric acid per 100 parts of sugar present in the molasses.

The present invention is not limited to the application of any predetermined working method, or of predetermined bodies for removing from the nourishing solution the superficially active substances, which modify the surface tension of the same, and on the contrary covers all suitable working methods and the utilization of any desired bodies by means of which at least a partial removal of the superficially more strongly active substances from the nourishing solution is secured. The expression "strongly superficially active substances" is used in this specification and in the claims to denote such substances which even in small amounts modify substantially the surface tension of the nourishing solution at the level of the same, that is lead to an increase of such tension, but incidentally may also lead to a reduction of the surface tension.

In addition to molasses other carbohydrate containing mashes, such as sugar solutions having various degrees of purity, pressed juices or sugared starch containing mashes which subsequently are fermented to citric acid, may be treated by the process of the present invention in a similar manner.

The provisions referred to hereinbefore, by means of which the superficially active substances are removed, have been used already in the fermentation art, but for a purpose quite distinct from the fermentation of carbohydrate containing mashes, such as molasses, to citric acid. Thus with yeast eharges for alcoholic fermentation, which by itself is connected with heavy foam formation due to the production of gases, the foam which is formed during boiling of the yeast charge is removed by a stripping-off operation. Also fermentation products, such as beer or wine, may be improved and made more durable by the addition of preformed clarifying agents or by precipitation of such agents from added salt solutions. However, all these provisions have a distinct purpose and are effected in a manner different from that which is applied in the present process.

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