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PROVISIONAL SPECIFICATION.

Improvements in the Bacterial Fermentation of Carbohydrates and in Bacterial Cultures for the same.

I, Dr. CHARLES WEIZMANN, of 30, Brunswick Road, Withington, Manchester, Chemist, do hereby declare the nature of this invention to be as follows:—

This invention relates to the production of acetone and alcohols by fermentation processes, and has for its object to obtain large yields by the fermentation of starchy bodies in a simple way.

Hitherto the production of acetone and alcohols by the fermentation of starchy bodies has been effected by means of anaerobic bacteria *inter alia* by bacteria defined as of the type of Fitz. Fermentation of this kind has always been effected under strictly anaerobic conditions.

Now I have found certain bacteria will grow in starchy bodies under aerobic conditions, *i.e.*, with free access of air, and that a strain of aerobically resistant facultative anaerobes can be readily prepared.

My invention therefore consists in the fermentation of starchy bodies with the production of acetone and alcohols under practically aerobic conditions.

My invention also consists in fermenting starchy bodies by means of bacteria, preferably the bacillus found on cereals, as *e.g.* maize, flax, *etc.*, preferably cultured as below indicated.

My invention further consists in a method of preparing an aerobically resistant culture for industrial fermentation processes according to which the bacteria are cultured, the culture highly heated for a short period, and again cultured, these steps being preferably repeated many times.

In carrying this invention into effect in the preferred manner I prepare a culture in the usual way from a mash of maize meal and I take a portion of this culture and heat it up to from 90° to 100° for a period of one to two minutes. Many of the bacteria are destroyed, and the resistant spores remain. The culture so treated is used for inoculating on a sterilised maize mash, and a sub-culture is thus prepared. The sub-culture is treated in the same way and the process repeated say 100 to 150 times. In this way a strain of bacteria is obtained which is very resistant to aerobic conditions. I believe the bacteria so produced are principally if not wholly the bacillus granulobacter pectinovorum.

I take then some 100 parts by weight of maize meal and suspend in 1500 to 2000 parts of water, or in the case of potatoes, say 100 parts are suspended in 300 to 500 parts of water, and the whole sterilised for three to four hours at a temperature of 130° C. to 140° C. and a pressure of 2 to 3 atmospheres. The mass, when cooled, is inoculated with a fresh culture prepared as above. Fer-

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mentation sets in after five to ten hours. The optimum temperature of the fermentation lies about 35° C. to 36° C.

The fermentation proceeds vigorously for about 36 hours gradually falling off after this period, and is completed after a period varying between 48 to 60 hours. The mash is then distilled, and the products isolated by fractional distillation in the usual way.

In some cases another bacillus is found, this type slightly varying in form which is also active but not so good.

I find that the bacteria above indicated attack maize or potatoes without any addition of nutritive materials or stimulants.

Dated this 29th day of March, 1915.

MARKS & CLERK.

COMPLETE SPECIFICATION.

Improvements in the Bacterial Fermentation of Carbohydrates and in Bacterial Cultures for the same.

I, Dr. CHARLES WEIZMANN, of 30, Brunswick Road, Withington, Manchester, Chemist, do hereby declare the nature of this invention and in what manner the same is to be performed, to be particularly described and ascertained in and by the following statement:—

This invention relates to the production of acetone and alcohols by fermentation processes, and has for its object to obtain large yields by the fermentation of starchy bodies with or without admixture of other carbohydrates in a simple way.

Hitherto the production of acetone and alcohols by the fermentation of starchy bodies has been effected by means of bacteria *inter alia* by bacteria defined as of the type of Fitz. Fermentation of this kind has always been effected under strictly anaerobic conditions in closed vessels.

I have found that certain heat-resisting bacteria, which are identified by the fact that they will convert the greater part of maize or other grain starch into acetone and butyl alcohol, and will also liquefy gelatine, can be used for the purpose of obtaining large yields of acetone and alcohols by the fermentation of solutions or suspensions of natural substances rich in starch or other carbohydrates mixed with such substances under aerobic or anaerobic conditions, *i.e.*, with free access of air as in yeast fermentation, or without.

My invention consists in the fermentation of solutions or suspensions of natural substances rich in starch or of other carbohydrates mixed with such substances by means of the aforesaid heat-resisting bacteria, under aerobic or anaerobic conditions, substantially as hereinafter indicated, with the production of large yields of acetone and alcohols.

The bacteria in question are found in soil and cereals, *e.g.*, maize, rice, flax, *etc.*

A convenient method of obtaining the bacteria above referred to is as follows:

I prepare a number, (say 100), of cultures in the usual way by inoculating *e.g.*, hot (say 90° C. to 100° C.) dilute, (say 2%), sterile maize mash with some maize meal, and then allowing it to ferment at about 35° C. to 37° C. for about four to five days.

From these tubes I select those which shew the most vigorous fermentation, and have a pronounced smell of butyl alcohol. These selected tubes I now heat up to from 90° C. to 100° C. for a period of one to two minutes. Many of the

bacteria are destroyed, but the desired resistant sports remain. I next inoculate a sterilised maize mash with the culture which has been heated as aforesaid, and so obtain a subculture. I then heat this subculture up to 90° C. to 100° C. for one to two minutes, and use it to inoculate another sterilised maize mash, and repeat the foregoing subculturing operation a number of times, say 100 to 150 times. In these operations no special precautions need be taken for the exclusion of air. I believe the bacteria so produced are probably chiefly, if not wholly, the bacillus granulobacter pectinovorum, though in some cases a bacillus of another type, slightly varying in form is found which is also active, but not so good.

The bacteria above indicated can then be used in the production of acetone and alcohol under aerobic conditions (which I prefer) by inoculating with the final culture a cooled solution or suspension of the selected substrate, *e.g.*, maize, which has been previously sterilised for three to four hours at a temperature of 130° C. to 140° C., and a pressure of 2 to 3 atmospheres.

I find that in the case of maize meal a suitable solution or suspension for inoculation can be formed by 100 parts by weight of maize meal, and 1500 parts by weight of water. Fermentation sets in after five to ten hours. The optimum temperature of the fermentation lies about 35° C. to 36° C.

The fermentation proceeds vigorously for about 36 hours, falling off rapidly after this period, and is completed after a period of about 48 hours. The mash is then distilled, and the products isolated by fractional distillation in the usual way.

I can carry the fermentation into effect under anaerobic conditions in the following manner:—The cooled sterilised maize mash for instance is run into closed tanks in which it can be contained under anaerobic conditions at about 35° C. to 36° C. It is then inoculated with the hereinbefore described final culture, and allowed to ferment. When the fermentation is completed, the mash is distilled, and the products are isolated as before.

I find that the bacteria above indicated will operate successfully on rice, wheat, oats, rye, darrhi, and potatoes, as well as maize, and in all these cases without any addition of nutritive materials or stimulants.

I am aware that it has been proposed to use heat-resisting bacteria the spores of which are capable of resisting for half an hour the heat of saturated steam having the temperature of boiling water for the purpose of production of fusel oil by the fermentation of appropriate substances, such as starch, carbohydrates, cellulose and the like, and I make no claim to the use of all heat-resisting bacilli for the production of acetone and alcohols.

Having now particularly described and ascertained the nature of my said invention, and in what manner the same is to be performed, I declare that what I claim is:—

1. The fermentation of solutions or suspensions of natural substances rich in starch or of other carbohydrates mixed with such substances under aerobic or anaerobic conditions, by the use substantially in the manner hereinbefore described, of heat-resisting bacteria, which are found in soil and cereals, such as maize, rice, flax, *etc.*, and which will convert the greater part of maize or other grain starch into acetone and butyl alcohol, and will also liquefy gelatine.

2. The fermentation of solutions or suspensions of natural substances rich in starch, under aerobic or anaerobic conditions, by the use substantially in the manner hereinbefore described, of heat-resisting bacteria, which are found in soil and cereals such as maize, rice, flax, *etc.*, and which will liquefy gelatine and will also convert the greater part of maize or other grain starch into acetone and butyl alcohol, without any addition of nutritive material of stimulents.

3. For the purpose of obtaining acetone and alcohol, the culture from soil and cereals such as maize, rice, flax, *etc.*, by repeated culture processes, of

heat-resisting bacteria capable of converting the greater part of maize, or other grain starch into acetone and butyl alcohol, and also liquefying gelatine, and the fermentation of natural substances rich in starch or of other carbohydrates mixed with such substances by the use of such bacteria cultured as aforesaid, all substantially as hereinbefore described.

Dated this 29th day of October, 1915.

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