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PRODUCTION OF BUTANOL AND ACETONE BY THE FERMENTATION OF WHEY

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Our invention concerns the production of bu-
tanol and acetone by the fermentation of whey,
that is to say the serum containing milk sugar
(lactose) obtained from milk after removal of the
milk fat and albumen (casein).

Butanol and acetone have already been obtained
by fermentation of sugar-containing solutions, for
example solutions of cane sugar (saccharose) or
waste liquors from the sulphite pulp manufac-
ture, or of wood sugar, or also starch-containing
natural products in suitably mashed condition
have been directly fermented. The varieties of
sugar used or developed in this way, essentially
glucose and/or fructose, shows no difficulties in
fermentation to butanol and acetone.

From the literature it is to be gathered that pure
milk sugar (lactose) shows at the most only half
the capacity for fermentation by butanol-forming
bacteria as do the usual varieties of sugar which
are employed in practice. Lactose is built up
from glucose and galactose and it therefore ap-
peared that merely the glucose portion of lactose
can be fermented to butanol while the galactose
portion remains unfermented.

It was therefore to be expected that in the
working up of whey (the serum from milk con-
taining milk sugar) derived in large quantities
from milk, up to half could at the most be fer-
mented to butanol.

Our investigations which have been conducted
with whey were however at first wholly negative.
We used for this purpose a whey of the following
composition:

Milk sugar (lactose)-----per cent--	4.17
Albumen -----per cent N--	0.113
Acid value -----	0.03
pH-----	7.08

The albumen materials or nitrogen compounds
in the whey are in the following expressed as ele-
mentary nitrogen. From this figure the actual
quantity can be obtained by the multiplication of
the nitrogen quantity by the factor 6.25. The
acid value is the number of cubic centimeters of
NNaOH required by 20 cubic centimeters.

Three samples of this whey were provided with
an addition of bacterial culture which, as previ-
ously stated, ferments glucose, saccharose or mal-
tose with outstanding yields to butanol and ace-
tone. In the investigations it appeared that no
butanol or acetone were formed; some acid merely
resulted. Bacteriological examination showed
that the added butanol bacteria had not de-
veloped.

We then found that the fermentation of whey
to butanol and acetone was in a considerable
degree prevented or hindered by the presence of
certain dissolved impurities.

Our invention resides in so purifying the whey
in advance that the development of the butanol

bacteria is not prevented. In particular our in-
vention envisages a reduction of the soluble nitro-
gen compounds (albumen); it has been found to
be satisfactory if the nitrogen compounds have
been reduced to a proportion of less than 0.08% N.
In general the proportion is reduced to 0.04 to
0.07% N. This whey is, in known manner, inocu-
lated with a culture of butanol bacteria and fer-
mented, whereupon the resulting butanol and
acetone are recovered from the fermented whey,
for example by distillation. The reduction of the
albumen content can be effected by precipitation
by means of known albumen-precipitating chemi-
cals.

The precipitation of the albumen can be effected
after a preceding neutralization of the acids present
in the milk, operation being equally good with
only a slightly acid whey or a renneted or sour
whey is treated.

We have further established that the dissolved
albumen in the whey which interferes with the
fermentation of butanol and acetone can also be
precipitated by heating.

Accordingly a further feature of our invention
consists in a process of heating whey and in this
manner reducing the dissolved albumen material
to an insignificant quantity. We may utilize for
this purpose temperatures of 80–90° C.

A slow increase of temperature is advantageous
during the heating as in this way the deleterious
albumen materials are precipitated in finely
divided form, a stirring with gases being advan-
tageous.

We have further found that this precipitate can
be left in the whey and that, similarly to turbid-
ity-producing materials, it exerts a good action
upon the development of the bacteria. By "turbid-
ity-producing materials" are to be understood
in general certain natural solid materials which
are suspended in syrups, mashes, wines, etc. Such
suspended materials prevent the sinking of the
bacteria and facilitate therefore a uniform dis-
tribution of the bacteria in the liquid to be fer-
mented.

The precipitated albumen in finely divided form
acts in a similar manner and improves the devel-
opment of the butanol-forming bacteria.

The precipitated albumen material then re-
mains in the liquid to be fermented. It can in
known manner, either before or after the dis-
tilling off of the acetone and butanol be separated
and recovered from the liquid or the distillation
residue respectively. At the same time also the
bacteria are separated and their albumen together
with the precipitated albumen of the whey can be
utilized in any suitable manner.

According to the present process, a practically
complete fermentation of the milk sugar in the
whey to butanol and acetone is obtained.

This result is surprising, following our initial

investigations with whey of a wholly negative character, and since also according to the literature, it was to be expected that at the most half of the milk sugar of the whey, corresponding to the glucose portion, could be fermented substantially to butanol.

So far as the reduction of the solid albumen material content by heating of the whey is concerned, it is further surprising that even a temperature of 90° C. suffices in order so far to reduce the impurification of the whey that this no longer hinders the development of the butanol-forming bacteria. In carbohydrate-containing mashers or mashers from extracted beets, temperatures up to about 90° C. are not sufficient by far to overcome the impurification.

Instead of leaving the precipitated albumen material in the whey it can also be removed by decantation, centrifuging or filtering. It has then, however, appeared advantageous to add to the cleared whey extraneous turbidity-producing materials for example peat dust, residues as obtained in wine production, or the like.

Further features of our invention will be evident from the subsequent examples which however, have no limiting significance. Further embodiments will be apparent to those skilled in the art by simple modification of the foregoing description.

Example 1

Whey pre-treatment.—In an enamelled container 1000 liters of whey are heated to 84–90° C., care being taken that the temperature shortly before the commencement of precipitation is only slowly increased. When the precipitation temperature is reached, air, nitrogen or fermentation gas (a gas or gaseous mixture developed by the microorganisms during fermentation) is forced in finely divided form through the whey by means of a suitable device. In this manner the customary large flocks or curded precipitates of whey albumen are wholly prevented. The precipitation is completed in a short time. The albumen exists for the greater part in finely divided form in the serum. Clear-filtered samples show after further heating no additional precipitation. After the precipitation of the albumen the lactic acid is neutralized. With an acid value of 1.09 (ccs. *n*-NaOH for 20 ccs. filtrate), about 2.2 kgs. of dry calcium hydrate to 1 cubic meter are necessary in order to set up the initial acid content to favor the fermentation. The neutralization of the lactic acid is effected directly after the albumen precipitation, the whole being stirred for about 5 minutes as above stated. Thereupon the whey, after reaching the fermentation temperature, is inoculated. Nitrogen content in the filtrate: 65.60 mg. N per 100 ccs.

Hours	Milk sugar, percent	Acid value in ccs. $\frac{n}{1}$ NaOH per 20 ccs. whey	pH	Albumen mgs. nitrogen per 100 ccs.
0.....	5.23	0.06	6.23	65.78
48.....	0.69	0.64	4.94	40.87

Degree of fermentation.....per cent... 86.5
 Acetone.....kgs. per cubic meter... 3.2
 Butanol.....do... 15.8
 Yield.....per cent... 90
 Proportion of acetone to butanol..... 1:5

Example 2

1000 liters of whey during heating to about 80–90° C. are brought to an acid value 0.06 by addition of 2.2 kgs. of calcium hydrate. The precipitation of the albumen is then effected as described in Example 1.

Hours	Milk sugar, percent	Acid value in ccs. $\frac{n}{1}$ NaOH per 20 ccs. whey	pH	Albumen mgs. nitrogen per 100 ccs.
0.....	5.23	0.06	6.23	65.78
48.....	0.79	0.57	5.00	43.15

Degree of fermentation.....per cent... 85
 Acetone.....kgs. per cubic meter... 2.9
 Butanol.....do... 15.4
 Yield.....per cent... 86
 Proportion of acetone to butanol..... 1:5

Example 3

1000 liters of whey free from precipitated albumen and neutralized are filtered clear and then sterilized after the addition of 10 kgs. of peat dust and are then fermented.

Hours	Milk sugar, percent	Acid value in ccs. $\frac{n}{1}$ NaOH per 20 ccs. whey	pH	Albumen mgs. nitrogen per 100 ccs.
0.....	5.10	0.07	6.42	67.20
32.....	2.42	0.90	4.98	-----
48.....	0.60	0.64	5.14	46.68

Degree of fermentation.....per cent... 88
 Acetone.....kgs. per cubic meter... 3.3
 Butanol.....do... 15.7
 Yield.....per cent... 85
 Proportion of acetone to butanol..... 1:5

Under "degree of fermentation" is to be understood the proportion of fermented to unfermented sugar and under yield the resulting amount of butanol or acetone compared with the highest proportion which can be obtained calculated upon the basis of the fermentation equation.

We claim:

1. Process for the technical production of butanol and acetone by fermentation, which includes the steps of treating whey having a content of dissolved albumen material of less than 0.08% N with a butanol-producing bacteria and recovering butanol and acetone from the treated material.

2. Process for the technical production of butanol and acetone by fermentation which includes the step of reducing soluble albumen content of whey to a value of below 0.08% N, fermenting the whey so treated by addition of butanol-producing bacteria to yield butanol and acetone.

3. Process for the technical production of butanol and acetone by fermentation which includes the step of treating whey with albumen-precipitating agents to bring the content of soluble albumen material to 0.08% N at the most and thereupon fermenting the whey by addition of butanol forming bacteria.

4. Process for the technical production of butanol and acetone by fermentation which includes the step of heating whey until the content of dissolved albumen materials falls by precipitation to below 0.08% N, and then fermenting the whey by addition of butanol forming bacteria.

5 5. Process for the technical production of butanol and acetone by fermentation which includes the step of heating whey slowly to temperatures between 80-90° C., to form a finely divided suspended precipitate of albumen material and to give a whey the dissolved albumen content of which amounts to less than 0.08% N, adding butanol forming bacteria to the whey containing the precipitate of albumen material and recovering the acetone and butanol from the fermented material.

10 6. Process for the production of butanol and acetone by fermentation which includes the step of reducing the soluble albumen content of a whey by precipitation to below 0.08% N separating the precipitated albumen material from the whey and fermenting the clear whey by addition of butanol-forming bacteria.

15 7. Process for the production of butanol and acetone by fermentation which includes the step of reducing the soluble albumen material content of whey to below 0.08% N by precipitation, add-

ing to the whey containing the precipitated albumen material butanol-forming bacteria, and allowing the whey to ferment, the fermented whey being then essentially freed by distillation from butanol and acetone, and the precipitated albumen material together with the bacteria separated from the distillation residue.

5 8. Processes for the production of butanol and acetone by fermentation which includes the step of slowly heating whey to a temperature between 80°-90° C. and at the same time stirring the whey by passing non-oxidizing gases therethrough, the heating being continued until the content of soluble albumen material amounts to less than 0.8% N, and albumen material is precipitated in finely divided form, and thereafter adding butanol forming bacteria to the whey containing the precipitated albumen material and permitting the whey to ferment.

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