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(54) **METHODE POUR L'ELIMINATION DE LA RIBOFLAVINE  
DANS LES SUSPENSIONS DE FERMENTATION**  
(54) **REMOVAL OF RIBOFLAVIN FROM FERMENTATION  
SUSPENSIONS**

(57) Riboflavin is removed from fermentation suspensions by them being heated at from 50 to 90 °C for from 1 to 3 hours, then cooled to from 0 to 30 °C over a period of from 1 to 5 hours, and subsequently being centrifuged to give a sediment fraction and liquid fraction in such a way that the sediment fraction contains predominantly riboflavin crystals as solid, and the liquid fraction contains virtually no crystalline riboflavin, and, where appropriate, resuspending the sediment fraction in from 0.5 to 2 parts by volume of water per part by volume of sediment fraction and repeating procedure c.

Abstract of the Disclosure: Riboflavin is removed from fermentation suspensions by them being heated at from 50 to 90°C for from 1 to 3 hours, then cooled to from 0 to 30°C over a period of from 1 to 5 hours, and subsequently being centrifuged to give a sediment fraction and liquid fraction in such a way that the sediment fraction contains predominantly riboflavin crystals as solid, and the liquid fraction contains virtually no crystalline riboflavin, and, where appropriate, resuspending the sediment fraction in from 0.5 to 2 parts by volume of water per part by volume of sediment fraction and repeating procedure c.

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The removal of riboflavin from fermentation  
suspensions

The present invention relates to an improved process for removing riboflavin from fermentation suspensions by centrifugation.

DE-C 2 920 592 discloses a process for removing riboflavin from fermenter suspensions in which the fermentation suspensions are diluted with from 25 to 100 % by volume of water and subsequently heated at from 50 to 65°C for from 15 to 45 minutes. After the suspensions have been cooled they are centrifuged twice to concentrate the riboflavin. The consequence of the dilution is that a larger volume of fermenter suspension has to be processed, which increases the costs of processing and the losses of riboflavin owing to some dissolving in the added water.

It is an object of the present invention to remove riboflavin from fermenter suspensions with minimal loss of riboflavin to give a solid with a maximum riboflavin content.

It has been found that this object is achieved by a process for removing riboflavin from a fermentation suspension by centrifugation, which comprises the fermentation suspension

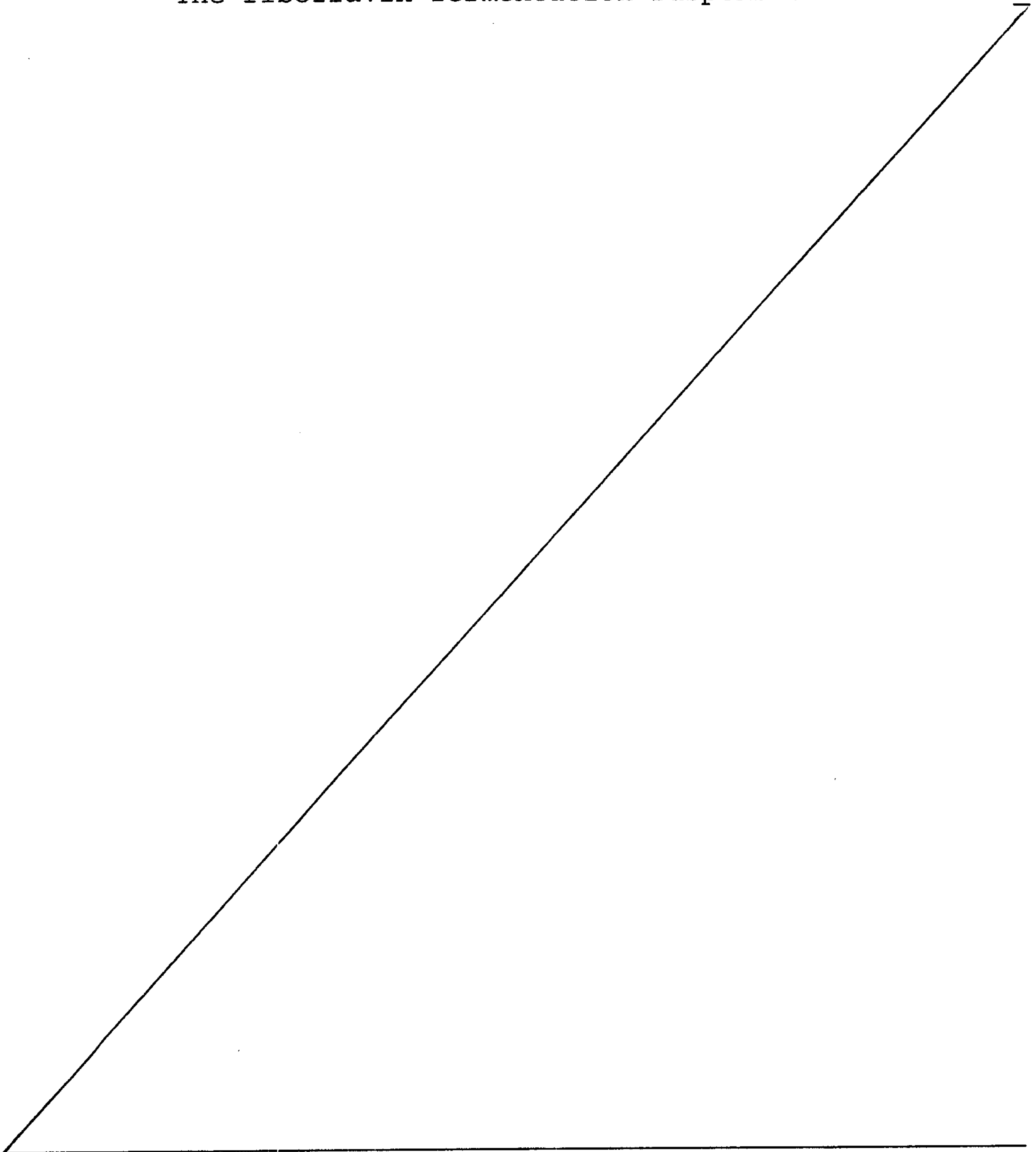
- a) being heated at from 50 to 90°C for from 1 to 3 hours and then
- b) being cooled to from 0 to 30°C over a period of from 1 to 10 hours, and subsequently
- c) being centrifuged in a decanting centrifuge operated by the classification principle to give a sediment fraction and a liquid fraction in such a way that the sediment fraction contains mainly riboflavin crystals

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as solid, and the liquid fraction contains virtually no crystalline riboflavin,

- d) if desired resuspending the sediment fraction with form 0.5 to 2 parts by volume of water per part by volume of sediment fraction, and repeating procedure (c) one or more times.

The riboflavin fermentation suspensions can be





obtained by conventional processes (see, for example, EP-A 231 605, EP-A 211 289; T. Szczesniak et al., Acta Microbiologica Polonica Ser. B, 3 (1971) 29-34 and 91-95), for example using mutants of yeast cells of the genus *Saccharomyces*, mutants of the strains *Candida flareri* GA 18Y8-6#2 and 6A 18Y8-6#2#11 and mutants of the strain *Ashbya gossypii*.

These fermentation suspensions contain up to 20 % by weight of riboflavin based on the total solids content of the suspensions. The remaining solids are essentially composed of complex cellular constituents.

It is essential for the process according to the invention that the fermenter suspension is heated, preferably for from 1 to 3 hours, in particular for from 1 to 2 hours. This brings about a transformation in the riboflavin crystals in which predominantly larger crystals are formed at the expense of smaller ones.

The transformation is preferably carried out at from 55 to 80°C, in particular from 60 to 75°C.

The fermentation suspension is cooled to from 0 to 30°C preferably over a period of from 1 to 8 hours, in particular 1 to 5 hours. This achieves a further optimization in the form of the riboflavin crystals.

The characteristics of the riboflavin crystals produced in this way make it possible, when suitable equipment is used, to separate the crystals optimally from the complex constituents of the cells and the media, which have lower specific gravities, in the fermentation suspensions, ie. to fractionate into a sediment fraction predominantly containing riboflavin crystals as solid and into a liquid fraction which contains virtually no crystalline riboflavin but does contain a large part of the complex cellular constituents.

Suitable equipment for removing riboflavin from the fermenter suspensions comprises decanter-type centrifuges which allow separation into two fractions when operated on the classification principle. Classification

means the separation of a slurry only into a more or less dewatered cake and an overflow containing the fine sediments (cf. Winnacker, Richter, Chemische Technologie, 1984, volume 1, pages 73 et seq.).

5           A particularly advantageous design of the process to be used for the centrifugation of the heat-treated fermenter suspension is a decanter-type centrifuge with full casing and a helical conveyor, as depicted in the figure, where the numbers (1) to (10) have the following meanings:

- 10           (1) bowl  
            (2) helical conveyor  
            (3) cylindrical sedimentation part  
            (4) conical dewatering part  
15           (5) suspension feed  
            (6) adjustable overflow height  
            (7) overflow diameter  
            (8) sediment discharge diameter  
            (9) liquid drain  
20           (10) sediment discharge.

            The geometry and the operation are optimized for the suspension of riboflavin crystals recrystallized according to the invention. The important parameters are the shape and speed of rotation of the bowl, the differential speed of rotation of the helical conveyor, the overflow height (6) and the suspension throughput, ie. the surface loading.

25           In order to compensate for variations in the riboflavin suspension with regard to solids content and the ratio of riboflavin to biomass and other constituents of the media in the suspension, the centrifuge ought to have the largest possible active classification area. This is achieved, on the one hand, by using a bowl (1) with a high slenderness ratio (slenderness ratio = length/diameter of the centrifuge), ie. a slenderness ratio of from 3 to 6, preferably of 4 or above, and, on 35 the other hand, by shifting the ratio of the cylindrical



sedimentation part (3) to the conical dewatering part (4) in favor of the sedimentation part by designing the conical part with an angle of, advantageously, from 10 to 25°, especially from 10 to 17°.

5           The suspension feed (5) is preferably sited approximately at the junction between the cylindrical and the conical parts of the centrifuge.

10           The overflow height (6) of the decanter must also be suited to the riboflavin crystal suspension. This preferably entails using an overflow diameter (7) which is about  $\pm 10$  mm different from the sediment discharge diameter (8). If the selected height (6) is too great (when the overflow diameter < sediment discharge diameter), there may be a short circuit leading to emergence  
15 of feed suspension at the sediment discharge, which reduces product purity. If the height is too low (when the overflow diameter > sediment discharge diameter), piling up of solid in the dewatering part leads to increased losses in riboflavin in the overflow.

20           In order to achieve an optimal classification between the riboflavin crystals on the one hand and the cell material and the constituents of the media on the other hand, ie. to have an optimal residence time in the decanter, it is necessary to match the speed of rotation  
25 of the bowl, the differential speed of rotation of the helical conveyor and the suspension throughput for a given decanter size. For example, if the selected speed of rotation of the bowl is too low at a given suspension throughput, the insufficient centrifugal force results in  
30 an increased loss of riboflavin crystals in the overflow. On the other hand, if the selected speed of rotation is too high, the increased sedimentation of cell material and constituents of the media results in a smaller improvement in product purity.

35           Hence the present invention also relates to a process, as defined above, for removing riboflavin from fermentation suspensions, which comprises the

fermentation suspension being centrifuged in step c) to give a sediment fraction and a liquid fraction so that at least 60 % of the solids in the sediment fraction is composed of riboflavin crystals, and the liquid fraction still contains a large part of the complex cellular constituents. This can advantageously be achieved by carrying out the centrifugation in step c) in a decanting centrifuge operated by the classification principle. It is particularly advantageous for the centrifugation in step c) to be carried out in a decanter-type centrifuge with full casing and a helical conveyor and with a slenderness ratio of 4 or greater and a conical part with an angle of from 10 to 25°, and operating it on the classification principle, with the overflow diameter being equal to the sediment discharge diameter  $\pm 10$  mm.

It is particularly advantageous for the differential speed of rotation of the helical conveyor to be from  $\pm 0.1$  % to  $\pm 1$  % of the speed of rotation of the bowl, and for the surface loading (= ratio of the suspension throughput to the equivalent settling area) to be from 0.8 to 1.8 l/(m<sup>2</sup> · h), preferably from 1 to 1.5 l/(m<sup>2</sup> · h), in the first decantation, and from 0.2 to 0.8 l/(m<sup>2</sup> · h), preferably from 0.4 to 0.6 l/(m<sup>2</sup> · h), if the decantation is repeated.

The centrifugation conditions are usually selected so that the resulting sediment fraction still contains from 65 to 90 % by weight, preferably 70 to 85 % by weight, of water.

The solid in the sediment fraction is more than 60 % by weight riboflavin. It is possible to resuspend and recentrifuge the sediment fraction to increase the proportion of riboflavin in the total solids content further.

The sediment fractions are preferably resuspended in from 0.5 to 2 parts by volume, in particular 0.7 to 1.5 parts by volume, of water per part by volume of sediment.



The sediment fractions containing more than 60 % by weight of riboflavin can be employed directly after the dewatering as animal feed additives or, after further purification, for pharmaceutical purposes.

5           The sediment fraction can be dried, for example, by fluidized bed spray granulation.

10           The process according to the invention can be used to obtain in a straightforward manner and with low riboflavin losses from fermentation suspensions up to about 60 % pure riboflavin with a single decantation and about 75 to 88 % pure riboflavin with repetition of the decanting procedure.

#### EXAMPLE 1

15           A fermentation suspension which was composed of about 85 % by weight water and 15 % by weight solid which contained about 17 % by weight riboflavin was heated at 60°C for two hours (h). The fermentation suspension was then cooled to 20°C over the course of 5 hours. The suspension treated in this way was centrifuged in a  
20           centrifuge with full casing and a helical conveyor and with a slenderness ratio of 4, a conical part with an angle of 17°, an overflow diameter of 3 mm less than the sediment discharge diameter, a suspension feed approxi-  
25           mately at the junction of the cylindrical and conical parts of the centrifuge and a surface loading of 1.3 l/(m<sup>2</sup> · h) in such a way that the sediment fraction was composed of 20 % by weight solid and 80 % by weight water.

30           The solid in the sediment fraction contained 63 % by weight riboflavin, and the riboflavin losses were 1.8 % by weight.

#### EXAMPLE 2

35           A fermentation suspension of the composition described in Example 1 was heated at 75°C for one hour. Centrifugation was then carried out as in Example 1.

          The resulting sediment fraction was composed of 66 % by weight riboflavin, and the riboflavin losses were

1.9 % by weight.

#### EXAMPLE 3

The sediment fraction obtained as in Example 1 was diluted with 0.8 part by volume of water per part by volume of sediment fraction and, for further concentration, centrifuged in a centrifuge with full casing and a helical conveyor and with a slenderness ratio of about 4, a conical part with an angle of  $17^\circ$ , equal overflow and sediment discharge diameters, a suspension feed approximately at the junction of the cylindrical sedimentation part with the conical dewatering part and with a surface loading of  $0.5 \text{ l}/(\text{m}^2 \cdot \text{h})$ . The differential speed of rotation was adjusted to the rate of feed so that piling up of solid was thus prevented. The solid in the resulting sediment fraction contained 88 % by weight riboflavin with the riboflavin losses being 1.0 % of the crystal suspension employed in Example 1.

#### EXAMPLE 4

(Comparative Example: thermal treatment and centrifugation not according to the invention)

As in Example 1 of DE 29 20 592, a fermentation suspension of the composition described in Example 1 was heated to  $60^\circ\text{C}$  over the course of 30 minutes (min), kept at  $60^\circ\text{C}$  for 10 min and cooled to  $20^\circ\text{C}$  over the course of 1 h.

This was followed by concentration in a centrifuge with full casing and with a slenderness ratio of about 3, a conical part with an angle of  $10^\circ$ , a dry section of 115 mm (caused by difference of 20 mm between the overflow and sediment discharge diameters), a suspension feed approximately at the cylindrical/conical junction and a surface loading of  $0.8 \text{ l}/(\text{m}^2 \cdot \text{h})$ . The solid in the resulting sediment fraction contained 46 % by weight of riboflavin, and the riboflavin losses were 6.5 % of the initial suspension.

## EXAMPLE 5

(Comparative Example: only thermal treatment not according to the invention)

5       A fermentation suspension with approximately the composition described in Example 1 was heat-treated as in Comparative Example 4.

10       This was followed by concentration in a centrifuge with full casing and with a slenderness ratio of about 3, a conical part with an angle of  $10^\circ$ , overflow diameter = sediment discharge diameter, suspension feed approximately at the cylindrical/conical junction and with a surface loading of  $1.1 \text{ l}/(\text{m}^2 \cdot \text{h})$ . The differential speed of rotation was adjusted to the feed rate so that no solid residue was produced.

15       The solid in the resulting sediment fraction contained 58 % by weight riboflavin, and the riboflavin losses were 4.4 %.



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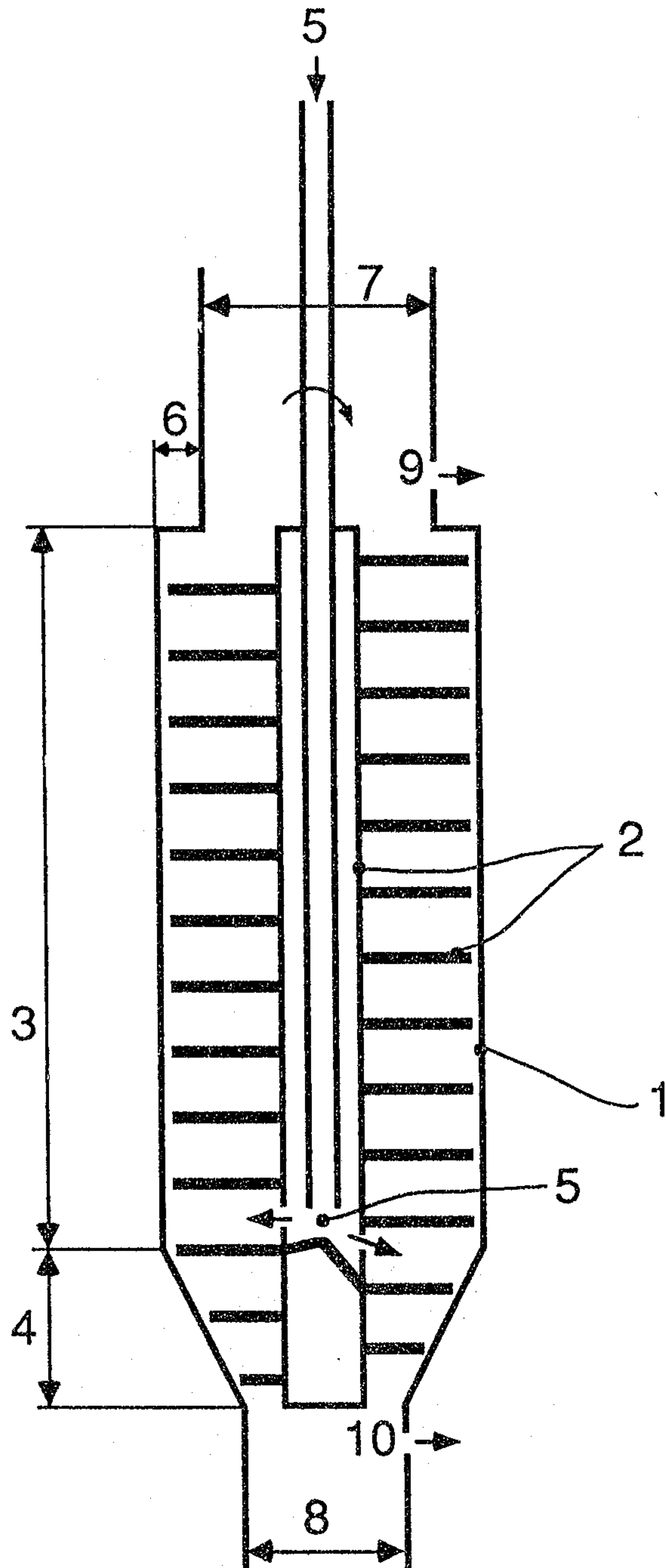
CLAIMS

1. A process for removing riboflavin from a fermentation suspension by centrifugation, which comprises the fermentation suspension
  - a) being heated at from 50 to 90°C for from 1 to 3 hours and then
  - b) being cooled to from 0 to 30°C over a period of from 1 to 10 hours, and subsequently
  - 10 c) being centrifuged in a decanting centrifuge operated by the classification principle to give a sediment fraction and a liquid fraction in such a way that the sediment fraction contains mainly riboflavin crystals as solid, and the liquid fraction contains virtually no crystalline riboflavin.
2. A process for removing riboflavin from fermentation suspension as claimed in claim 1, wherein the sediment fraction is resuspended with from 0.5 to 2 parts by volume of water per part by volume of sediment fraction,  
20 and procedure (c) is repeated one or more times.
3. A process for removing riboflavin from a fermentation suspension as claimed in claim 1 or 2, which comprises the fermentation suspension being centrifuged in step c) to give a sediment fraction and a liquid fraction so that at least 60% of the solids in the sediment fraction is composed of riboflavin crystals, and the liquid fraction still contains a large part of the complex cellular constituents.

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4. A process for removing riboflavin from a fermentation suspension as claimed in claim 1, 2 or 3, wherein the decanting centrifuge used in step c) has a full casing and a helical conveyor and has a slenderness ratio of 4 or greater and a conical part with an angle of from 10 to 25°, and said centrifuge is operated by the classification principle with a overflow diameter being equal to a sediment discharge diameter  $\pm$  10 mm.

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