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(54) **Method for batchwise enzymatic retting of flax or other rettable plants.**

(57) In the method the cut flax or other rettable plant is treated with an SPS-ase preparation producible by means of a strain belonging to the genus Aspergillus, preferably to Aspergillus japonicus or Aspergillus aculeatus, in an aqueous medium until the retting is completed or substantially completed, whereafter, if desired, the retted flax or other rettable plant is decolourized by being exposed to a moderate bleaching procedure only. In this manner a number of technical advantages are obtained, especially a tremendous time saving, a higher yield, a higher strength, a lighter colour and a better reproducibility.

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Description

METHOD FOR BATCHWISE ENZYMATIC RETTING OF FLAX OR OTHER RETTABLE PLANTS

The invention comprises a method for batchwise enzymatic retting of flax or other rettable plants in regard to fibre release.

For the sake of brevity, usually the above group of plants will be referred to in the following as flax, even if it also comprises all other rettable plants, e.g. jute, sisal, hemp and ramie.

After the cutting of the flax, it is usually dried and then subjected to the retting process, which is actually a rotting of the flax or the first step in the normal decomposition or degumming of flax stalks, whereby the pectic material binding the flax fibres together is dissolved to separate the fibres.

Retting is usually carried out in the following ways :

- "field retting" or "dew retting"
- "pool retting"
- "tank retting" or "warm water retting"
- "enzymatic retting"

By field retting, the cut flax is spread in the fields and exposed to dew, rain, etc. The time required for complete retting is around 4 to 7 weeks, and also microbiological attack on the flax will impair the strength and colour of the flax fibres. Subsequent bleaching for improving the colour will further impair the strength of the flax fibres.

By pool retting, flax and water is filled into crates, together with crate weights for immersion of the flax, whereafter microbiological retting agents will produce a retted flax in 1 to 3 weeks. The method is not reproducible and is now abandoned.

By tank or warm water retting, the flax is kept in tanks with approx. 40° C hot water under partially controlled conditions. This method is expensive and requires several exchanges of water, the advantages being that retting is completed in a relative short time, i.e. 4 to 10 days.

By enzymatic retting, the microbiological enzymes produced by the retting agents, in relation to pool or tank retting, are replaced by cell wall splitting agents, especially pectolytic enzymes. No satisfactory retting time reduction has been obtained with enzymatic retting methods so far.

Thus, a need exists for a method for retting of flax, which is cheap, simple, reproducible, easily adaptable to existing equipment, and fast, thereby giving rise to flax fibres of superior strength and colour.

Now, according to the invention, it has been found that a batchwise enzymatic retting with a specially selected enzyme fulfills this purpose, inasmuch as specially the retting time can be reduced to a few hours.

Thus, the method according to the invention for batchwise enzymatic retting of flax or other rettable plants in regard to fibre release is characterized by the fact that the cut flax or other rettable plant is treated with an SPS-ase preparation producible by means of a strain belonging to the genus Aspergillus, preferably to Aspergillus japonicus or Aspergillus aculeatus, in an aqueous medium until the retting is completed or substantially completed, whereafter, if desired, the retted flax or other rettable plant is decolourized by being exposed to a moderate bleaching procedure only.

For the purpose of this specification with claims, it is intented that the term SPS-ase preparation is understood to mean a preparation of SPS-ase, as described and defined in UK patent application No. 2 115 820 A, provided that the SPS-ase preparation is producible by means of a strain belonging to the genus Aspergillus. It has been found that a similar retting time reducing effect does not appear if a similar treatment is carried out with PECTINEX pectinase or CELLUCLAST cellulase and thus, it is assumed that the active principle pertaining to the treatment of the flax fibres with an SPS-ase preparation is the SPS-ase enzyme itself. However, the applicant does not want to be bound to this hypothesis.

By a moderate bleaching procedure we mean a bleaching procedure with a maximum bleaching capability corresponding to sodium perborate in a concentration of 0.3 % weight/volume, at 45° C and pH 9 for 1.5 hours.

Surprisingly, according to the invention it has been found also that the retting with the SPS-ase preparation opens up the possibility of performing an excellent decolourization by means of moderate bleaching only, whereby the reduction of strength due to the conventional highly aggressive bleaching agents is completely eliminated.

The treatment is carried out in a suitable container or tank, and usually without any significant movement of the flax or the aqueous medium ; however, agitation in the container could be carried out, if wanted.

It is easy for the worker skilled in the art to evaluate when the retting is completed or substantially completed. Reference is made to e.g. Dujardin, A. (1940), Vlasroten, De Westvlaamsche Boekhandel, Kortrijk, Belgium.

In French patent No. 2 516 555, a continuous retting process for flax by means of pectinolytic enzymes is described. In the first place this process is not well adaptable to the already existing batchwise plants, and also, it had been found that conventional pectolytic enzymes do not fulfill the above indicated purpose of the invention.

Also, in the brochure "Enzylin Vlastechnologie" from Ir. D.M. van der Sar and Ir. M. van Hoorn, Postbus 435, 6700 AK Wageningen, the Netherlands, another continuous retting process for flax is described. As before, in the first place this process is not well adaptable to the already existing batchwise plants, and furthermore, according to our investigations, the time saving with the enzyme used in this continuous process is negligible.

US patent No. 1 842 024 describes the retting of fibres of flax and similar plants by means of enzymes capable of degrading ligneous material. However, the use of the SPS-ase in this prior art retting process is not described.

For summarizing purposes, the method according to the invention offers the following unobvious combination of advantages in comparison to the prior art methods :

1) a tremendous time saving, as the method can be completed in a few hours as compared to at least several days

2) a better yield of retted fibres

3) a better strength of the retted fibres

4) a lighter colour

5) less pollution, if the aqueous medium is reused, which it usually will be in order to meet financial requirements

6) a well controlled method giving rise to retted fibres of reproducible quality from batch to batch

7) easy adaptability to existing equipment, as the only modification required is the addition of the SPS-ase preparation to the retting tank

8) a higher fineness number, i.e. thinner fibres

9) a higher average degree of polymerization of the cellulose in the retted fibres.

It is to be understood that some of these technical advantages are interrelated. Thus, the better strength (3) and the lighter colour (4) are direct results of the time reduction (1), because the microbiological decomposition due to the short retting period will not be able to provide any major strength reduction or unwanted decolourization.

In a preferred embodiment of the method according to the invention, the cut flax is dried before the treatment. In this way a stock of dry flax is provided, thereby facilitating a smooth subsequent batchwise retting procedure.

In a preferred embodiment of the method according to the invention, the SPS-ase activity in the aqueous medium is between 25 to 350 SPS-ase units/1, preferably between 90 and 250 SPS-ase units/litre. The SPS-ase unit (SPSU) is as defined in UK patent application GB 2 115 820 A, page 14, lines 12-14. With an activity below 25 SPS-ase units/l the retting time is not reduced satisfactorily, and with an activity above 350 SPS-ase units/l the retting is uneconomic at the present time.

In a preferred embodiment of the method according to the invention, the SPS-ase preparation is a liquid preparation with an SPS-ase activity of between 10 and 100 SPS-ase units/g, preferably between 25 and 50 SPS-ase units/g. A liquid preparation is easily distributed in the aqueous medium, and thus the retting process starts immediately after addition of the liquid SPS-ase preparation. The activity interval indicated is convenient for application on an industrial scale, as a volume of the SPS-ase preparation has to be added which is small enough for easy handling and large enough for accurate dosage.

In a preferred embodiment of the method according to the invention, a biocide is added to the aqueous medium in a bacteriocidal amount. In this way the reproducibility of the process is improved.

In a preferred embodiment of the method according to the invention, an antifoam agent is added in an amount sufficient for foam prevention. In this way a smooth retting process is secured.

In a preferred embodiment of the method according to the invention, the pH value is not adjusted artificially. This embodiment is simple and yet effective.

In a preferred embodiment of the method according to the invention, the initial pH value is adjusted to a value between 3.5 and 6.5, preferably between 4.5 and 5.5 In this way an initial pH is secured, at which the SPS-ase exhibits its maximum activity.

In a preferred embodiment of the method according to the invention, the temperature is not adjusted artificially. This embodiment is simple and yet efficient.

In a preferred embodiment of the method according to the invention, the initial temperature is adjusted to a value between 15 and 45°C, preferably to around 40°C. In this way an initial temperature is secured, at which the SPS-ase exhibits both a satisfactory activity and a good stability. In case the method is carried out at around 40°C, the container in which the retting is carried out is preferably closed in order to keep the temperature from dropping.

In a preferred embodiment of the method according to the invention, the aqueous medium contains a pectolytic enzyme besides the SPS-ase preparation. In relation to certain rettable plants, e.g. hemp, the combination of SPS-ase and a pectolytic enzyme gives rise to an improved retting efficiency.

In a preferred embodiment of the method according to the invention, the weight proportion between flax (dry matter) and aqueous medium is between 1:5 and 1:15, preferably around 1:10. With a proportion of more than 1:5 it has been found that it is difficult to provide a sufficient soaking of the flax, and with a proportion of less than 1:15 it has been found that the process is too costly due to an enzyme consumption which is too large.

In a preferred embodiment of the method according to the invention, the aqueous medium is separated and used for retting of another batch of unretted flax after completion or substantial completion of the retting. In this way the economy of the process is improved tremendously, and it has been found that the stability of the SPS-ase is sufficient for satisfactory reuse at least around 10-15 times under normal conditions. As a certain amount of SPS-ase solution is removed with the batch of retted flax, a minor amount of SPS-ase solution has to be added each time another batch of unretted flax is to be treated for replenishing purposes.

In a preferred embodiment of the method according to the invention, a docolourisation is carried out by exposing the retted flax fibre or other rettable plant fibre to a moderate bleaching procedure only. In this manner a fibre with an especially good combination of satisfactory appearance and fibre strength is obtained.

In a preferred embodiment of the method according to the invention, the decolourisation is carried out by immersing the fibre in a solution of 0.5-2.5 % by weight of citric acid or acetic acid and 1-5 % by weight of sodium carbonate or sodium hydroxide at 40-60° C for 0.5-2 hours, whereafter the fibre is washed 1-3 times in hot water at 50-60° C. Hereby a cheap and yet effective decolourisation is obtained.

In a preferred embodiment of the method according to the invention, the decolourisation is carried out by immersing the fibre in a solution containing 0.3-1.5 % by weight of sodium perborate. 0.1-0.5 % by weight of sodium tripolyphosphate and 0.05-0.25 % by weight of an anionic surfactant, preferably sodium alkyl ether sulphate, 3EO for 20-60 minutes at 40-50° C, whereafter the fibre is washed 1-3 times in hot water at 50-60° C. Hereby a cheap and yet very effective decolourisation is obtained.

In order to present documentation for the superior reduction of retting time of an SPS-ase preparation in comparison to prior art pectolytic enzymes, reference is made to the following table, in which all enzymes listed below the SPS-ase are prior art pectolytic enzymes.

Enzyme name	Enzyme concentr.	pH at start of retting	Result of Fried test (1)	Breaking test (2)	Comments
SPS-ase	3 g/l	4.95	3	2	Fibres easily released
		6.0	3	2	" "
Novozym 234	3 g/l	5.5	0	not possible	Fibres not released
Panzyme Combi	3 g/l	4.9	0	"	" "
Rapidase C80-P	3 g/l	5.5	0	"	" "
Klerzyme 40	3 g/l	5.8	0	"	" "
Rapidase CPE	3 g/l	5.5	0	"	" "

Retting was performed at 40°C for 24 hours using 50 g flax and a flax to enzyme solution ratio of 1:10.

(1) Fried test :

Three pieces of retted flax stalks approximately 5 cm long are put into a test tube and 8 ml of boiling water is poured over them. The test tube is stoppered and vigorously shaken. The degree of retting is then evaluated on a scale of 0 to 3 and is expressed as follows :

0 : no retting
1 : retting started
2: medium retting
3: complete retting

Reference : Dujardin, A. (1940), Vlasroten, De Westvlaamsche Boekhandel, Kortrijk, Belgium.

(2) Breaking test :

This measures the number of times the flax stalk has to be squeezed between two serrated opposing surfaces to release the fibres. The smaller this number is, the better is the retting.

In the following, the method according to the invention will be illustrated by some examples and embodiments.

Example 1 :

This example illustrates a most simple enzymatic flax retting method on pilot plant scale.

Required equipment and materials :

a. Dimensions of pilot plant retting tank : 1.3 m x 1.3 m x 2.2 m. Useful contents of tank : 3.5 m^3.

b. Rippled flax per run : 250 kg.

c. Enzyme : SP 249 SPS-ase preparation (Novo Industri). batch PPS 1717, as described in NOVO ENZYME INFORMATION, IB-297d-GB, with an SPS-ase activity of 34 SPSU/g is used in concentrations from 3 to 5 g/liter, corresponding to from 102 to 170 SPSU/litre.

d. Temperature : 40°C.

e. pH : 5.0 (at the start the pH value is adjusted to 5.8).

f. A suitable pump.

Procedure :

250 kg of flax is placed in the retting tank and subsequently the proper amount of enzyme solution, i.e. 2800 litres of SP 249 (3.5 g/litre in one experiment) containing 0.1 % Thymol biocide with fungicidal activity is pumped from a special container or neighbouring tank (used for the preparation of the desired enzyme concentration) over the flax bushels into the retting tank. After 20 to 24 hours at 40°C the flax is nicely retted and can, after removal from the tank, be dried in the usual way. After replacement of the volume of enzyme solution absorbed by the flax (1.6 litre/kg of flax), by a solution of 4 g/litre of SP 249 (the slightly higher enzyme concentration compensates for possible enzyme denaturation), the restored volume of 2800 litres (from the first tank) is pumped over a new batch of flax (placed for retting in a neighbouring tank), and again the retting process is continued at 40°C for another 24 hours. This is repeated six times.

In all cases the retting was perfect, and the retted flax exhibited excellent properties.

Example 2 :

The example illustrates the combination of enzymatic stepping of the flax followed by a decolourisation of the extracted fibre.

In this example the retting procedure is carried out exactly the same as in example 1, except that the enzyme dose used was 5 g/l of SP 249, no biocide was added and the treatment period used was 16-19 hours.

After retting, the flax was dried and the fibre was obtained in the normal manner.

Samples of 10 g of the flax fibre prepared in the above manner were then subjected to different decolourisation procedures as detailed below. For comparison 10 g samples of flax fibre obtained by conventional pit retting of flax straw from the same harvest were subjected to the same procedures.

Procedure A :

10 g of fibre is added to 200 ml of a solution containing 1 % by weight citric acid and 2 % by weight sodium carbonate at 50°C. After 1 hour of incubation the fibre is washed twice with hot water (50-60°C) and then dried.

Procedure B :

10 g of fibre is added to 200 ml of a 2 % w/w solution of sodium hydroxide at 60° C for 30 minutes. The fibre is then washed twice in hot water at 50-60°C. After this the washed fibre is immersed in 200 ml of a solution containing 0.3 % by weight sodium perborate, 0.1 % by weight sodium tripolyphosphate and 0.05 % anionic surfactant (i.e. sodium alkyl ether sulphate, 3EO) at 45°C for 15 minutes. The decolourised fibre is washed twice with hot water (50-60°C) and then dried.

Procedure C :

10 g of fibre is added to 200 ml of a solution containing 0.6 % by weight sodium perborate, 0.2 % by weight sodium tripolyphosphate and 0.1 % sodium alkyl ether sulphate, 3EO at 45°C for 30 minutes. The fibre is then washed twice with hot water at 50-60°C. After washing, the fibre is then immersed in 200 ml of a solution of sodium perborate, sodium tripolyphosphate and sodium alkyl ether sulphate, 3EO as described in procedure B. The decolourised fibre is washed twice with hot water (50-60°C) and then dried.

After drying the washed and dried fibres are compared to fibre that has been washed with hot water only and the degree of decolourisation assessed on a scale of 1 to 10 where 1 is the colour of the untreated fibre and 10 equivalent to magnesium oxide standard white.

The results of the assessment tests of the degree of decolourisation are shown in the following table :

Decolourisation Procedure	Source of fibre: Pit retted	Source of fibre: SPS-ase retted
A	2	2
B	8	8
C	5	9

Example 3 :

This example illustrates the combination of enzymatic steeping of the flax followed by after-retting in a moist tank or chamber, on a laboratory scale. It is intended that the term "after-retting" is referring to the retting effect produced by the enzyme absorbed by the plant tissues. The process is performed without any further water supply in a moist tank or chamber at 40° to 45° C.

In this example, simple steeping is combined with after-retting in a separate moist tank or chamber at 40° C.

50 g of flax is steeped for two hours at 40° C and atmospheric pressure in 500 ml of a solution of 4.0 g/litre of SP 249 SPS-ase preparation as in example 1. During this period, 1.55 kg of the enzyme solution is absorbed by 1 kg of flax. After removal of the steeped flax from the enzyme solution, it is placed during 22 hours for after retting in a humid tank or chamber kept at 40° C (total retting time = sum of steeping time (2 h) and after-retting time (22 h) = 24 hours). Subsequently, the amount of enzyme solution absorbed by the flax is replaced by a fresh and equivalent amount of 4.5 g/litre of SP 249 SPS-ase preparation as in example 1. In this way a new batch of flax can be steeped. The latter batch must be kept ready in a neighbouring container before the enriched solution of the first container can be pumped over the flax in the second container. After steeping again for two hours, after-retting can once more be performed as described above. The above procedure is repeated 10 times, under normal laboratory conditions.

In all cases the retting was perfect, and the retted flax exhibited excellent properties.

Example 4 :

This example illustrates how the use of enzyme may be reduced further than that described in examples 1 and 2.

In this case the procedure followed is the same as that in example 2 except that after 8 hours of incubation the enzyme solution is pumped to another tank containing flax straw and the volume restored to 2800 l as described in example 1. The wetted, partially retted flax in the first tank is covered to reduce evaporation and cooling and left for 18 hours. At the end of this period it is dried and the fibre extracted in the normal manner.

The flax straw in the second tank is then allowed to ret for 16 hours and then the enzyme solution is transferred back to the first tank which has been filled with fresh flax straw. The retted flax from the second tank is then dried and extracted in the normal manner. This double cycle is repeated 10 times.

In all cases the retting was perfect and the retted flax exhibited excellent properties.

Example 5 :

this example illustrates the combination of enzymatic steeping of flax followed by squeezing of the retted flax between rollers, on a laboratory scale.

50 g of flax is steeped for 16 hours in a solution of 4 g/l SP 249 SPS-ase as in example 1. After retting the flax straw is passed between two opposing rubber rollers to squeeze out absorbed enzyme solution. Normally 1.0 kg of flax straw will absorb 1.5-1.8 kg of enzyme solution and by squeezing through rollers approximately 50 % of this can be recovered.

The recovered enzyme solution may be added back to the enzyme solution remaining in the retting apparatus and the above procedure may be repeated 10 times, under normal laboratory conditions.

Further embodiments :

As an alternative maximum exploitation of enzymatic activity combined with after-retting in a special moist tank or chamber at 40° C can be carried out. Hereby, more enzyme can be saved. Indeed, when several retting tanks (preferably not too big) are used in combination, then at least 50 % of the original enzyme solution can be totally used for retting.

Indeed, by combining after each run the remnants of the steeping solutions of the different tanks into one or more of the tanks, a further saving of enzyme can be achieved, while the fresh enzyme, which always has to be added in order to replace the enzyme absorbed by the retting flax, can easily be added to each of the tanks under consideration. This alternative has been tested successfully on a laboratory scale.

As an other alternative, evacuation of the retting tank e.g. at 1/5 of the atmospheric pressure can be carried out, thus providing maximal exploitation of the enzymatic activity combined with after-retting in a special moist tank or chamber at 40° C. In this embodiment a suitable vacuum pump (for example a good Leybold pump) has to be used as an extra requirement. With this embodiment the preheated (40° C) retting tank is first filled with flax. Thereafter, the preheated (42° C) enzyme solution prepared in a heated (45° C) neighbouring container is pumped into the retting tank until all flax is submerged by the enzyme solution. Subsequently, the latter tank is covered with a hood, whereafter a vacuum is applied by means of the vacuum pump.

During the evacuation (e.g. around 1 h), air from the plant tissues is rapidly exhanged with enzyme solution. Then the vacuum is released and the remaining enzyme solution is pumped from the first retting tank into the following tank, which beforehand has been filled with a smaller amount of flax. This following retting tank is now evacuated etc. High humidity is necessary in order to allow the enzyme absorbed by the plant tissues to degrade the pectins. Laboratory experiments have shown that after 60 minutes evacuation with an enzyme solution containing 3 g/litre of the enzyme in example 1, followed by 23.00 h after-retting, the retting of the flax is complete.

The features disclosed in the foregoing description, and in the following claims may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Claims

1. Method for batchwise enzymatic retting of flax or other rettable plants in regard to fibre release, wherein the cut flax or other rettable plant is treated with an SPS-ase preparation producible by means of a strain belonging to the genus Aspergillus, preferably to Aspergillus japonicus or Aspergillus aculeatus, in an aqueous medium until the retting is completed or substantially completed, whereafter, if desired, the retted flax fibre or other rettable plant fibre is decolourized by being exposed to a moderate bleaching procedure only.

2. Method according to claim 1, wherein the cut flax is dried before the treatment.

3. Method according to claim 1 or 2, wherein the SPS-ase activity in the aqueous medium is between 25 and 350 SPS-ase units/l, preferably between 90 and 250 SPS-ase units/l.

4. Method according to claim 1-3, wherein the SPS-ase preparation is a liquid preparation with an SPS-ase activity of between 10 and 100 SPS-ase units/g, preferably between 25 and 50 SPS-ase units/g.

5. Method according to claim 1-4, wherein a biocide is added to the aqueous medium in a bacteriocidal amount.

6. Method according to claim 1-5, wherein an antifoam agent is added in an amount sufficient for foam prevention.

7. Method according to claim 1-6, wherein the pH value is not adjusted artificially.

8. Method according to claim 1-6, wherein the initial pH value is adjusted to a value between 3.5 and 6.5, preferably between 4.5 and 5.5.

9. Method according to claim 1-8, wherein the temperature is not adjusted artificially.

10. Method according to claim 1-8, wherein the initial temperature is adjusted to a value between 15 and

45°C, preferably to around 40°C.

11. Method according to claim 1-10, wherein the aqueous medium besides the SPS-ase preparation contains a pectolytic enzyme.

12. Method according to claim 1-11, wherein the weight proportion between flax (dry matter) and aqueous medium is between 1:5 and 1:15, preferably around 1:10.

13. Method according to claim 1-12, wherein the aqueous medium after completion or substantial completion of the retting is separated and used for retting of another batch of unretted flax.

14. Method according to claim 1-13, wherein a decolourisation is carried out by exposing the retted flax fibre or other rettable plant fibre to a moderate bleaching procedure only.

15. Method according to claim 14, wherein the decolourisation is carried out by immersing the fibre in a solution of 0.5-2.5 % by weight of citric acid or acetic acid and 1-5 % by weight of sodium carbonate or sodium hydroxide at 40-60°C for 0.5-2 hours, whereafter the fibre is washed 1-3 times in hot water at 50-60°C.

16. Method according to claim 14, wherein the decolourisation is carried out by immersing the fibre in a solution containing 0.3-1.5 % by weight of sodium perborate, 0.1-0.5 % by weight of sodium tripolyphosphate and 0.05-0.25 % by weight of an anionic surfactant, preferably sodium alkyl ether sulphate, 3EO for 20-60 minutes at 40-50°C, whereafter the fibre is washed 1-3 times in hot water at 50-60°C.