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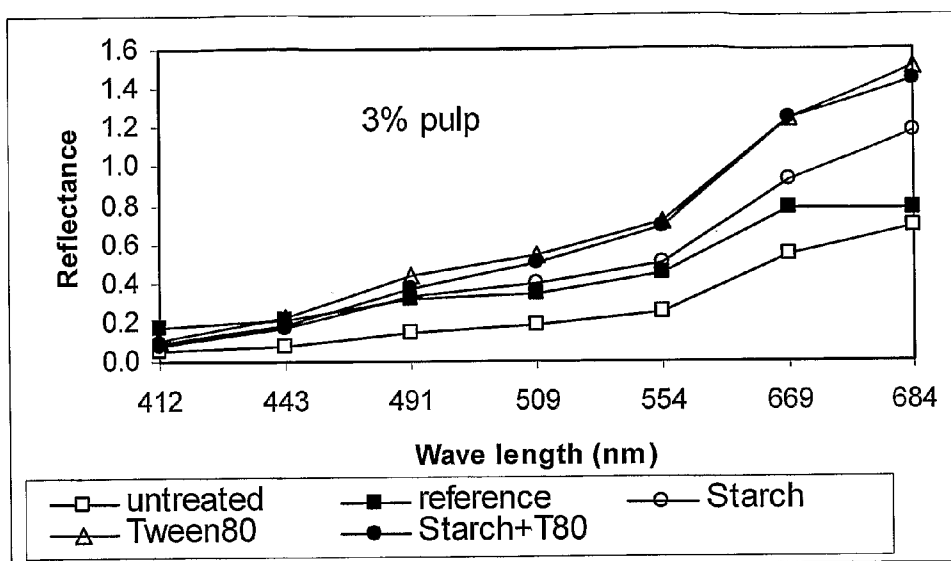
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(54) Title: A PROCESS FOR ENZYMATIC DEINKING OF PRINTED PAPERS



(57) Abstract: The present invention provides a process for enzymatic deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink using the cell free culture supernatant of *Vibrio alginolyticus* or immobilized cells of the same bacterium having the accession number NIO/DI/32 and deposited at ARS Patent Culture Collection, USA, under the Number NRRL-B-30638. Further, this invention relates to decolorization of office waste paper pulp within 72 h after contacting the said enzymes from the said bacterium in the pulp of 3-12% consistency in seawater. Decolorization is achieved by using extracellular enzymes produced by the bacterium NIO/DI/32 grown in nutrient broth supplemented with starch or Tween 80.

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A PROCESS FOR ENZYMATIC DEINKING OF PRINTED PAPERS

Field of invention:

The present invention relates to process for enzymatic deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink.

More particularly, it relates to a process for enzymatic deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink using the cell free culture supernatant of *Vibrio alginolyticus* or immobilized cells of the same bacterium having the accession number NIO/DI/32 and deposited at ARS Patent Culture Collection, USA, under the Number NRRL-B-30638.

Background of the invention:

Paper manufacturing is a major industry and a continuously growing one. Increased production of paper imposes a severe demand on plant raw material and thus harms the environment. Recycling of used paper is an alternative that can alleviate the stress that is exercised on the environment. The three major sources of raw material for such recycling are newsprint, photocopied paper and inkjet - printed papers. Recycling of paper requires the removal of the printing ink from the used paper, called deinking, so that the processed material is brighter. Printing on paper is accomplished by using two types of inks, the impact and the non-impact inks. In impact inks, used for newsprints, the ink does not fuse with the paper and is, therefore, easy to remove or disperse during the deinking, or recycling process. Such recycling is now well known and has been carried out for years. On the contrary, non-impact inks used in photocopying, ink-jet printing and laser printing results in the ink fusing with the paper and makes it non-dispersible, thus rendering the deinking process much more difficult (Jeffries, 1996, website "Bioenergy"). The toners used for photocopying generally contain iron oxide (about 55 %), olefins (about 5%) and plastic or polymer (Website:<http://www.eng-tips.com>).

Various hydrolytic enzymes such as cellulase, lipase, amylase of bacterial or fungal origin, individually or in combination have been used for deinking of office waste paper.

(i) A reference may be made to a publication wherein, a method for deinking of xerographic and printed paper, collectively called office waste, includes applying deinking solution to the paper to be deinked, the deinking solution being composed of a cleaning solution and a surfactant, abrading the paper to remove the ink from paper, and washing the paper to remove the deinking

solution from the paper (US6022423 dtd 2000-02-08). However, this patent involves using a special device for deinking of whole sheets and cannot be used on pulp.

(ii) A reference may be made to a publication wherein, a mono component cellulase is used for removal of ink and toners from printed paper (US5525193). The technique of isolating mono component cellulase from microorganisms by various purifications, chromatographic steps which is time consuming and expensive.

(iii) A reference may be made to a publication wherein, endogluconases and hemicellulases from several fungi are used effectively for deinking of xerox and laser-printed paper (Gubitz et al.,1998). However, the pulp needs to be treated with surfactants and washed to remove the floating ink particles.

(iv) A reference may be made to a publication, wherein deinking of laser printed or xerographic paper is done using enzyme containing a resistant binder. Enzyme is added in the specified form to improve speck removal (Patent No. GB 2304741 dated 26.03.97). As with the above references, the technology to remove the ink by floatation remains a problem.

(v) A reference may be made to a publication, wherein deinking of office waste paper is carried out by contacting the pulp with a deinking agent containing enzymes cellulase, preferably active at pH 4 to 8. The treated paper is used in the production of newsprint and high quality paper (Patent No. EP 717144 dated 19.06.96). As with the above references, the technology to remove the ink by floatation remains a problem.

(vi) A reference may be made to a publication, wherein deinking of waste paper is achieved by incubating the paper pulp with cellulase at 40°C for 1 hour under pressure. Following dissociation of ink particles, the resulting washed pulp is brighter (Patent No. JP 06346390 dated 20.12.94). The technology for incubation under pressure and high temperature is an expensive process.

(vii) A reference may be made to a publication, wherein deinking is achieved by using lipase to remove ink from used paper. The procedure is carried out under neutral or alkaline conditions which produces regenerated pulp having enhanced brightness (Patent No. JP 2160984 dated 20.06.90). The ink particles are removed by washing and floatation.

(viii) A reference may be made to a publication, wherein deinking of waste paper is done by using the yeast *Hansenula polymorpha*, which degrades the ink-based resin (Patent No. KR

9303705 dated 08.05.93). However, the ink floats on the surface and the pulp has to be extensively washed to remove the detached ink.

(ix) A reference may be made to a publication, wherein decolorization of Indian ink is achieved by using thermostable alkaline cellulase from a *Bacillus* sp (Patent number JP10313859). However, this process is very specific to Indian ink.

(x) A reference may be made to a patent filed, wherein decolorization of office waste is achieved by inoculating the paper pulp with the bacterium *Vibrio alginolyticum* (US Patent application Number 20030178162). However, handling the live bacterium is a big disadvantage in this process and may not be acceptable to many.

Objects of the invention:

The main object of the present invention is to provide a process for enzymatic deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink.

Another object of the present invention is to provide a process for enzymatic deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink using the cell free culture supernatant of *Vibrio alginolyticus* or immobilized cells of the same bacterium having the accession number NIO/DI/32 and deposited at ARS Patent Culture Collection, USA, under the Number NRRL-B-30638.

Summary of the invention:

The present invention relates to a process for enzymatic deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink using the cell free culture supernatant of *Vibrio alginolyticus* or immobilized cells of the same bacterium having the accession number NIO/DI/32 and deposited at ARS Patent Culture Collection, USA, under the Number NRRL-B-30638. Further, this invention also relates to decolorization of office waste paper pulp within 72 h after contacting the said enzymes from the said bacterium in the pulp of 3-12% consistency in seawater. Decolorization is achieved by using extracellular enzymes produced by the bacterium NIO/DI/32 grown in nutrient broth supplemented with starch or Tween 80.

Detailed description of the invention:

Deinking of paper is a limiting factor in recycling of paper. Newsprint and office waste paper is generally deinked using chemicals which go into the effluent and cause pollution of the water

bodies. The newsprint or offset printing is done using dispersible or non-impact ink whereas laser, xerographic and ink-jet printer ink does not disperse and is also called impact ink. There are several patents describing various methods of deinking by using hydrolytic enzymes such as cellulase, hemicellulase and lipase of microbial origin which release toner particles from fibers. However, enzyme action is affected by the paper constituents in the deinking condition. The chemically pulped fibers are more susceptible than mechanically pulped fibers. Office waste paper is high in laser and toner content and the technology for taking the toner particles out is not very good at the moment (Jefferies, 2002). We report here a process for deinking of office waste paper comprising xerographic and ink-jet printed paper by cell free culture filtrate of a bacterium isolated from marine sediment of an estuary in Goa, India. The salinity of the water at the time of isolation was around 15 parts per thousand which is roughly equivalent to half-strength sea water.

The organism given in the present invention is *Vibrio alginolyticus*, a gram-negative, coccoid bacterium isolated from coastal marine sediment and given the accession number NIO/DI/32 and is deposited in ARS patent culture collection center having accession number NRRL--B-30638. The said bacterium is grown in nutrient broth containing beef extract, peptic digest of animal tissue, prepared with sea water with salinity diluted to 50% and having a pH of at least 7.5 and supplemented with 1% starch or 1% Tween-80 solution or both. The bacterium when grown in nutrient agar medium appears as circular colonies off-white in color, having 2 mm diameter in the beginning and growing to a size of 5 mm within 3-4 days. The bacterium produces esterase/lipase and amylase in the presence of Tween-80 and starch respectively. It is fermentative and catalase-negative bacterium. The bacterium is grown at room temperature (30° C) for about 4 days, the culture supernatant is collected by centrifugation under sterile conditions. Xerographic, ink-jet printed paper and other papers printed with impact ink are pulped by soaking in hot water for a minimum of 2 hrs, macerated in a domestic mixer with surfactant such as Tween-80. An example for the process for deinking involves suspending such a pulp at least at 3-12% consistency in seawater with salinity diluted to 50%, incubating with cell-free culture supernatant of the said bacterium. The treated pulp is incubated at room temperature or 37°C for a minimum of 2-3 days to get completely decolorized pulp without traces of ink in the supernatant. The pulp is diluted, aerated from bottom to remove free contaminating particles and salts. Soap solution is added at 1% concentration for flotation of any

residual ink particles. The pulp is filtered over a Buchner funnel under suction, pressed with flat stainless steel plates to make the pulp sheet uniform in thickness. The radiance of the resultant sheet is read from 412 to 684 nm and the radiance expressed in Lux units (Lu). One LU is equivalent to $\mu\text{W}/\text{cm}^2/\text{nm}/\text{Sr}$. The ratio of radiance between enzymatically deinked and recycled paper purchased from the market (hereafter called the reference paper) is taken as a measure of brightness.

Most of the methods used for enzymatic deinking release ink particles from the fibers and they need to be subsequently washed off from the pulp. In the process described in the present invention, this problem does not arise as the ink and toner particles are totally decolorized. The method is very cost effective as the only step involved is raising the bacterial inoculum in any conventional nutrient broth containing assimilable carbon and nitrogen source and supplemented with starch or Tween-80. A comparative statement given in Table 1 explains the novelty of our invention in comparison with prior art known in this field.

Table 1. Comparative statement

Reference (Prior art)	Type of paper or ink	Source of deinking agent	Remarks
Patent US6022423	Impact ink and office waste	Chemical & a surfactant, abrading the paper and washing the deinked paper using a special device to put a whole sheet	Not practical on a large scale
US5525193	Toner, impact ink from mixed office waste	Monocomponent cellulase purified from cellulolytic enzymes of various microorganisms or genetically engineered organism which produces only monocomponent cellulase	The ink particles get freed from the pulp, but they need to be removed by mechanical methods such as floatation and washing.
Gubitz et al. 1998	Xerox and laser-printed	Endogluconases and hemicellulases from fungi	The ink particles get freed from the pulp, but they need

	paper		to be removed by mechanical methods such as floatation and washing.
GB2304741	Laser-printed or xerographic paper	Enzyme containing a resistant binder	The ink floats on the surface and the pulp has to be extensively washed
Patent EP 717144	Office waste paper	Cellulase	The ink floats on the surface and the pulp has to be extensively washed
JP06346390	Office waste paper	Contacting with cellulase at 40°C for 1h under pressure	Enzyme high temperature and pressure will make the cost of technology not very practical.
JP2160984	Office waste paper	Contacting pulp with lipase enzyme under neutral or alkaline conditions	The ink floats on the surface and the pulp has to be extensively washed.
KR9303705	Office waste paper	Contacting pulp with the culture of the yeast <i>Hansenula polymorpha</i> which degrades the ink-based resin.	The ink floats on the surface and the pulp has to be extensively washed
JP10313859	For decolorization of Indian ink	Thermostable alkaline cellulase from a bacterium	Recommended for surfactant composition, as a fiber treatment

			agent and as an Indian ink decolorization agent.
US Patent Application No. 20030178162	For deinking of office waste	Live bacterial culture of <i>Vibrio alginolyticus</i>	Recommended for deinking of Xerox and ink jet-printed paper pulp. Using live bacterium may not be acceptable to many.
Present invention	For Xerox and inkjet-printed paper pulp	Direct contact of the pulp slurry with the cell free culture supernatant of the bacterium NIO/DI/32 for 48-72h at room temperature to get complete decolorization of the pulp and clear waste water.	No enzyme, purification, no temperature or pH adjustment, the pulp and the water are clear and washing is only optional.

Accordingly the present invention provides a process for enzymatic deinking of printed papers inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink comprising:

- growing the bacterial isolate *Vibrio alginolyticus* having accession number NIO/DI/32, the culture being deposited at ARS Patent Culture Collection under NRRL Number NRRL-B-30638, in a novel medium at a temperature ranging from 27⁰C to 37⁰C for 3-4 days;
- removing the bacterial cells by centrifugation under sterile condition to obtain cell free culture supernatant;
- incubating the xerox or inkjet-printed paper pulp diluted to 3-12 % consistency in sea water with salinity diluted to 50% with the resulting cell free culture supernatant obtained from step (b) at 20% concentration for at least 72 hours at room temp followed by

diluting the pulp ten fold in water to obtain completely decolorized pulp without traces of ink in the supernatant;

- d) filtering the resulting pulp obtained from step (c) under suction to get the desired deinked paper.

In an embodiment of the present invention, the medium is prepared by dissolving nutrient broth contains beef extract, peptic digest of animal tissue in sea water with salinity diluted to 50% and supplemented with tween 60 or 1% starch or both.

In another embodiment of the present invention, the bacterium is grown for a minimum of 4 days in nutrient broth containing beef extract, peptic digest of animal tissue in sea water with salinity diluted to 50% and supplemented with tween 60 or 1% starch or both.

In yet another embodiment of the present invention, the sodium chloride at a minimum concentration of 1.5% can be used in the place of sea water for growing the bacterium.

In yet another embodiment of the present invention, cell free supernatant contains the extracellular enzymes such as esterase, lipase and amylase etc, used for deinking of papers.

In still another embodiment of the present invention, the xerographic or inkjet-printed paper is soaked in hot water for 1-2 hours after addition of 1% surfactant followed by macerating it to pulp using any domestic mixer.

In still another embodiment of the present invention, the pulp sheet is pressed to make it uniform and dried at about 60° C for a minimum of 4-5 hours.

In still another embodiment of the present invention, the radiance of the resultant sheet is read from 412 to 684 nm and the radiance expressed as Lux units (LU). One LU is equivalent to $\mu\text{W}/\text{cm}^2/\text{nm}/\text{Sr}$.

In still another embodiment of the present invention, the pulp sheet made out of recycled paper available in the market is used as a reference.

In still another embodiment of the present invention, the same batch of cell free culture supernatant can be effectively used for two-three cycles of deinking of xerox paper/inkjet-printed paper pulp.

In still another embodiment of the present invention, the lyophilized culture supernatant can also be effectively used for deinking of the said pulp.

In still another embodiment of the present invention, bacteria immobilized in sodium alginate beads can also be used for deinking the xerox paper/inkjet printed paper pulp to get completely bleached pulp within 48-72 hours.

The following examples are given by way of illustration of the present invention and should not be construed to limit the scope of present invention.

EXAMPLE 1

The bacterial isolate *Vibrio alginolyticus* NIO/DI/32 was isolated from the coastal estuarine sediment, Dona Paula, Goa, India in nutrient agar medium containing beef extract and peptic digest of animal tissue and agar in sea water with its salinity diluted to 50%. The culture was maintained in the slants of nutrient agar medium for all further experiments.

The said bacterium is grown in nutrient broth containing beef extract, peptic digest of animal tissue in sea water with its salinity diluted to 50%. Besides, the medium is amended either with starch or Tween-80 or a both together used at 1% concentration. The bacterium is grown at room temperature (30° C) for about 4 days, the bacterial cells are removed by centrifugation and the cell-free culture supernatant is used for deinking purposes. Xerographic paper printed with impact ink from HP or Epson or any other printer are pulped by soaking in hot water for a minimum of 2 hrs, macerated in a conventional domestic mixer with surfactant such as Tween 80. An example for the process for deinking involves suspending such a pulp at 3, 6 and 9% consistency (g wet pulp in 100 ml of water) incubating with 50 ml of cell free culture supernatant prepared as described above and incubating at room temperature for a minimum of 3 days to get completely bleached pulp with the ink totally disappearing from the supernatant water. The pulp is diluted, aerated from bottom to remove free contaminating particles. Soap solution is added at 1% concentration for flotation of any residual ink particles. The pulp is filtered over a Buchner funnel under suction, pressed with flat stainless steel plates to make the pulp sheet uniform in thickness. The radiance of the resultant sheet is read from 412 to 684 nm and the radiance expressed in Lux units (Lu). One LU is equivalent to $\mu\text{W}/\text{cm}^2/\text{nm}/\text{Sr}$. The ratio of reflectance between recycled paper available in any stationery shop (reference paper) and biologically deinked paper is taken as a measure of brightness. The reflectance of untreated pulp is the negative control.

Accordingly, Figure 1A-1C show the reflectance of ink jet-printed paper pulp (untreated) is lower than that of reference paper pulp (recycled paper purchased from market). The printed

paper pulp treated with culture supernatant containing starch, Tween –80 or both showed higher radiance than the untreated pulp. This brightness was achieved 3 days after incubation with the culture supernatant. Accordingly Fig. 1A, 1B and 1C show that treatment with culture filtrate containing the fatty acid ester Tween 80 yielded highest brightness. Treatment with culture filtrates of starch or Tween 80-grown cultures or both together increased the brightness much more than the reference paper pulp (the target brightness). Ratio of radiance of untreated printed paper pulp to recycled paper and ratio of radiance of enzymatically deinked paper to that of recycled reference paper pulp (Table 1) showed that, pulp at 6% concentration treated with culture supernatant containing Tween 80 showed the maximum brightness.

Table 1: Ratio of reflectance achieved using various treatments.

Treatment*	Reflectance ratio (Lux Units)***	% increase in reflectance
3 % Pulp		
Untreated pulp (negative control)	0.54	
Pulp treated with CF containing Starch**	1.04	48
Pulp treated with CF containing Tween 80**	1.39	61
Pulp treated with CF containing Starch+Tween 80	1.29	58
6 % Pulp		
Untreated pulp (negative control)	0.57	
Pulp treated with CF containing Starch	1.03	45
Pulp treated with CF containing Tween 80	1.24	64
9 % Pulp		
Untreated pulp (negative control)	0.68	
Pulp treated with CF containing Starch	0.88	23
Pulp treated with CF containing	1.05	35

Tween 80		
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*Culture filtrate (CF) of the bacterium NIO/D!/32 grown in nutrient medium for 4 days was added to paper pulp of various concentrations (W/V).

**Starch or Tween 80 were added to the medium at 1% concentration.

*** Reflectance ratio was calculated by dividing average reflectance of untreated or treated pulp at different wave lengths by average reflectance of reference (target) paper pulp.

EXAMPLE 2

The aim of the present experiment was to compare deinking of pulp using the bacterial culture supernatants containing starch and Tween 80 at room temperature and at 37°C.

The printed pulp at 3% consistency was incubated with bacterial culture supernatant containing starch or Tween 80 for 72 hours at 37°C. At the end of the incubation period, the slurry was filtered, washed and dried as described in the Example 1. The reflectance was measured as detailed above.

Accordingly, Figure 2A and 2B show that no significant improvement in brightness occurred at 37°C. Table 2 shows the comparative average reflectance ratios in various treatments. The results indicate that effective deinking can be achieved by treating at room temperature and thereby reducing energy utilization.

Table 2. Ratio of reflectance achieved using various treatments

Treatment* (3 % pulp)	Reflectance ratio (Lux Units)***	% increase in reflectance
Untreated pulp (negative control) at RT	0.54	
Pulp treated with CF containing Starch** incubated at RT	1.04	48
Pulp treated with CF containing Tween 80** incubated at RT	1.39	61
Pulp treated with CF containing Starch+Tween 80 incubated at RT	1.29	58
Untreated (negative control) incubated at 37°C	0.54	

Pulp treated with CF containing Starch** incubated at 37°C	1.19	54
Pulp treated with CF containing Tween 80** incubated at 37°C	1.14	53
Pulp treated with CF containing Starch+Tween 80 Incubated at 37°C	1.09	51

*Culture filtrate (CF) of the bacterium NIO/DI/32 grown in nutrient medium for 4 days was added to paper pulp of various concentrations (W/V).

**Starch or Tween 80 were added to the medium at 1% concentration.

*** Reflectance ratio was calculated by dividing average reflectance of untreated or treated pulp at different wave lengths by average reflectance of reference (target) paper pulp.

EXAMPLE 3

In order to avoid direct contact with bacterial inoculum, the ability of the immobilized bacterial isolate NIO/DI/32 to deink ink-jet printed paper was carried out as follows:

Bacterial cells were immobilized in sodium alginate. Briefly, this involved mixing 8 ml of cells (containing 10^8 cell ml⁻¹) with 25 ml of 2% alginic acid (Sigma Chemicals, USA). The beads were prepared in 0.2 M calcium chloride solution and were cured for overnight. The same batch of immobilized bacterial cells were used in two cycles to achieve deinking. The other procedures used were the same as described in the Example 1.

Accordingly, Figure 3 shows radiance of decolorized paper pulp after 72 hours incubation with two cycles of immobilized cells. The average reflectance ratios in these two cycles of immobilized cells are shown in Table 3. It can be concluded that immobilized cells were as effective as the free bacterial cells in deinking. The repeated use of immobilized cells is an added advantage.

Table 3. Ratio of reflectance achieved using various treatments.

Treatment* (3 % pulp)	Reflectance ratio (Lux Units)***	% increase in reflectance
Untreated (negative control) at RT	0.54	

Free cells*	1.13	52
Immobilized cells, cycle I**	1.24	56
Immobilized cells, cycle II	1.09	50

*Immobilized cells/ free cells of the bacterium NIO/D!/32 grown in nutrient medium for 4 days were added to paper pulp of various concentrations (W/V).

**Same batch of Immobilized cells were used in two cycles.

*** Reflectance ratio was calculated by dividing average reflectance of untreated or treated pulp at different wave lengths by average reflectance of reference (target) paper pulp.

EXAMPLE 4

The deinking of inkjet-printed paper was carried out using heat-killed bacterial cells and heat-inactivated culture supernatants (containing starch or Tween 80). For this purpose the bacterial cells and the culture supernatants were autoclaved at 121°C for 15 minutes at 15 lb.

The preparation of pulp, inoculum and experimental set up and incubation were same as in the previous examples.

Accordingly, Figure 4 shows that by day 3 no deinking could be achieved by using killed bacterial cells and heat-inactivated culture supernatants indicating that the active principle responsible for deinking is heat sensitive.

EXAMPLE 5

The effect of storage of culture supernatant is one of the important criteria for its effective use. Therefore, the crude culture supernatant stored for 2 days at room temperature was used for deinking of the pulp. For comparison, the fresh culture filtrate was used for deinking the pulp. The procedure for deinking was the same as described in examples 1-4.

Accordingly, Figure 5A and 5B show that the stored supernatants lost its efficiency as compared to the fresh culture filtrate in deinking, indicating that the active principle involved in deinking is unstable when stored at room temperature.

We also compared the efficiency of the freeze-dried culture filtrate on deinking. Accordingly, Table 4 shows that the freeze-dried culture filtrate from medium containing starch or Tween 80 could effectively deink the pulp.

Table 4. Reflectance ratio of the pulp treated with freeze-dried culture supernatant.

Treatment* (3 % pulp)	Reflectance ratio (Lux Units)***	% increase in reflectance
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Untreated (negative control) at RT	0.54	
Pulp treated with freeze-dried CF containing starch	1.25	57
Pulp treated with freeze-dried CF containing Tween 80	1.25	57

The freeze-dried culture filtrate (CF) was thawed and diluted to the original volume with 50% diluted sea water and incubated with pulp. The negative control was incubated with 50% diluted sea water.

EXAMPLE 6

The active principles involved in deinking of the pulp using culture supernatant of media containing starch or Tween 80 were investigated. Accordingly, amylase and lipase activities were measured in these supernatants. Amylase activity was measured using 1% starch, iodine and potassium iodide (Medda and Chandra 1980). Lipase activity was measured using model substrate 4-methylumbelliferyl-butyrates (Sigma Chemicals, USA). The fluorescent MUF product released is measured at excitation and emission wave length of 364 and 445 nm respectively (Hoppe H-G. 1993). The use of Tween 80 in the medium will induce esterase as well as lipase activity (Gupta et al. 2003).

Accordingly the 5-day old culture filtrate containing starch and Tween 80 showed 49 and 33 U ml⁻¹ activity respectively. These were the minimum concentrations required for effective deinking because diluting the enzyme solutions did not result in effective deinking. Figure 6 shows the untreated control and enzyme treated pulp.

Advantages:

The main advantages of the present invention are:

1. Cell free supernatant can be reused for deinking of office waste paper inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink.
2. There is no need of enzyme purification in the present process.
3. No temperature and pH adjustment is required.

We Claim:

1. A process for enzymatic deinking of printed papers inclusive of xerographic and inkjet-printed paper printed with non impact and non dispersible ink comprising:
 - a) growing the bacterial isolate *Vibrio alginolyticus* having accession number NIO/DI/32, the culture being deposited at ARS Patent Culture Collection under NRRL Number NRRL-B-30638, in a novel medium at a temperature ranging from 27⁰C to 37⁰C for 3-4 days;
 - b) removing the bacterial cells by centrifugation under sterile condition to obtain cell free culture supernatant;
 - c) incubating the xerox or inkjet-printed paper pulp diluted to 3-12 % consistency in sea water with salinity diluted to 50% with the resulting cell free culture supernatant obtained from step (b) at 20% concentration for at least 72 hours at room temp followed by diluting the pulp ten fold in water to obtain completely decolorized pulp without traces of ink in the supernatant;
 - d) filtering the resulting pulp obtained from step (c) under suction to get the desired deinked paper.
2. A process as claimed in claim 1, wherein the strain used has the following characteristics:
 - a) it is a gram negative bacterium;
 - b) it is a coccoid bacterium isolated from the coastal marine sediments of Dona Paula, Goa.
3. A process as claimed in claim 1, wherein the said novel medium is prepared by dissolving the nutrient broth in sea water with salinity diluted to 50% and supplemented with tween 60 or 1% starch or both.
4. A process as claimed in claim 1, wherein sodium chloride at a minimum concentration of 1.5% can be used in the place of sea water for growing the said bacterium.
5. A process as claimed in claim 1, wherein the xerographic or inkjet-printed paper is soaked in hot water for 1-2 hours after addition of 1% surfactant followed by macerating the pulp.
6. A process as claimed in claim 1, wherein the pulp sheet is pressed to make it uniform followed by drying at temperature ranging from 25⁰C to 70⁰C for a period of 4-5 hours.

7. A process as claimed in claim 1, wherein the same batch of cell free culture supernatant can be effectively used for two-three cycles of deinking of xerox paper/inkjet-printed paper pulp.
8. A process as claimed in claim 1, wherein the lyophilized culture supernatant can also be effectively used for deinking of the said pulp.
9. A process as claimed in claim 1, wherein bacteria immobilized in sodium alginate beads can also be used for deinking the xerox paper/inkjet printed paper pulp to get completely bleached pulp within 48-72 hours.

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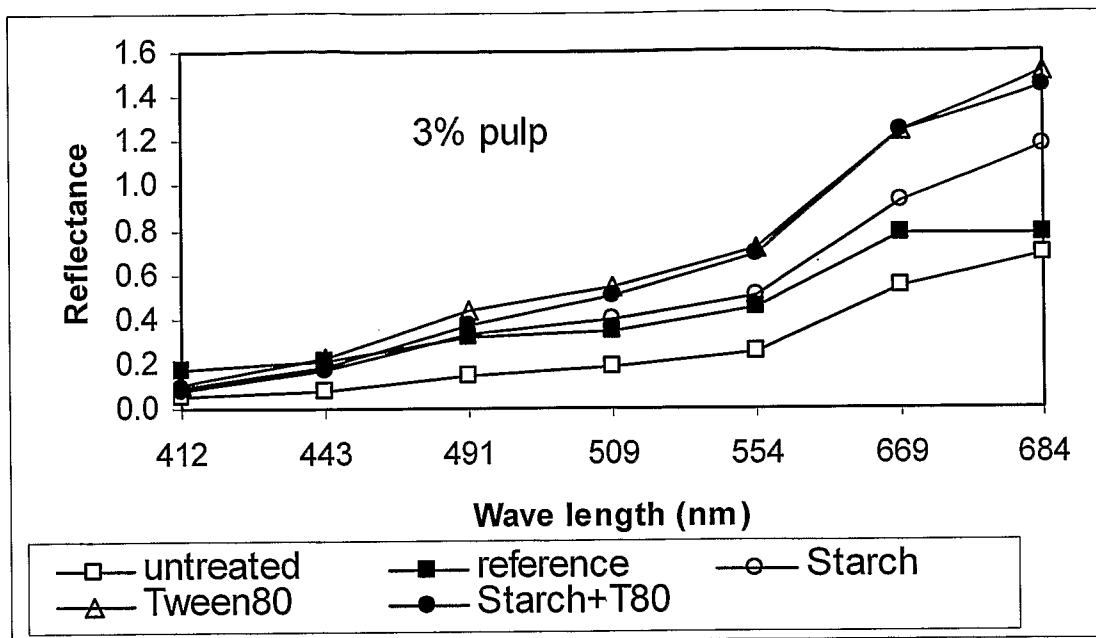


Fig. 1 A

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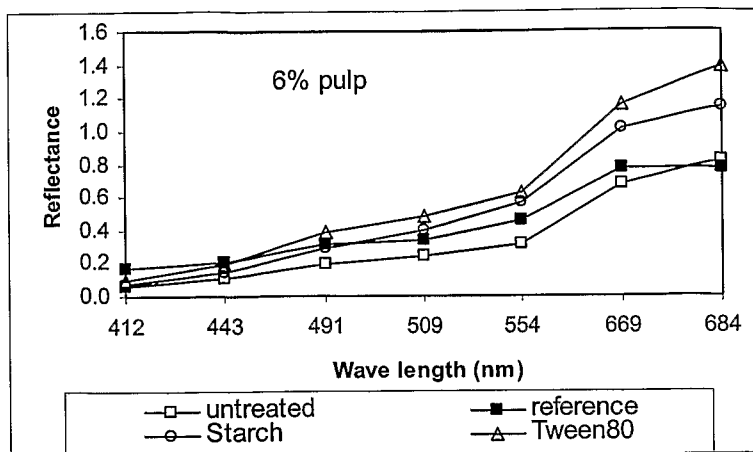


Fig-1 B

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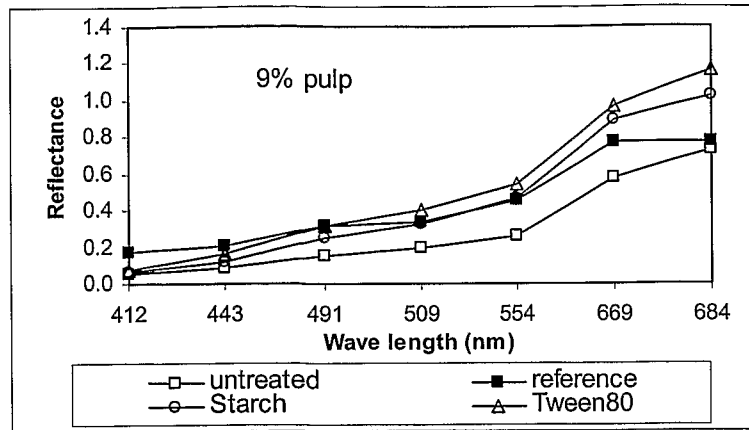


Fig 1C

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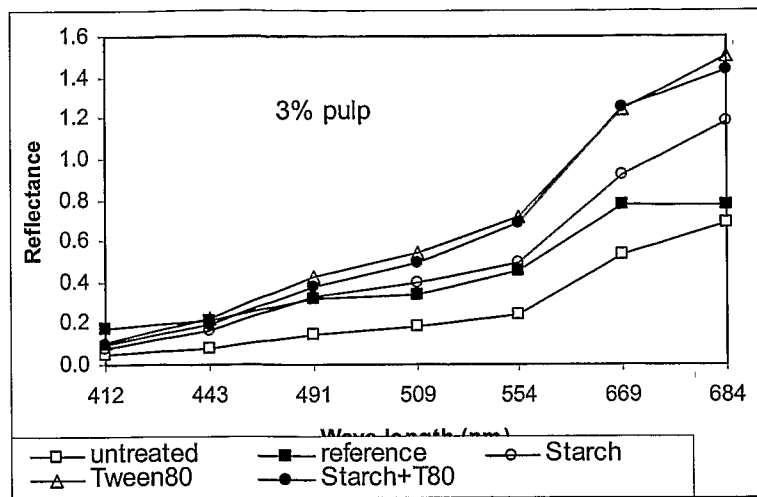


Fig 2A

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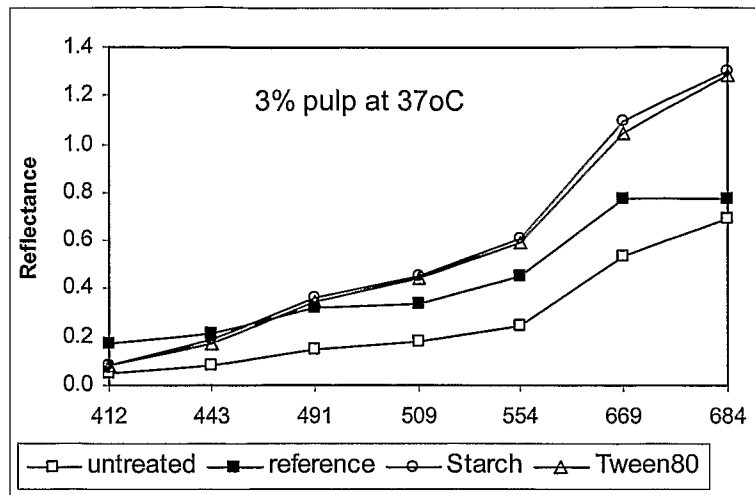


Fig 2B

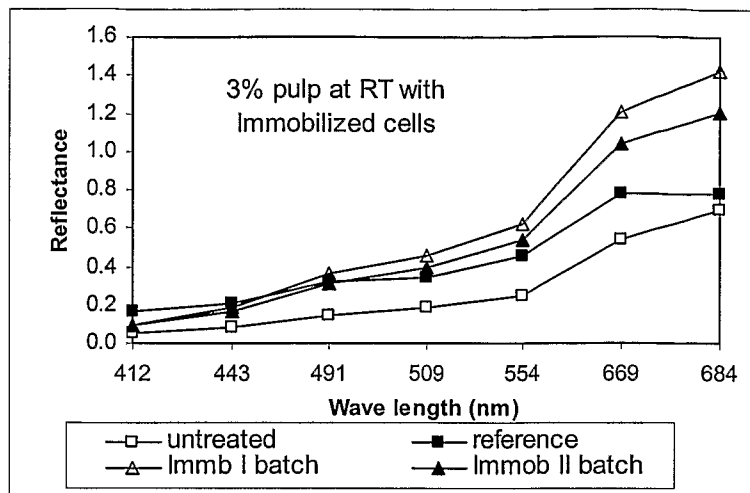


Fig 3

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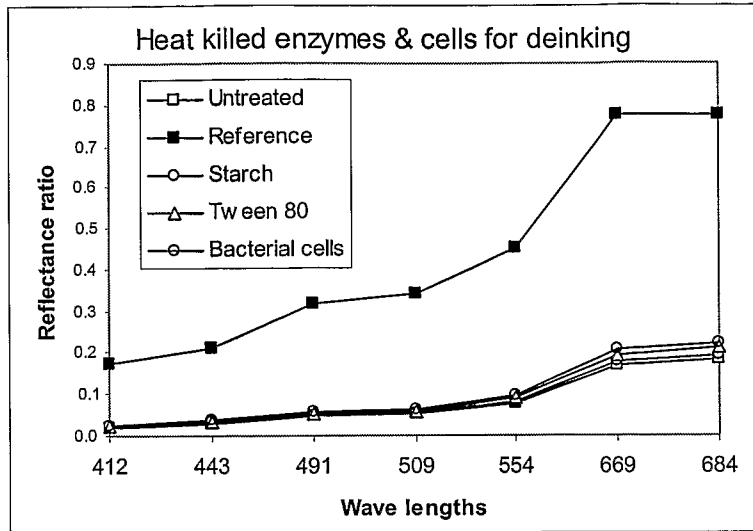


Fig 4

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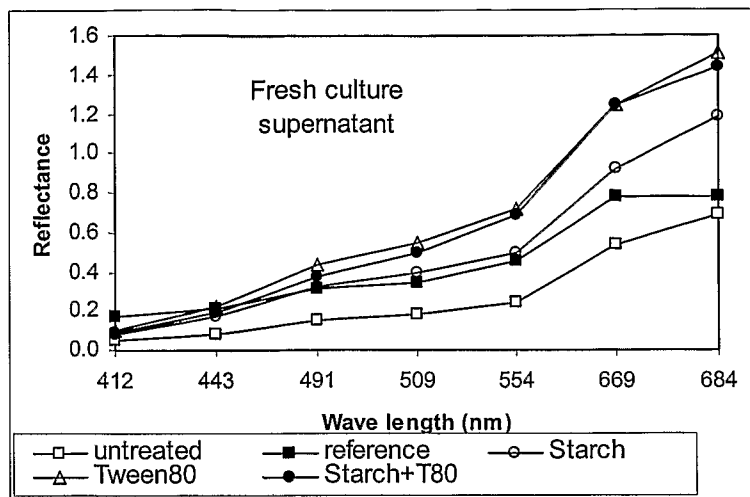


Fig 5A

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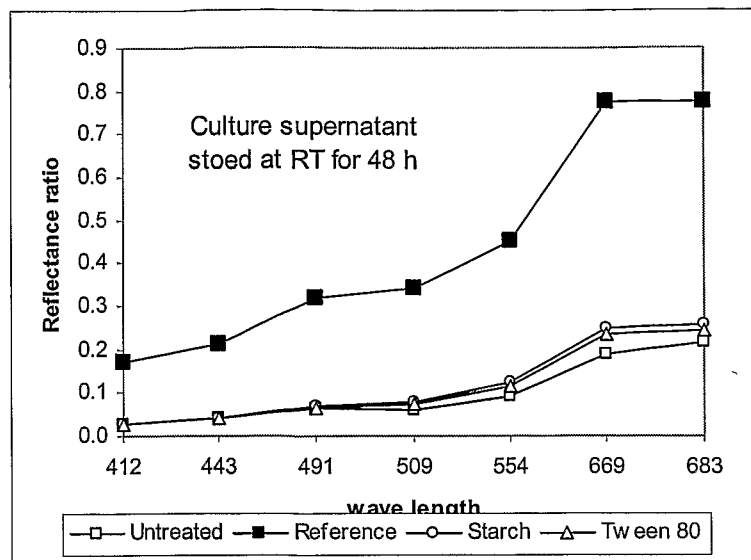


Fig 5 B

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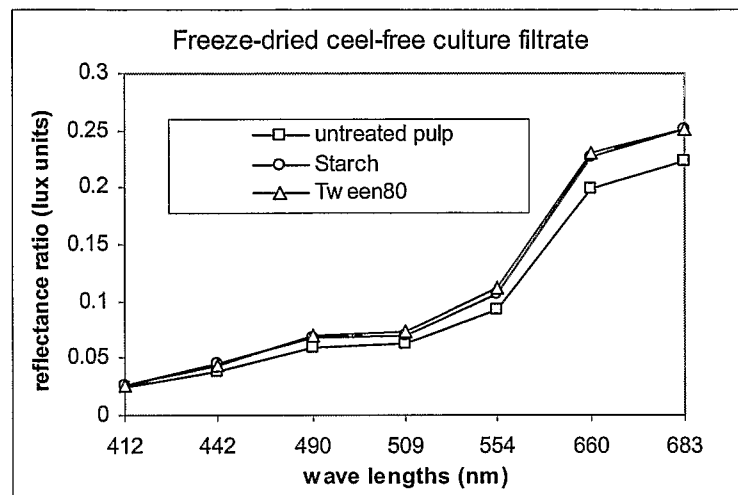


Fig 5C

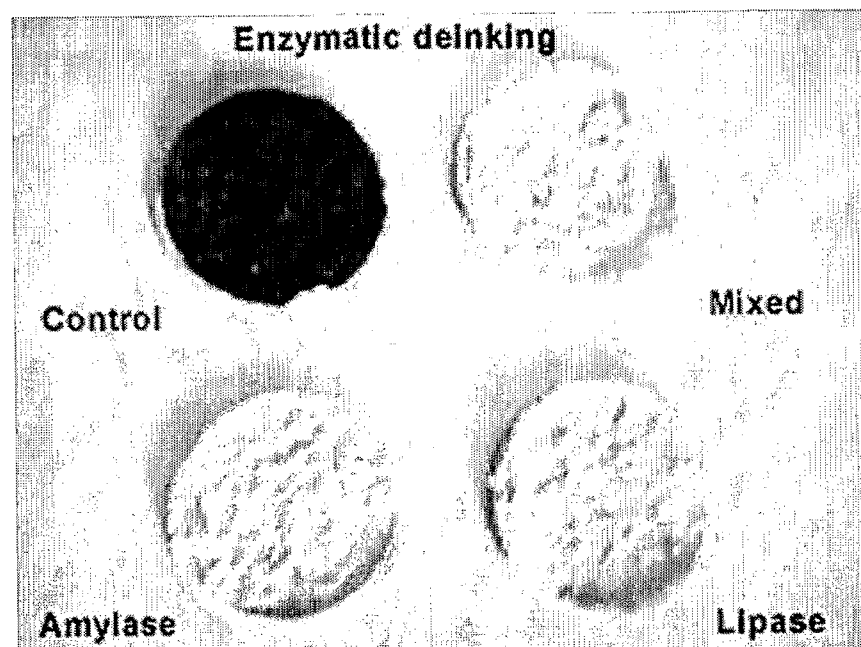


Fig 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2005/000403

A. CLASSIFICATION OF SUBJECT MATTER

INV. D21C5/02
ADD. D21C5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
D21C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/178162 A1 (RAGHUKUMAR CHANDRALATA ET AL) 25 September 2003 (2003-09-25) cited in the application page 1, paragraph 1 page 1, paragraph 13 - page 2, paragraph 14 page 2, paragraph 16 - paragraph 32 claims 1-12	1-9
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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- * & * document member of the same patent family

Date of the actual completion of the international search

18 April 2006

Date of mailing of the international search report

26/04/2006

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No

PCT/IN2005/000403

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