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MICROBIAL DEINKING OF PRINTED PAPER

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ABSTRACT

In this study we report the deinking of printed paper using microorganisms isolated from oil contaminated soil. The microorganisms producing lipase and esterase enzymes were screened using tributyrin agar. Deinking of the pulp was carried out using screened microorganisms and the preliminary identification of the microorganism that showed effective deinking was carried out by biochemical tests such as Gram straining ,TSI, MR-VP, Indole test, catalase test which indicated the presence of *Pseudomonas sp*. which was further confirmed by 16s-rRNA sequencing. The obtained sequence was analysed by bioinformatics tools which showed 98% sequence alignment with *Pseudomonas aeruginosa.* The microbially deinked pulp was subjected to SEM analysis to examine the fibre surface because an intact surface of individual fibres is desirable in paper making.

KEYWORDS: Deinking, Lipase , esterase, *Pseudomonas sp*., SEM.

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INTRODUCTION

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[1]. 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 impact and non impact ink. Newsprint paper made of impact ink is easy to remove as it does not fuse with the paper and so deinking of this paper is well established. Non impact ink used in photocopying, inkjet and laser printing results in fusing of ink with paper and therefore, non dispersible and difficult to remove[2]. The chemical deinking process is widely used for deinking of printed paper. However, the widespread technology using thermoplastic toner in printing process presents a special challenge in the conventional deinking process. Furthermore the process requires the use of large quantities of chemical agents such as sodium hydroxide, sodium carbonate, sodium silicate, hydrogen peroxide , surfactants , resulting in a costly water treatment to meet environmental regulation[3].This makes the process highly damaging to the environment and treatment of the effluent to meet environmental regulations is costly. As an alternative to conventional deinking process, enzymatic deinking of wastepaper has received increasing attention during the last few years. Various hydrolytic enzymes such as cellulases, glucanases, amylase, lipase and xylanase of bacterial or fungal origin and more recently oxidative enzyme laccase have been used, individually or in combination for deinking of office waste paper.Enzyme usage has been reported to be a potentially efficient and less polluting solution to overcome disposal problem[4]. Removal of oil-carrier based inks can be facilitated by treatment with lipases and esterases[5] as lipase hydrolyze long chain fatty acid esters whereas esterase cleave down ester bonds of short chain fatty acids[6]. The main objective of our work is to find a cost effective and eco-friendly method to recycle the printed paper using lipase and esterase producing microbes and to examine the paper making state of the deinked pulp.

MATERIALS AND METHODS

Collection of soil sample

Soil from sites rich in oil could be a potential source of lipase and esterase bacteria. Thus soil sample was collected from an automobile workshop, located near Mahindra city, Chengalpet, Tamilnadu and stored at 4° C until further use (Fig 1).

Figure 1
SOIL SAMPLE

This article can be downloaded from www.ijpbs.net

Isolation of microorganisms by serial dilution method

Labelled six test tubes 1 through 6 and with a 10 ml pipette dispensed 9 ml of saline into each tube. Weighed out 1 g of soil and deposited it into tube 1.Vortex mixed tube 1 until all soil was well dispersed throughout the tube. A tenfold dilution was made from tube 1 through tube 6 by transferring 1 ml from tube to tube. Labelled three Petri plates from 4-6 and the serial dilution was performed. From each of the last three tubes transferred 1 ml to a plate of nutrient agar. The organisms were spread over the agar surfaces on each plate with an L-shaped glass rod that has been sterilized each time in alcohol and open flame. The plates were observed for the appearance of bacterial colony[7].

Screening of lipase and esterase producing microorganisms

For screening of lipase and esterase producing bacteria, tributyrin agar was used. Bacteial colonies formed on serial diluted sample were inoculated in tributyrin agar plates and kept for incubation at 37° C for 24 hours and examined for the zone of clearance[8].

Preparation of paper pulp

Printed paper from HP printer (printed with single line spacing on both side) were pulped by soaking in hot water for 2 hours and ground using kitchen blendor. The pulp was oven dried at 50° C and stored in sterile container at 4° C until further use[1].

Deinking trials using bacterial cells

The bacterial colonies which showed zone formation in tributyrin agar were inoculated in nutrient broth overnight at room temperature. Pulp at a consistency of 3 % (3 g air dried pulp in 100 ml of distilled water) was sterilized by autoclaving. After cooling it to room temperature it was transferred to flasks containing overnight incubated bacterial culture and incubated at room temperature for 3 days. The washed and treated pulp was pressed between two stainless steel plates and oven-dried at 50˚ C for 12 hours .The paper pulp without bacterial culture treatment was used as control[8].

Characterization of bacteria showing effective deinking of printed paper

Preliminary identification of the bacteria that showed effective deinking was carried out by biochemical tests such as Gram straining ,Triple sugar iron (TSI) test, Methyl Red and Voges-Proskauer (MR-VP) Test, Indole test and catalase test [10].The species identified by preliminary biochemical tests was further confirmed by 16S rRNA sequencing[9].

Study on fibre surface of microbially deinked paper pulp

The microbially deinked pulp was subjected to High Resolution Scanning Electron Microscopic analysis to examine the fibre surface because an intact surface of individual fibres is desirable in paper making[11].

RESULTS AND DISCUSSION

Screening of lipase and esterase producing bacteria

Eleven bacterial colonies were isolated from soil sample by serial dilution (Fig 2). Among the bacterial isolates from nutrient agar, 8 colonies were found to produce lipase and esterase using tributyrin agar test.

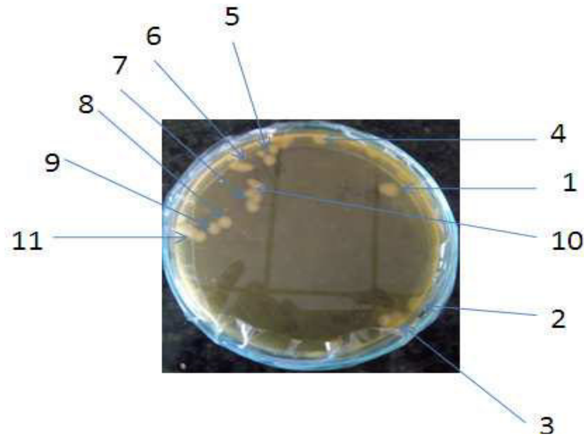


Figure 2
Serial diluted plate observed for bacterial colonies

Among the bacterial colonies from serially diluted plate it was found that 8 colonies Colony 1, 4 to 10 were showing zone of clearance in tributyrin agar indicating the presence of lipase and esterase enzymes (Fig 3) and were used further for deinking studies. Other colonies (Colony 2,3 and 11) from serially diluted plate did not show any zone of clearance in tributyrin agar indicating the absence of lipase and esterase activity.

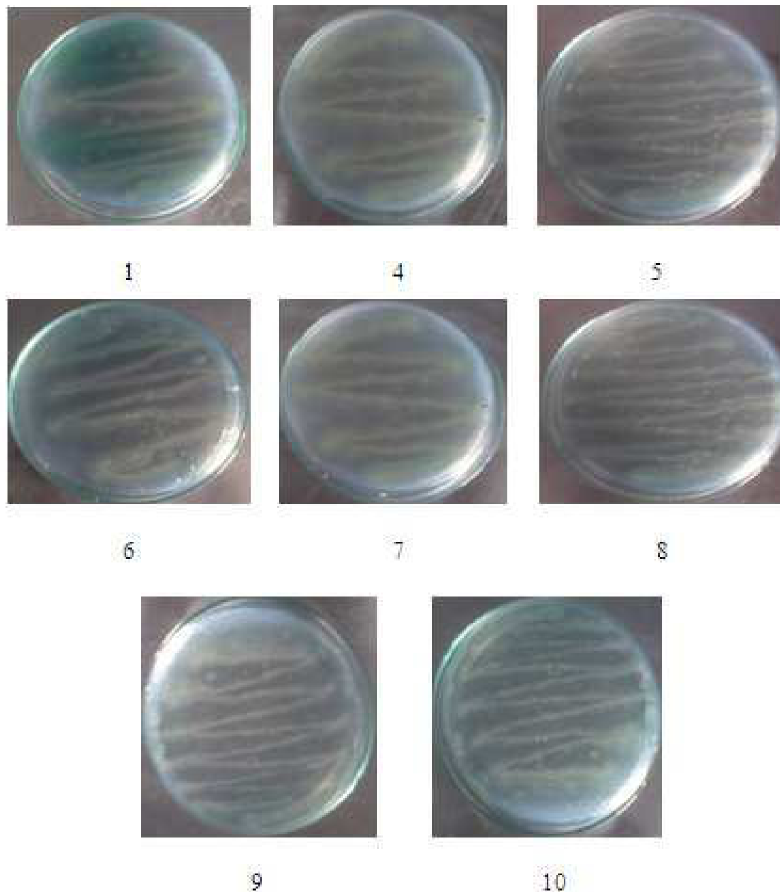


Figure 3
Colonies 1, 4 to 10 from serially diluted plate formed zone of clearance in tributyrin agar

Deinking trial using bacterial cell

The pulp inoculated with cultures of bacterial colonies number1, 4-10 were decolourized by day 3. Bacteria of colony 1 showed effective deinking than other colonies by comparing the brightness of the manually pressed pulp before and after treatment with bacterial culture (Fig 4).

Figure 4
Manually pressed pulp before and after treatment with bacterial culture

Characterization of bacteria showing effective deinking of printed paper

The biochemical test results of the bacteria of colony 1 was shown in table 1. Thus, based on the results of biochemical tests performed it was identified that the bacterial colony that shown effective deinking belongs to *Pseudomonas* species[12].

Table 1
Results of biochemical tests for identification of bacteria showing effective deinking.

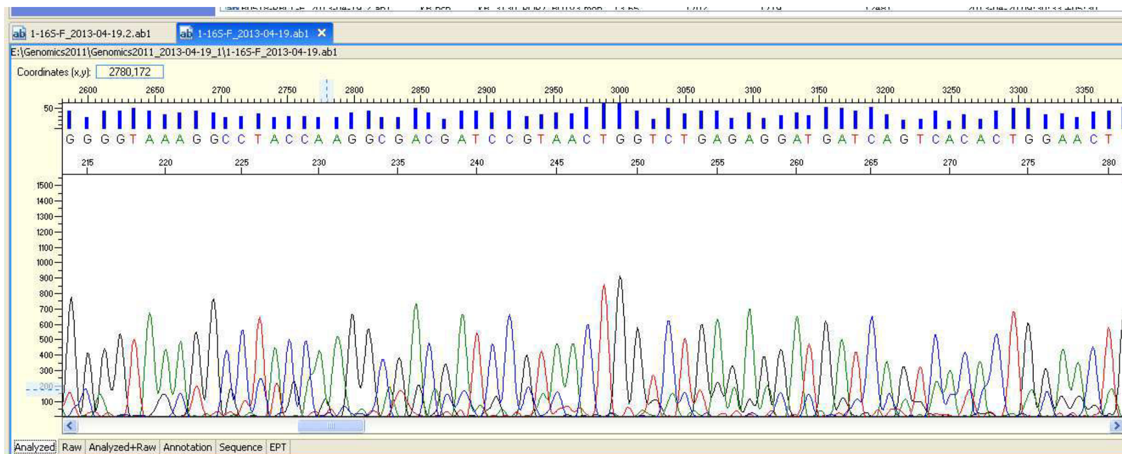
S.NO	BIOCHEMICAL TESTS	RESULT
1	Indole Test	Negative
2	Triple sugar iron Test	Negative
3	Citrate Test	Positive
4	Catalase Test	Positive
5	Gram Staining and Morphology	Gram negative, Rod shape

16S rRNA sequencing

Further the Pseudomonas sp. was genetically confirmed by 16s rRNA sequencing which resulted in 98% alignment with *Pseudomonas aeruginosa* (Fig 5).

Pseudomonas-16S-F

TGCAGTCGagCGGatgAaGGGaGCTTGcTCCTGGAtTCAGCGGCGGAcGGgTGAgTAATGCCTA
GGAATCTGCCTGGtAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTG
AGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTA
GTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGT
CACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGgGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGC
ACTTTAAGTTGGGAGGAAGGGCAGtaAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATA
AGCACCGGCTAACTTCGTGCCAgCAGCCGCGGTAATACGAAGGGTGCAAGCGTTaATCGGAA
TTACTGggCGTAAAGCGCGCGtAGGTGGtTCAGCaAGTTGgAtGTGAaATCaccGGGCTCAAcCT
GGGAaCTGCaTCCaAAACTACTGAGCTAGAtTACGGtAgAGGGTGGTGGAATTCcTGTGTAGCG
gttcAATGcgtAGATATAGGaagGAACGCcAGtGCGAAGGcGAcCTcCctggACTGttaCTGaCaCTGag
GTTGCG



Pseudomonas-16S-R

TCTGGAGCAACCCACTCCCATGgtGtGACGGGCGGtGTGTACAAGGCCCGGGAACGTATTCAC
CGTGACATTCTGATTCACGATTACTAGCGATTCCGACTTCACGCAgtCGAGTTGCAGACTGCgA
TCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGgCAACCCTTTGTACCG
ACCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCAC
CTTCCTCCGGtTTGTCACCGGCAGTCTCCTTAgAGTGCCCACCCGAGGTGCTGGTAACTAAGG
ACAAGGGTTGCGCTCGTTACGGgACTTAACCCAACATCTCACGACACGAGCTGACGACAGCC
ATGCAGCACCTGTGTCTGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCAGCATG
TCAAGGCCAGGTAAGGTTCTTCGCGTTGCTTCGAATTAaACCACATGCTCCACCGCTTGTGCG
GGCCCcCGTCAATTCATTTGAGTTTTAACCttGCGGcCGtACTCcCCAGGCGGTCGACTTATCgC
GTTAGCTGCGcCAcTAAGATCTCAAGGATCCCAACggCTAGTCgAcATCGtTTaCGGCGTGgaCT
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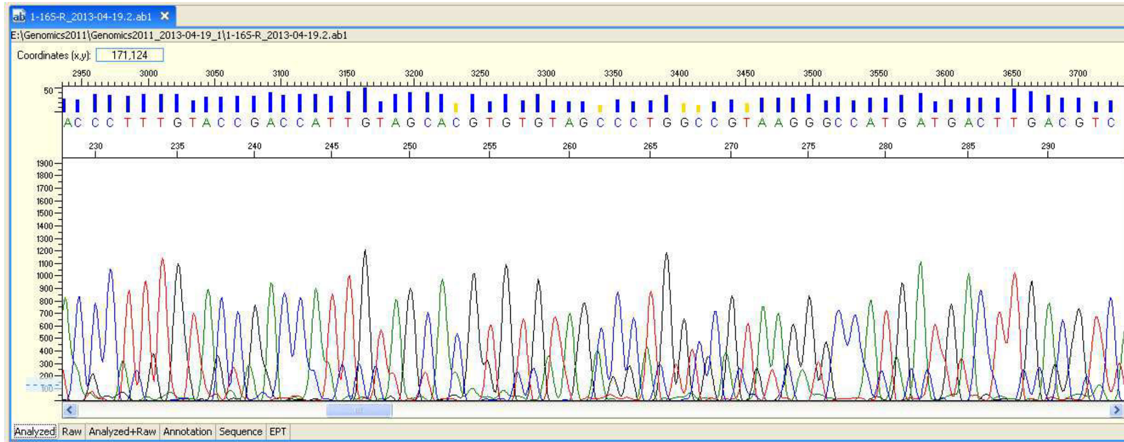
gCCtTtcgCcACT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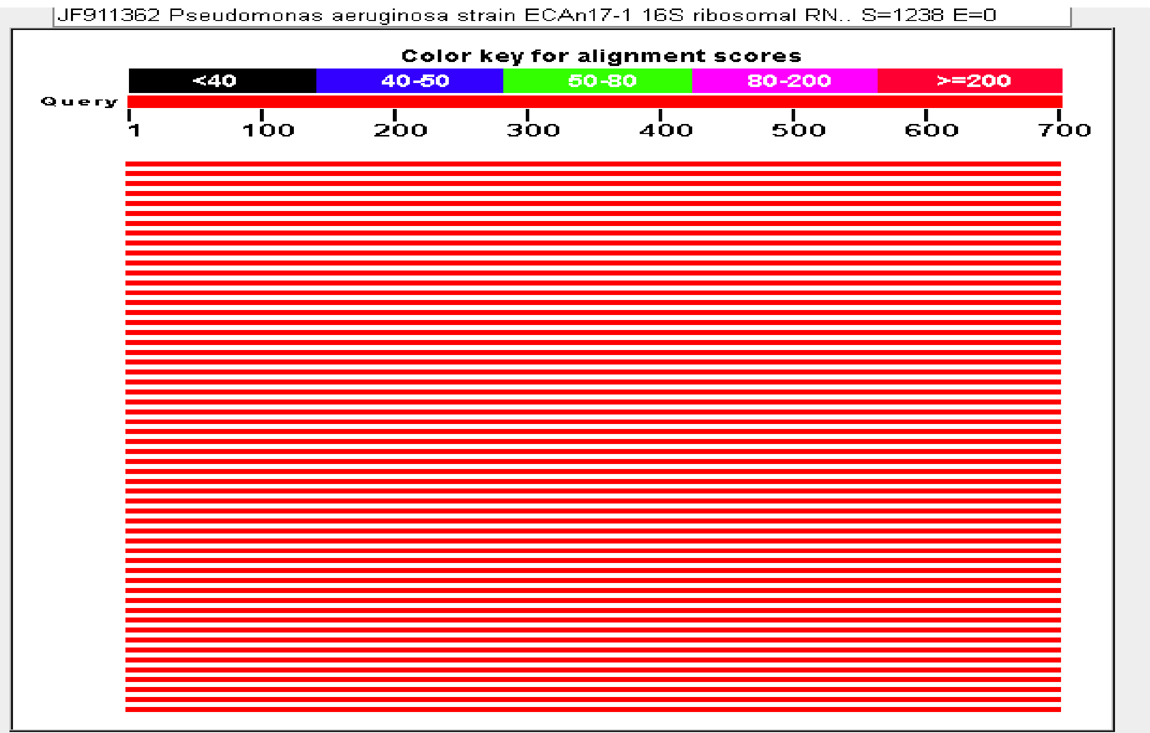
JF911362 Pseudomonas aeruginosa strain ECAn17-1 16S ribosomal RN.. S=1238 E=0
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<40
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80-200
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Query
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100
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300
400
500
600
700

Sequences producing significant alignments:

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Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
☐	Pseudomonas aeruginosa partial 16S rRNA gene, isolate F-MF18_Kn	1238	1238	100%	0.0	98%	HF675136.1
☐	Pseudomonas aeruginosa partial 16S rRNA gene, isolate F-MF10_In	1238	1238	100%	0.0	98%	HF675134.1
☐	Pseudomonas aeruginosa partial 16S rRNA gene, strain F4	1238	1238	100%	0.0	98%	HF572851.1
☐	Pseudomonas aeruginosa strain ALK319 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	KC456534.1
☐	Pseudomonas aeruginosa strain ALK318 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	KC456533.1
☐	Pseudomonas aeruginosa strain ALK317 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	KC456532.1
☐	Pseudomonas aeruginosa strain ALK316 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	KC456531.1
☐	Pseudomonas sp. JN16 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	KC121042.1
☐	Pseudomonas aeruginosa strain ED43 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX391983.1
☐	Pseudomonas aeruginosa strain 11.2 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX286673.1
☐	Pseudomonas aeruginosa strain 10.3 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX286672.1
☐	Pseudomonas aeruginosa strain 9.1 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX286671.1
☐	Pseudomonas aeruginosa strain 47.2 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX286670.1
☐	Pseudomonas aeruginosa strain T5 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX484842.1
☐	Pseudomonas sp. Mexd37 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX436404.1
☐	Pseudomonas sp. Hex35 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX436400.1
☐	Pseudomonas sp. Hex318 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX436399.1
☐	Pseudomonas aeruginosa strain SRF2.8 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX232378.1
☐	Pseudomonas sp. B6(2012) 16S ribosomal RNA gene, partial sequence	1238	1238	100%	0.0	98%	JX073565.1

Figure 5
16S rRNA Sequence of the bacteria showing 98% alignment with Pseudomonas aeruginosa.

SEM analysis

The SEM image showed intact fibre surface which indicated that the individual fibres were in their "Paper making state" because an intact , wet fibre surface is desirable for paper making (Fig 6).

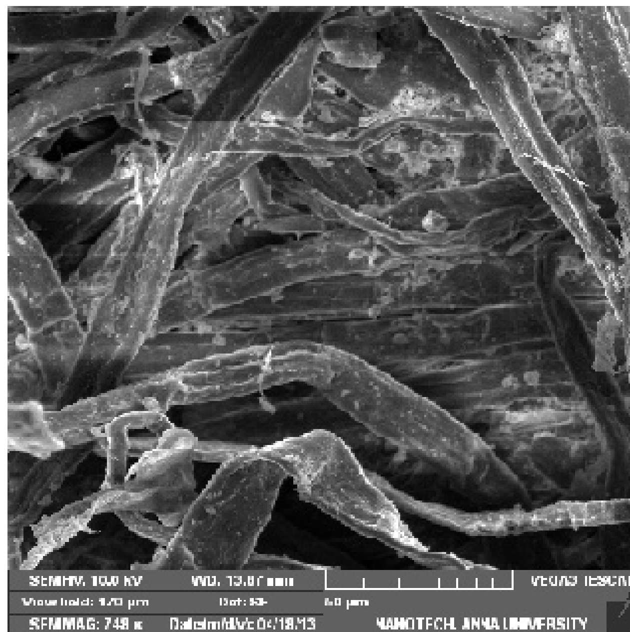

Figure 6
Analysis of fibre surface of microbially deinked pulp using HR-SEM

CONCLUSION

The deinking of paper pulp was carried out using micro organisms isolated from oil contaminated soil sample. From soil sample 11 bacterial colonies were isolated by serial dilution. 8 colonies that produced lipase and esterase enzymes were screened using tri butyrin agar. Bacterial cells of each screened colonies were used to deink the printed paper pulp and the bacterial cells of colony 1 shows effective deinking .The effectively deinked bacterial colony was screened to identify the species by using biochemical tests which showed that the bacteria was Gram Negative, catalase positive ,Indole negative, TSI Negative and citrate positive which indicated that the bacterial colony showing effective deinking belongs to *Pseudomonas* spp. Further the microorganism is genetically confirmed by 16s rRNA sequencing which resulted in 98% alignment with *Pseudomonas aeruginosa.* SEM analysis was carried out to view the fibre surface and the result showed the individual fibre surfaces of microbially deinked pulp were intact indicating that they are suitable for paper making.

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