Patent Search on Application of Cellulases in Denim Finishing

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ABSTRACT

Cellulose, the most abundant polymer in the earth crust, is a major attraction in the field of bio-innovation and cellulases are the major agent for mobilizing it as per human necessity. Cellulases have wide range of application in various industries such as textiles, bio-fuel, bioconversion, animal feed, detergent, bio-control, brewing, food etc. Thus, cellulases research has been able to gather large amount of research and development investment. Before any Research and Development investment, patent search is essential to determine novelty, patentability, technology trends and business potential. Patent search on *application of cellulases on denim finishing* performed by keyword search strategy on Free Patent Online search engine revealed 38 search results. Of them most of the patents and patent applications were documented in United States Patent and Trademark Office databases, World Intellectual Property Databases, European Patent Documents. Results show that among 38 search results 35 patents or patent applications were owned by biotech companies where as only 3 are solely by the inventors. This shows that possibility of obtaining license of a patent relevant to cellulases application on denim finishing by a biotech company from a biotech company is minimal. In the field of denim finishing by cellulases, AB Enzymes O/Y, Finland holds majority of the relevant patents. From the patenting trends in this field, most of the researches were carried out to find produce better cellulases rather than on mode of application of cellulases.

1 BACKGROUND

Bioinnovation provides more eco-friendly and sustainable solutions to human needs while safeguarding the existing natural resources. In pursuit of meeting human needs while maintaining sustainability, cellulose has been found to be the only foreseeable renewable resource in earth (Lynd, Wyman, & Gerngross, 1999). So, global leaders in bio-innovations; Novozymes, Verenium, Metabolix, Cargil etc are in a race to develop a promising strategy for commercial exploitation of cellulose (Office of Industries, U.S. International Trade Commission, 2008). For this purpose, cellulolytic enzymes are central to exploitation activities. Among various areas of application, cellulases have their wide application in multibillion dollar business of denim industry for denim finishing. Cellulases are used for the removal of excess dyes from the denim fabrics, removal of excess micro fibrils from the surface, softening and enhancing the color brightness of the denim fabrics etc. (Bhat & Bhat, 1997).

Furthermore, innovation in biotechnology is the outcome of large amount of research and development (R&D) investment. So, it needs to be commercialized in such a way that it provides sustainable solution to human needs while protecting the R&D investment, maintaining their dominating position in the research field and increasing the market share. But, before any research investment one should assure that such investment is in the novel field of research, outcome of investment can be protected and be used as intangible asset to have a dominating position in the world of commercialization. Such assurance can be obtained through patent search.

Cellulose being the most abundant polymer in earth crust and most can be utilized as major source of raw materials for various commercial commodities including denim fabrics. Cellulases are the major mobilizing agents for any raw materials that consist of cellulose. Thus, research in cellulases is a major concern for leaders in bio-innovation and patent search in cellulases is equally important too. In nutshell, following are the importance of patent search in application of cellulases in denim finishing,

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ABSTRACT

Cellulose, the most abundant polymer in the earth crust, is a major attraction in the field of bio-innovation and cellulases are the major agent for mobilizing it as per human necessity. Cellulases have wide range of application in various industries such as textiles, bio-fuel, bioconversion, animal feed, detergent, bio-control, brewing, food etc. Thus, cellulases research has been able to gather large amount of research and development investment. Before any Research and Development investment, patent search is essential to determine novelty, patentability, technology trends and business potential. Patent search on application of cellulases on denim finishing performed by keyword search strategy on Free Patent Online search engine revealed 38 search results. Of them most of the patents and patent applications were documented in United States Patent and Trademark Office databases, World Intellectual Property Databases, European Patent Documents. Results show that among 38 search results 35 patents or patent applications were owned by biotech companies where as only 3 are solely by the inventors. This shows that possibility of obtaining license of a patent relevant to cellulases application on denim finishing by a biotech company from a biotech company is minimal. In the field of denim finishing by cellulases, AB Enzymes O/Y, Finland holds majority of the relevant patents. From the patenting trends in this field, most of the researches were carried out to find produce better cellulases rather than on mode of application of cellulases.

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Cellulose being the most abundant polymer in earth crust and most can be utilized as major source of raw materials for various commercial commodities including denim fabrics. Cellulases are the major mobilizing agents for any raw materials that consist of cellulose. Thus, research in cellulases is a major concern for leaders in bio-innovation and patent search in cellulases is equally important too. In nutshell, following are the importance of patent search in application of cellulases in denim finishing,

- To determine novelty of R&D topic
- To determine the mechanism of return in R&D investment
- To identify business intelligence
- To determine patentability of invention
- To identify the trends in development of new technology (state of art search)
- To avoid infringement of existing patents
- To identify freedom to operate in the field of denim finishing
- To determine the validity of patents
- To reveal the possibility of licensing and cross licensing of the patent

The present study on patent search will focus on cellulases technologies applied for biostoning and biopolishing in denim industry.

2 THEORY

Cellulose, the most abundant polymer in the earth crust, forms the major component of plant material. It has straight unbranched chains of glucose molecules linked together by B-1-4 linkage which run parallel to each other allowing the formation of H-bonding, establishing cross linkage between the side chains and finally giving stiffness to the molecule. Thus, cellulose molecules are arranged in a compact fashion to form microfibrilar structure which in turn are arranged differentially to form fibers (Toole & Toole, 2004). In nature they occur in various forms with other polymers which give rise to structurally complex, crystalline and rigid nature. This nature of cellulose has given rise to diverse forms of degradative enzymes (Bayer, Chanzyt, Raphael, & Shoham, 1998).

2.1 Cellulases

Cellulose bioconversion to the soluble monomeric constituent, glucose molecule is catalyzed by a group of degradative enzymes collectively known as cellulases. Cellulases activity for complete digestion of cellulose consists of three different types of individual enzymes namely, endo 1,4 ß D glucanases, exo 1, 4 ß D glucanases and ß glucosidases. These enzymes may be found separately or as a complex in various cellulolytic fungi, bacteria, actinomycetes etc. (Bhat & Bhat, 1997). The endo enzyme cleaves internal bonds of the cellulose chain randomly in processive or non processive manner to produce oligomers, dimmers or monomer. exo enzyme cleaves the cellulose chain either from reducing or non reducing end produce dimer or the ß cellobiose. The exoenzyme which act in progressive manner are also known as cellobiohydrolases. The third enzyme in cellulolytic system is b glucosidases which convert the cellulobiose product from exo and end enzyme activity to glucose molecule (Horn, Kolstad, & Westere, 2012). In addition to above mentioned enzymes for cellulolytic activity, Horn, Kolstad, & Westere, (2012) have discovered new oxidative enzymes CBM33 (Carbohydrate Binding Modules) and GH61(Glycoside Hydrolase) which are capable of cleaving crystalline recalcitrant polysaccharide by oxidative mechanism and thus leveraging the cellulolytic activity.

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2.2 Production of Cellulases

Cellulases enzymes are produced by various fungi, bacteria, actinomycetes and protozoa. Cellulose digestion in higher animals relies on the symbiotic relationship between unicellular organisms that colonize in the hind gut and the organism. Though endogenous cellulases components have been reported from termites, univocal and unambiguous reports were lacking. But, identification of cellulases encoding gene of insects' origin by Watanabe, Noda, Tokuda, & Lo, (1998) has challanged the traditional view of cellulase origin and cellulose digestion in higher animals. Some of the common organisms producing cellulase can be presented in the **Table 1**.

Table 1 Common Cellulolytic Microorganisms, Protists and Animals (table modified after **(Kuhad, Gupta, & Singh, 2011)**)

S. No.	Organisms	Types of organisms	Examples
1	Fungi	Soft rot	*Aspergillus niger, Fusarum solani, Trichoderma harzianum, Neurospora crassa, Paecilomyces inflatus* etc. (Kuhad, Gupta, & Singh, 2011)
		Brown rot	*Coniophora puteana, Porea placenta* etc. (Kuhad, Gupta, & Singh, 2011)
		White rot	*Sporotrichum thermophile, Agaricus arvensis, Pleutotus ostreatus, Phanerochaete chrysosporium* etc. (Kuhad, Gupta, & Singh, 2011)
2	Bacteria	Aerobic	*Acidothermus cellulyticus, Bacillus subtillis, Acenitobacter junii, Cellulomonas biazotea* etc. (Kuhad, Gupta, & Singh, 2011)
		Anaerobic	*Ruminococcus albus, Clostridium thermocellum, Acetivibrio cellulolyticus* etc (Kuhad, Gupta, & Singh, 2011)
3	Actinomycetes	-	*Cellulomonas fimi, Streptomyces drozdowiczii, thermomonospora fusca* etc. (Kuhad, Gupta, & Singh, 2011)
4	Protists	-	*Diplodinium pentacanthum, Enoploplastron triloricatum, Diploplastron affine* etc. (Coleman, Laurie, Bailey, & Holdgate, 1976)
5	Animals	Termites	*Reticulitermis speratus* (Watanabe, Noda, Tokuda, & Lo, 1998)

2.3 Application of Cellulases

Cellulases have been used by human for long decades back for bioconversion of biomass. This capacity of cellulase made it to be used in various industrial applications such as biofuel, food and animal feed, pulp and paper industry, laundry and detergent, textile, biocontrol of diseases in agriculture, bioconversion of cellulosic material to useful products, caretonoid extraction etc. (Kuhad, Gupta, & Singh, 2011). Along with the depletion of nonrenewable resources in the earth crust, cellulose can be the major source of raw material for energy, fuel, food, and various other biotechnological industries. Cellulose is a very

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important renewable source of raw material associated with lignin as lignocelluloses. Thus, enzymatic conversion of cellulose can meet human needs in near future.

Various industrial applications of cellulases can be presented as follows in table 2.

Table 2 Industrial Application of Cellulases (table modified after (Kuhad, Gupta, & Singh, 2011))

S. No.	Industry	Application
1	Textiles	Textile industry use cellulases for biostoning, biopolishing, softening, improving fabrics stability, restoration of brightness, indigo redeposition , backstoning etc.
2	Paper and Pulp	Cellulases treatment improves fiber brightness, freshness and cleanliness, helps in the production of biodegradable products. It is also used to improve the drainage in paper and pulp industries
3	Biofuel	Bioethanol production from lignocellulosic material can be the most attractive alternative to mostly used petro-chemicals.
4	Bioconversion	By the application of cellulases in various raw materials, the low grade cellulose is converted to high value products such as ethanol, organic acids and solvents, single cell proteins.
5	Detergent industries	Cellulases are used by detergent industries for the superior cleaning and dirt removal with improved brightness of fabrics, prevention of redeposition of ink and improved texture without any damage to the fabrics.
6	Biocontrol	Field applied mulches containing cellulases can be use in the biocontrol of pathogenic fungi such as *Phytopthora cinnamomi.*
7	Brewing industry	Improved mashing, malting and improved fermentation products
8	Food industry	Cellulases are used in food industry is used to impart aroma, texture flavors etc.
9	Animal feed industry	Feed formulations containing cellulases are used in animal feed industry to increase the fodder quality and hence increasing the ruminant overall performance in digestion, absorption and finally on productivity.
10	Miscellaneous	For improved extraction of carotenoids, olive oils etc and their improved quality and extraction.

2.4 Application in Denim Finishing

Denims are cotton fabrics where cotton weft passes through two or more warp threads. The denim industry is largely driven by fashion industry. In addition, types of denim jeans preferred by customers depend upon physic-chemical properties and comfort they provide. Traditionally, denim jeans are dyed with indigo blue and are given faded and aged fashionable appearance with abrasive action of pumic stones (Arja , 2007). But the use of sodium hypochlorite and potassium permanganate treated stones has been inefficient and there was a need for sustainable alternative to it. Pumic dust endangers the health of

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workers, time consuming and expensive task of removing stones, creation of larger waste and their subsequent disposal, pollution, imperfection, variation in quality with loss of tensile strength of fibres, abrasion on machine, buttons, rivets etc takes place. Use of pumic stone occupy space, reduces the life of machine, and receives pressure from environmentalists and other statuary hurdles reduces the efficiency of the process. Thus, introduction of cellulases for denim finishing has been of significant importance (Chen, 2003).

Denim finishing mainly involves biostoning and biopolishing. Cellulases used in biostoning primarily attack on the surface of the indigo dyed cotton fibers while leaving the interior intact, thus creating light areas and imparting desired contrast. This process is further enhanced by application of mechanical action which makes the outermost portion of cellulose crystals accessible to enzyme activity. During the process of biostoning, indigo dye released from fabrics has a tendency to redeposit indirectly on white yarn of the fabrics. This effect is known as backstaining (Miettinen-Oinonen, 2004).

From their microassay analysis, Gusakov et al., 1998 found that major cause of backstaining is indigo cellulase affinity and adsorption of cellulases on faded part of yarn of the denim (Gusakov, et al., 1998). Back staining is a major problem with enzymes. However, it decreases with increase in the pH. Lower the pH greater will be the back staining problem. So, neutral to alkaline cellulases are highly desirable in denim industry (Novozymes, Rethink Tomorrow).

Removal of fuzz from cellulose fibers by the application of cellulases is known as biopolishing or biofinishing. Enzymatic treatment of denim causes reduction of pilling and frosted appearance, increases smoothness, imparts cleaner look, increased drapeability etc. (Heikinheimo, 2002).

2.5 Cellulases Technology in Denim Finishing

Denim industries take the advantage of both complete cellulases and individual cellulases components to achieve the partial hydrolysis of denim fabrics to improve the fabrics property to desired level. Indigo dyed denim fabrics have the indigo bound mostly on short cotton fabrics and outer surface of the yarn. During biostoning process cellulases act on the cotton fabric to break off the small fiber ends on the yarn surfaces thus attached dyes are loosened. These breaks off are later on released during the wash cycle by mechanical abrasion. Thus, dyes bound to the surfaces are removed and imparts the faded appearance (Karmakar & Ray, 2011). The fluffy and dull appearance of denim fabrics can occur after repeated washing biostoning. This is due to the attached presence of partially detached microfibrils. Defibriliation of these microfibrils is achieved by the action of endonuclease rich cellulases during the process of biopolishing and thus enhance whiteness, brightness and color characterstics of denim fabrics (Karmakar & Ray, 2011).

2.6 Aim of the Study

The aim of the study is to perform state of art search and technology activity search on most efficient and economic cellulases technologies that use cellulases in denim finishing (denim biostoning and denim biopolishing).

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3 METHODOLOGY

The patent search on application of cellulases in denim finishing was performed by keywords search strategy. Free Patent Online search engine was used to search the patents and patent applications in US Patent, US Patent Application, EP documents, Abstracts of Japan, WIPO (PCT), and German Patents. The patents and patent applications clearly describing the technology of denim finishing (biostoning and biopolishing) were considered relevant patent for the purpose of the study. The major steps followed during the patent search are summarized below.

3.1 Identification of Central Concept of the Technology

Based on various available online resources and intensive review of available literatures, the central concept related to application of cellulase on denim finishing was identified.

3.2 Identification of keywords and Their Synonyms Through Brainstorming

Major technical terms related to the cellulase application in denim finishing were identified through review of available literature. For the convenience of search in different databases, synonyms of those technical terms were identified.

3.3 Performing the First Search

Keyword search strategy was used to perform the patent search in Free Patent Online search engine. The major databases searched by Free Patent Online Search Engine were; US patents, US patent Application, EP documents, Abstracts of Japan, WIPO (PCT) and German Patent. The keywords and their synonyms used are; Cellulase, Denim, Biostoning, Biofinishing, Biopolishing, Backstaining, Redeposition, Formulation etc. Boolean Operators such as AND, OR, NOT were used to form a search string.

3.4 Refining the Search

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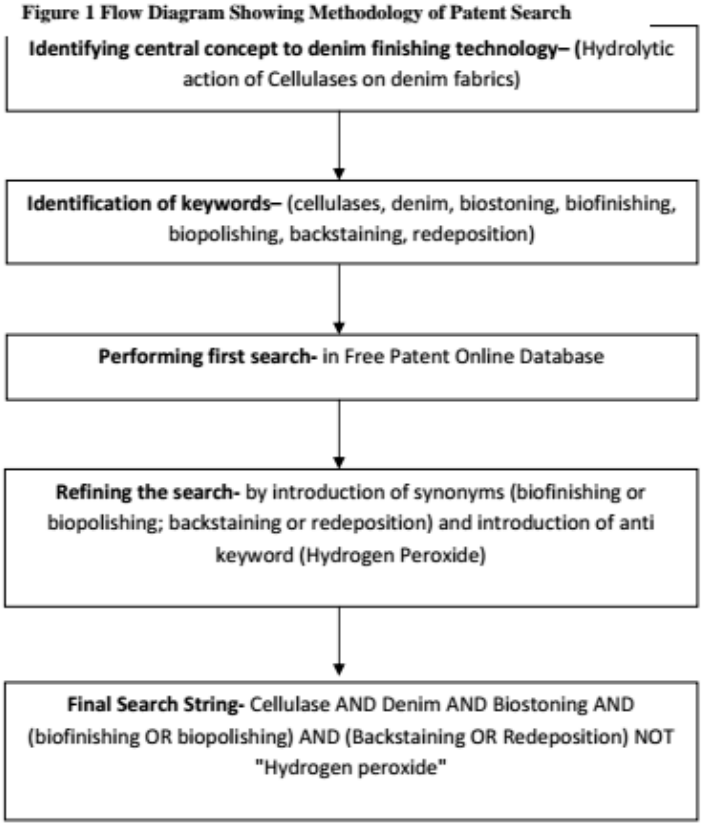
3.4 Refining the Search

Patent search by the use of keywords in first search yielded more patents. Many of them may be irrelevant to purpose of the study. In order to decrease the quantity while increasing the relevancy, anti-keywords were introduced. Hydrogen Peroxide was used as an anti keyword in the search string.

Figure 1 Flow Diagram Showing Methodology of Patent Search



4 RESULT AND DISCUSSION

Patents are made available to general public through various databases but single database does not sufficiently cover the patent documents worldwide. Thus it is essential to consult more than one database to find and access the document of interest (World Intellectual Property Organization, 2012). Addition of more than ten million patent documents from United States Patents and Trademark Office (USPTO), WIPO's PATENTSCOPE has expanded its access to more than 28 million searchable patent document including patents access to more than 30 national and regional patent collections. Since Free Patent Online search engine searches all the patent documents from US patents, US patent applications, EP

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Figure 1 Flow Diagram Showing Methodology of Patent Search

Identifying central concept to denim finishing technology– (Hydrolytic action of Cellulases on denim fabrics)

Identification of keywords– (cellulases, denim, biostoning, biofinishing, biopolishing, backstaining, redeposition)

Performing first search- in Free Patent Online Database

Refining the search- by introduction of synonyms (biofinishing or biopolishing; backstaining or redeposition) and introduction of anti keyword (Hydrogen Peroxide)

Final Search String- Cellulase AND Denim AND Biostoning AND (biofinishing OR biopolishing) AND (Backstaining OR Redeposition) NOT "Hydrogen peroxide"

4 RESULT AND DISCUSSION

Patents are made available to general public through various databases but single database does not sufficiently cover the patent documents worldwide. Thus it is essential to consult more than one database to find and access the document of interest (World Intellectual Property Organization, 2012). Addition of more than ten million patent documents from United States Patents and Trademark Office (USPTO), WIPO's PATENTSCOPE has expanded its access to more than 28 million searchable patent document including patents access to more than 30 national and regional patent collections. Since Free Patent Online search engine searches all the patent documents from US patents, US patent applications, EP

documents, abstracts of Japan, WIPO (PCT) and German Patents, it was used as the reliable search engine for wide patent coverage.

Patent search by keyword search strategy was performed using Free Patent Online search engine. For the purpose, keywords suitable for the topic, application of cellulases in denim finishing, obtained through literature review were; Cellulases, Denim, Biostoning, Biofinishing, Biopolishing, Backstaining and Redeposition. The anti-keyword, hydrogen peroxide, was also introduced. Thirty eight search results were obtained which are listed below in table 3.

Table 3 Patent Result Distribution and Ownership

S.No.	Assignee/Applicant	No. of Patents/ Application
1	AB Enzymes O/Y, Finland	17
2	AB Enzymes GmbH, Germany	4
3	Danisco US Inc., USA	4
4	Clariant Finance (BVI) Ltd, Tortola	3
5	Novozymes A/S, Denmark	3
6	Rohm Enzymes Finland O/Y	2
7	Primal Co. Ltd., Finland	1
8	IOGEN Bioproduct Co-Operation (Acquired by Novozymes A/S)	1
9	Miettinen-Oinonen et. al.	3
Total		**38**

Patent search on denim biostoning and finishing showed that most patents are owned by Biotech Company whereas only 3 patent applications were solely owned by inventors. If anyone whishes to obtain license for commercial production and uses, there is a little probability of obtaining a license from owner company because all the patent owner companies are involved in commercial production. However, the probability of obtaining license always remains from the inventors.

Among 38 search results, 10 patent/patent applications were found to be relevant. Clearly defined application, mechanism of cellulases action, effectiveness and efficiency of cellulases technology on denim industry form the basis for defining the relevancy of the patents. Ten most relevant patent/patent applications are listed as below in table 4.

Table 4 Summary of Results for most Relevant Patents

S. No.	Title of Patent/Patent Application	Patent Number/Patent Application Number	Publication Date	Inventor/s	Assignee/Applicants	Evaluation*

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