Cellulase

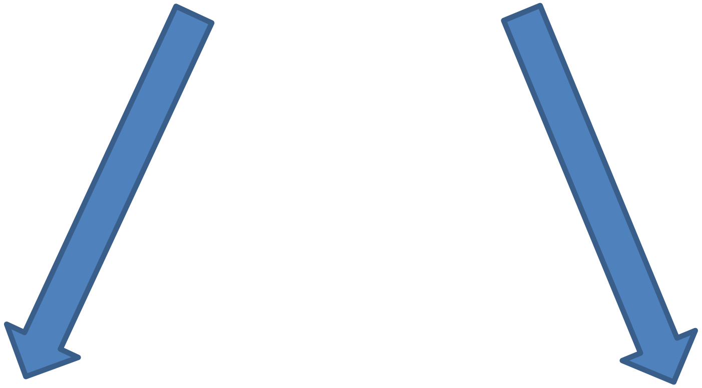
Introduction
(source)

Cellulase refers to an entourage of enzymes produced chiefly by fungi, bacteria and protozoans that catalyze cellulolysis (i.e. the hydrolysis of cellulose).

However, there are also cellulases produced by a few other types of organisms, such as some termites and the microbial intestinal symbionts of other termites.

Several different kinds of cellulases are known, which differ structurally and mechanistically.

Cellulase



Cellobiohydrolases

whose major activity involves the cleavage of cellobiose residues consecutively from the ends of the cellulose chains

Endoglucanases

whose major activity involves the cleavage of β-glycosidic bonds in the cellulose chain

they are necessary for the efficient hydrolysis of cellulose to soluble oligosaccharides

Complete vs. incomplete cellulases

- Some species of fungi and bacteria are able to exhaustively digest crystalline cellulose in pure culture are said to have complete or true cellulases.

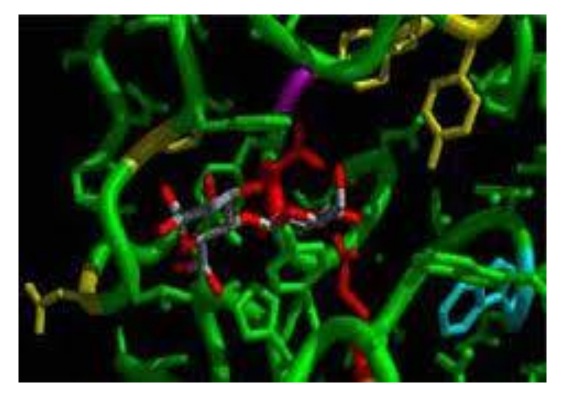
- The majority of organisms that produce cellulases can only hydrolyze the cellulose in their diets to certain extent. they are known as incomplete cellulases.

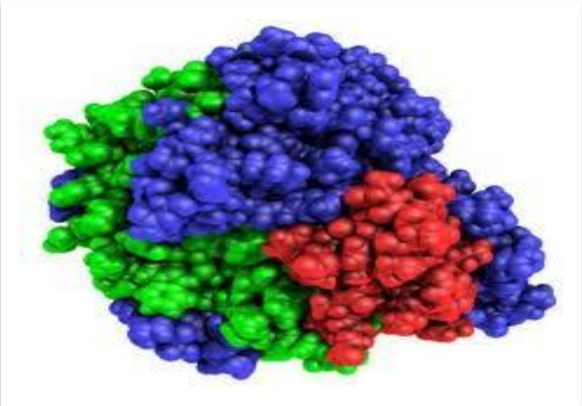
- These cellulases unable to digest cellulose exhaustively can still generate sufficient amount of glucose for their producers. Endogenous cellulases of termites belong to this category.

Other Names

Other names for 'endoglucanases' are:

endo-1,4-beta-glucanase, carboxymethyl cellulase (CMCase), endo-1,4-beta-D-glucanase, beta-1,4-glucanase, beta-1,4-endoglucan hydrolase, and celludextrinase. The other types of cellulases are called exocellulases.





Types of reactions/ Classification

General types of cellulases based on the type of reaction catalyzed:

1. Cleaves internal bonds at Endocellulase (EC 3.2.1.4) randomly amorphous sites that create new chain ends.
2. Cellobiase (EC 3.2.1.21)
   or beta-glucosidase hydrolyses the exocellulase product into individual monosaccharides.
3. Cellulose phosphorylases depolymerize cellulose using phosphates instead of water.

Choice of host organism

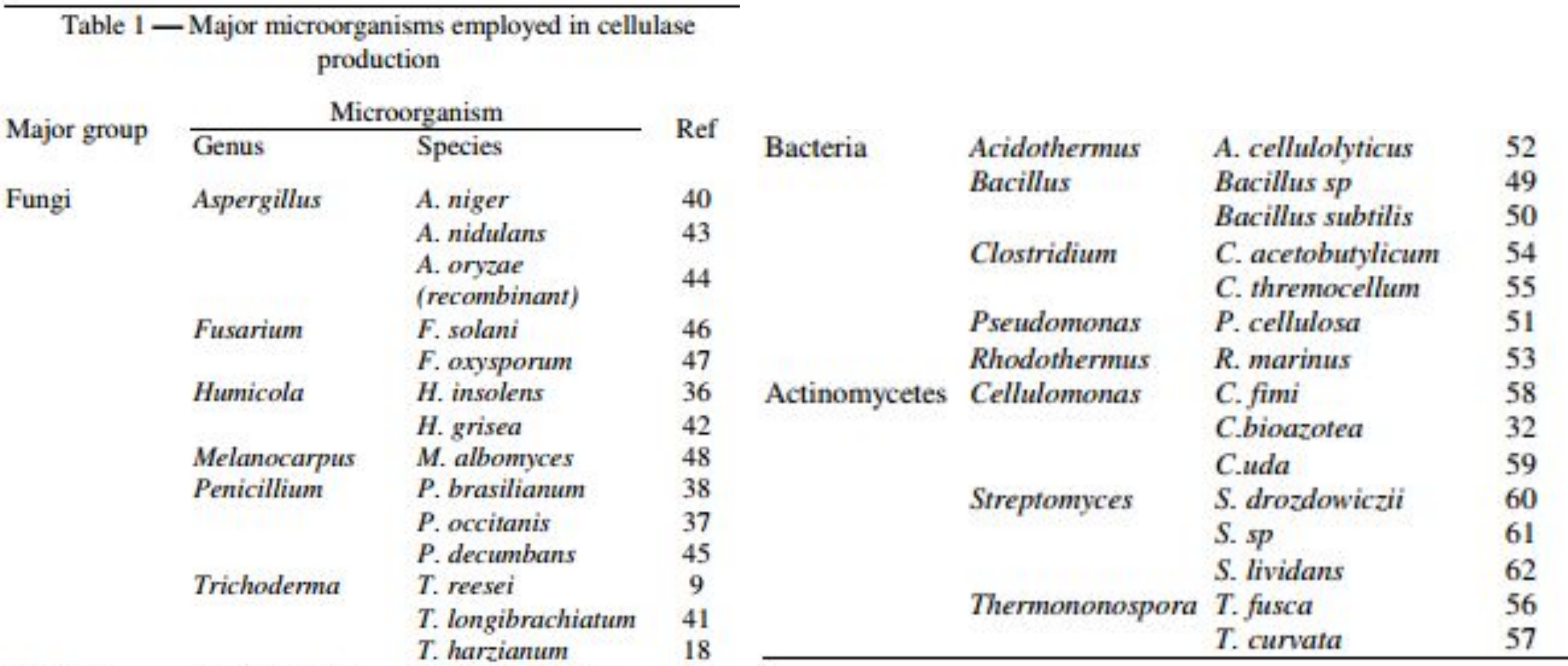


Table 1 — Major microorganisms employed in cellulase production

Major group	Microorganism		Ref
	Genus	Species	
Fungi	*Aspergillus*	*A. niger*	40
		A. nidulans	43
		A. oryzae (recombinant)	44
	Fusarium	*F. solani*	46
		F. oxysporum	47
	Humicola	*H. insolens*	36
		H. grisea	42
	Melanocarpus	*M. albomyces*	48
	Penicillium	*P. brasilianum*	38
		P. occitanis	37
		P. decumbans	45
	Trichoderma	*T. reesei*	9
		T. longibrachiatum	41
		T. harzianum	18
Bacteria	*Acidothermus*	*A. cellulolyticus*	52
	Bacillus	*Bacillus sp*	49
		Bacillus subtilis	50
	Clostridium	*C. acetobutylicum*	54
		C. thremocellum	55
	Pseudomonas	*P. cellulosa*	51
	Rhodothermus	*R. marinus*	53
Actinomycetes	*Cellulomonas*	*C. fimi*	58
		C.bioazotea	32
		C.uda	59
	Streptomyces	*S. drozdowiczii*	60
		S. sp	61
		S. lividans	62
	Thermononospora	*T. fusca*	56
		T. curvata	57

Strain engineering

- Thermostable cellulases production
- Nowadays, most of the studies about production of thermostable cellulases are focused on the utilization of cellulase-producing thermo/alkalophiles and also, on the improvement of cellulase
  production by optimizing its nutritional and environmental necessities or by engineering new highproducer recombinants or cellulase-producing transgenic plants, such as transgenic
  tobacco

Homologous overexpression in bacteria

- Some studies report the use of directed evolution techniques in combination with a rational design to overexpress cellulases in their own bacterial source. Genera such as *Bacillus* (*B. subtilis*)
  and *Clostridium* (*C. thermocellum*) were used as a homologous cellulases production system, their easy genetic modification and other proper features.
- However, the use of these bacteria has disadvantages such as low protein yields, high production costs or need of enriched media

Heterologous overexpression

- The strategies based in heterologous expression are focused in the use of non-cellulolytic micro/organisms that have high production ratio for expressing microbial cellulases
- Bacteria such as *E. coli*, different species from the genus *Bacillus, Pseudomonas fluorescens, Ralstonia eutropha* and *Zymomonas mobilis*;
- yeasts such as *Saccharomyces cerevisiae* and *Pichia pastoris* and filamentous fungi from genera *Aspergillus* and *Trichoderma* genera

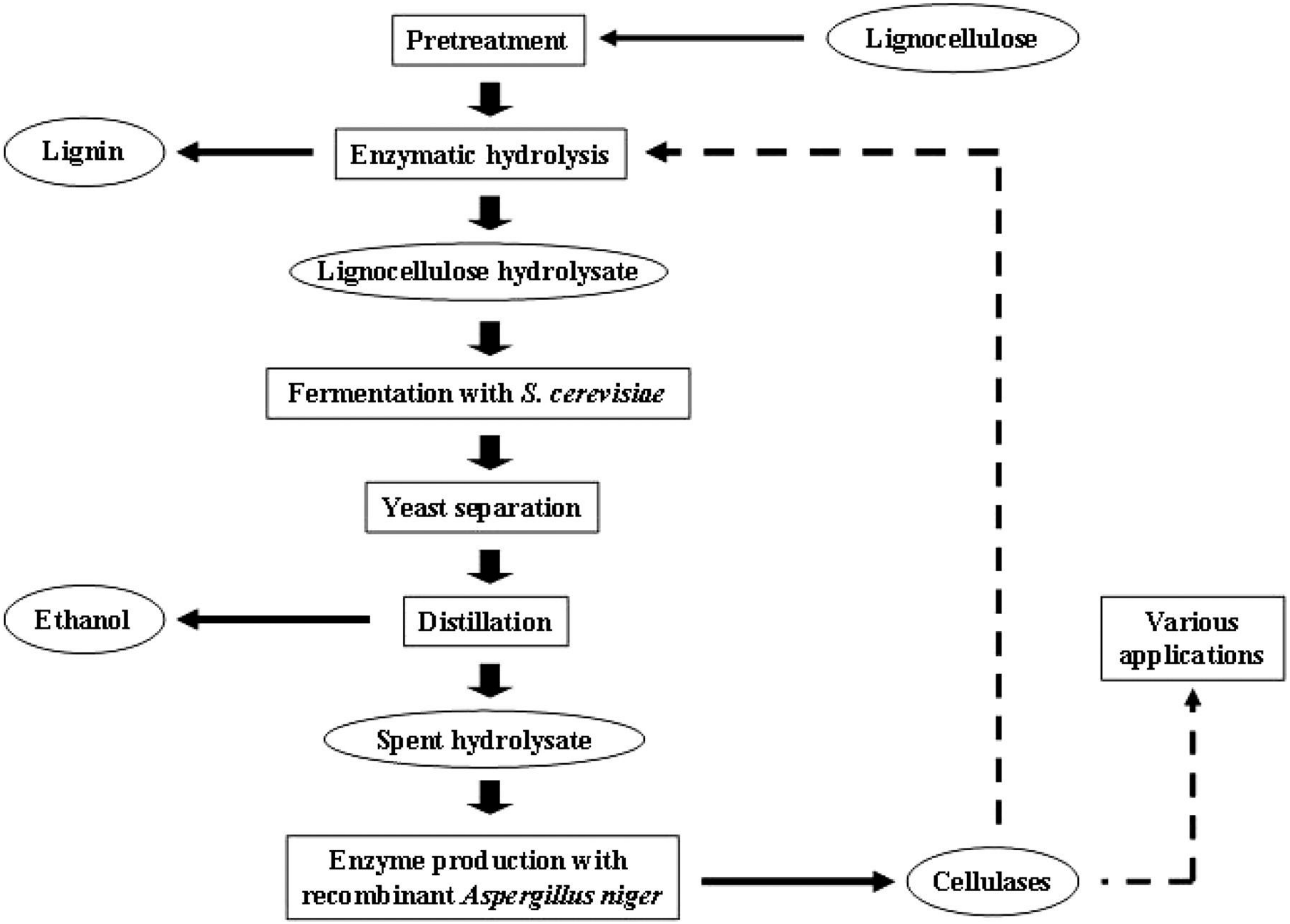
  are the most used in research and industry, considered as host systems for producing recombinant enzymes. Furthermore, cell cultures of mammals, plants or insects and transgenic plants and/or animals are used for protein expression

- Future targets for genetic manipulation and optimization will include the use of the cellulolytic system of *Clostridium thermocellum* for engineering new strains, depending of the concrete industrial application and the fully characterization of the promising thermophilic bacterium *Caldicellulosiruptor bescii*.

Table 2 — Cellulase production –Bioprocesses and organisms employed

Microorganism	Substrate	Method	Magnitude	Enzymes - Activity	Ref (s)
Aspergillus niger A 20	Cellulose	SmF	Shake flask	Cellobiase -27.5 U/ml	108
A. niger NRRL3	Wheat bran/Corn cob	SSF	Flask	Cellobiase-215 IU/g cellulose	117
Bacillus pumilus	CMCellulose/Glycerol	SmF	SF	CMCase-1.9 U/ml, Cellobiase - 1.2U.ml	109
Bacillus sp KSM N252	Carboxymethyl cellulose	SmF	Shake flask	CMCase - 0.17 U/mg protein	110
B. subtilis	Soybean industry residue	SSF	Cylindrical bioreactor	FPAse -1.08U/mg protein	50
B. subtilis	Banana waste	SSF	Shake flask	FPAse - 2.8 IU/gds CMCase - 9.6 IU/gds Cellobiase - 4.5 IU/gds	118
Chaetomium thermophilium CT2	Cellulose (sigma cell)	SmF	Shake flask	CMCase -2.7 IU/ml	111
Melnocarpus albomyces	Solka floc	SmF	700L fermentor	Cellulase -1160 ECU/ml, Endoglucanase -3290 ECU/ml,	48
Mixed culture: T. reesei, A. niger	Rice chaff/ Wheat bran (9:1)	SSF	Flask	FPAse -5.64 IU/g	119
Mucor circinelloidens	Lactose	SmF	Shake flask	EGL - 0.25 U/ml	112
Neurospora crassa	Wheat straw	SmF	Shake flask	FPAse - 1.33 U/ml CMCase - 19.7 U/ml BGL - 0.58 U/ml	94
Penicillium decumbans	wheatstraw/bran (8:2)	SSF	SSF bioreactor	Fpase -20.4 IU/g	120
P. occitanis	Paper pulp	SmF-Fed batch	20L fermentor	FPAse - 23 IU/ml CMCase - 21 IU/ml	13
P. janthinellum	Sugar cane bagasee	SmF	Shake flask	FPAse -0.55U/ml, CMCase - 21.5 U/ml, BGL - 2.31 U/ml	97
Phaenerocheate chrysosporium	Cellulose (Avicell)	SmF	100L fermentor	Cellulase - 29mg/g cellulose	113
Rhodothermus marinus	CM cellulose	SmF	150L fermentor	Endoglucanase-97.7 U/ml	53
Steptomyces sp T3-1	Carboxymethyl cellulose	SmF	50L fermentor	CMCase - 148 IU/ml Avicellase- 45 Iu/ml BGL- 137 IU/ml	114
S. drodowiczii	Wheat bran	SmF	Shake flask	CMCase - 595 U/L	60
Thermoascus auranticus	Wheat straw	SSF	Perforated Drum Bioreactor	FPAse - 4.4 U/gds CBH -2.8 U/gds Endoglucanase - 987 U/gds BGL- 48.8 U/gds	121
Thermotoga maritima	Xylose	SmF	Shake flask	Cellobiase-11 mU/ml, Avicellase - 0.3 mU/ml, Beta Glucosidase-30mU/ml	115
Trichoderma reesei	Xylose /Sorbose	SmF-Continuous	Bioreactor	FPAse - 0.69 U/ml/h	100
T. reesei	Steam treated willow	SmF	22L fermentor	FPAse- 108 U/g cellulose	26
T. reesei RUT C30	Cellulose (Avicell)	SmF	Microbubble dispersion bioreactor	FPAse- 1.8U/ml	116
T. reesei RUT C30	Corrugated cardboard	SmF	30L fermentor	FPAse- 2.27 U/ml	95
T. reesei ZU 02	Corn cob residue	SSF	Tray fermentor	FPAse - 158 U/gDS	122
T. reesei ZU-02	Corn stover residue	SmF	30L fermentor	Cellulase - 5.48 IU/ml, FPAse - 0.25 U/ml	96
T. viridae	Sugar cane bagasee	SmF	Shake flask	FPAse - 0.88 U/ml, CMCase - 33.8 U/ml, BGL - 0.33 U/ml	97

Schematic representation of the experimental approach and on-site enzyme production in a cellulose-to-ethanol process.



Pretreatment

Lignocellulose

Lignin

Enzymatic hydrolysis

Lignocellulose hydrolysate

Fermentation with *S. cerevisiae*

Yeast separation

Ethanol

Distillation

Various applications

Spent hydrolysate

Enzyme production with recombinant *Aspergillus niger*

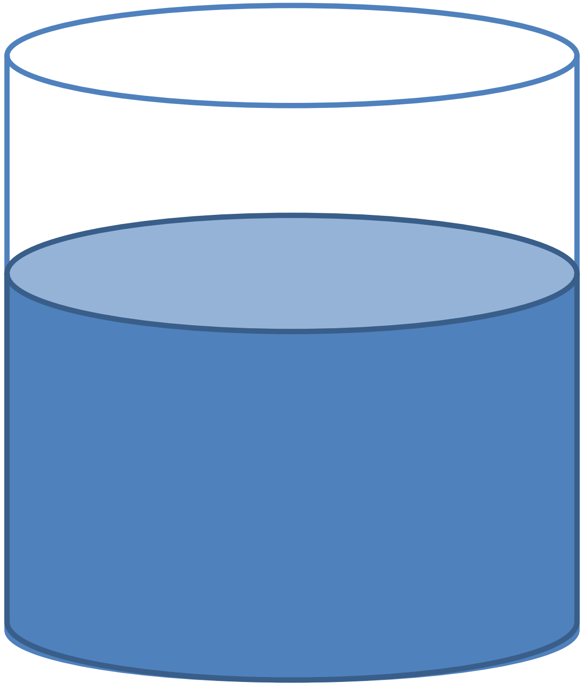
Cellulases

Björn Alriksson et al. Appl. Environ. Microbiol. 2009;75:2366-2374

Cultivation Media

Medium 1 ((without carbon source)

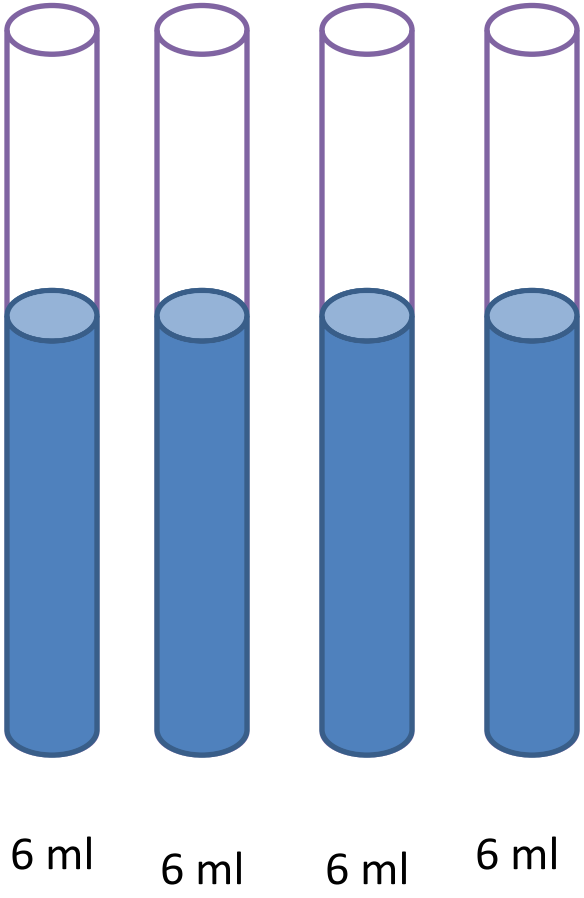
NaNO3, 2.0, KH2PO4, 1.0, MgSO4 · 7H2O, 0.5, and (mg.L-1) FeSO4, 10.0. The pH of the medium was adjusted to 6.5.

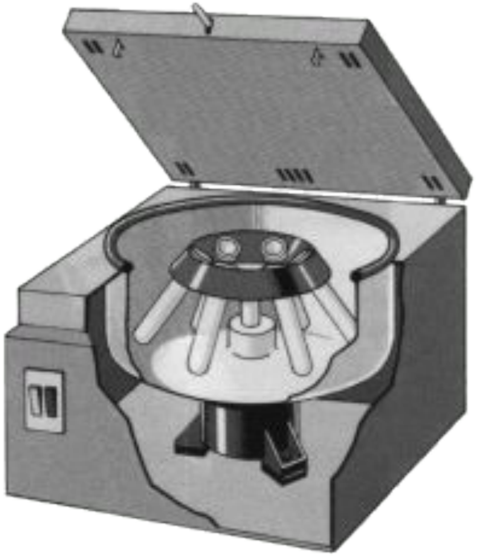


Medium 2

Peptone, 6.0, KH2PO4, 1.0, MgSO4 · 7H2O, 1.72, KCl, 0.5, and (mg.L-1) FeSO4, 10.0. The pH of the medium was adjusted to 5.5.

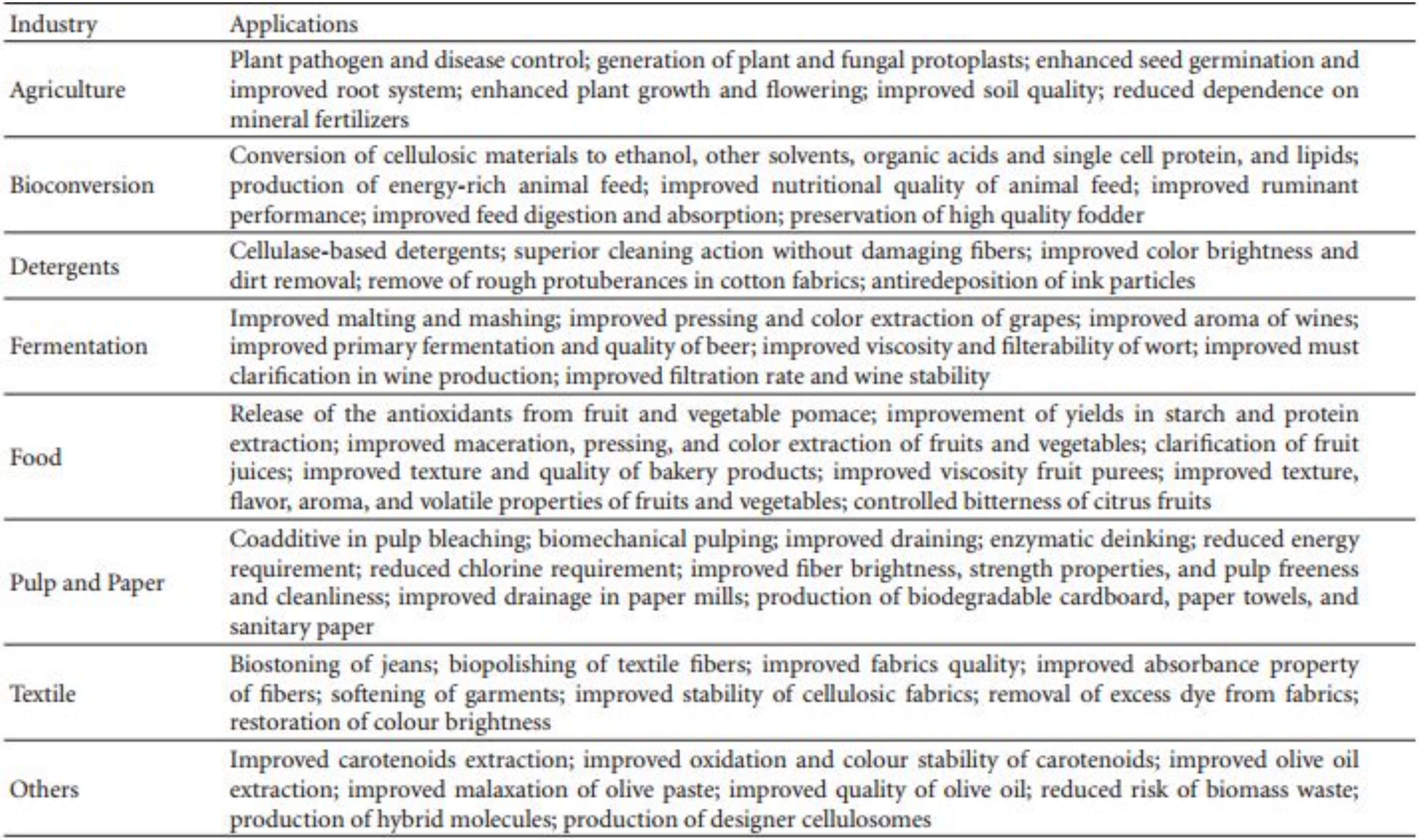
Harvest and Separation of Enzymes





These were then centrifuged at 5000 rpm for 15 minutes and the supernatant was collected to 10 mL sterile tubes
and stored at -20ºC for further use in enzyme assays

Uses



Industry	Applications
Agriculture	Plant pathogen and disease control; generation of plant and fungal protoplasts; enhanced seed germination and improved root system; enhanced plant growth and flowering; improved soil quality; reduced dependence on mineral fertilizers
Bioconversion	Conversion of cellulosic materials to ethanol, other solvents, organic acids and single cell protein, and lipids; production of energy-rich animal feed; improved nutritional quality of animal feed; improved ruminant performance; improved feed digestion and absorption; preservation of high quality fodder
Detergents	Cellulase-based detergents; superior cleaning action without damaging fibers; improved color brightness and dirt removal; remove of rough protuberances in cotton fabrics; antiredeposition of ink particles
Fermentation	Improved malting and mashing; improved pressing and color extraction of grapes; improved aroma of wines; improved primary fermentation and quality of beer; improved viscosity and filterability of wort; improved must clarification in wine production; improved filtration rate and wine stability
Food	Release of the antioxidants from fruit and vegetable pomace; improvement of yields in starch and protein extraction; improved maceration, pressing, and color extraction of fruits and vegetables; clarification of fruit juices; improved texture and quality of bakery products; improved viscosity fruit purees; improved texture, flavor, aroma, and volatile properties of fruits and vegetables; controlled bitterness of citrus fruits
Pulp and Paper	Coadditive in pulp bleaching; biomechanical pulping; improved draining; enzymatic deinking; reduced energy requirement; reduced chlorine requirement; improved fiber brightness, strength properties, and pulp freeness and cleanliness; improved drainage in paper mills; production of biodegradable cardboard, paper towels, and sanitary paper
Textile	Biostoning of jeans; biopolishing of textile fibers; improved fabrics quality; improved absorbance property of fibers; softening of garments; improved stability of cellulosic fabrics; removal of excess dye from fabrics; restoration of colour brightness
Others	Improved carotenoids extraction; improved oxidation and colour stability of carotenoids; improved olive oil extraction; improved malaxation of olive paste; improved quality of olive oil; reduced risk of biomass waste; production of hybrid molecules; production of designer cellulosomes

Thank you for attention!!!