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Determining the Biomedical Waste Degrading Property of Cellulase Enzyme Produced From Bacterial Isolates

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Abstract

Medical waste management (MWM) has been a very critical issue as it poses potential health risks and damage to the environment. It is also of greater importance due to its potential environmental hazards and public health risks with high propensity to result into epidemics. It continues to be a major challenge, particularly, in most healthcare facilities of the developing countries where it is hampered by technological, economical, social difficulties and inadequate training of staff responsible for handling of the waste. Poor conduct and inappropriate management and disposal methods exercised during handling and disposal of medical waste (MW) is an increasing significant health hazards and environmental pollution/hazards due to the infectious nature and unpleasant smell of the waste. Despite the fact that current medical waste management (MWM) practices vary from hospital to hospital, the problematic areas are similar for all healthcare units and at all stages of management.

This cutting-edge research aims to explore the production of cellulase from various bacterial isolates and investigate their effectiveness in degrading cotton. By understanding the cellulase production process and its impact on cotton degradation, we can unlock new possibilities for textile waste management, biofuel production, and even potential applications in other industries. Get ready to dive into the world of cellulase production and discover how this enzyme holds the key to a greener and more sustainable future.

Introduction

Bacterial isolates for cellulase production:

Cellulase production starts with the selection of bacterial isolates that have the potential to produce high yields of the enzyme. Bacteria are known to be a rich source of cellulase enzymes due to their ability to thrive in diverse environments. Scientists have identified various bacterial isolates that possess cellulase-producing capabilities. These isolates are often found in soil, plant materials, and even the digestive systems of certain animals. By isolating and studying these bacteria, researchers hope to uncover the secrets behind their cellulase production and potentially utilize this knowledge for industrial, Medical and commercial applications.

Once potential cellulase-producing bacterial isolates are identified, the next step is to screen and select the most promising candidates. This involves testing the isolated bacteria for their ability to produce cellulase under specific conditions. Screening methods include measuring the cellulase activity through various assays. Bacterial isolates that exhibit high cellulase activity are then selected for further optimization and characterization. This initial screening process is crucial in identifying bacterial isolates that can be used for large-scale cellulase production.

After the screening process, selected bacterial isolates must undergo optimization to maximize cellulase production. This involves manipulating various factors such as temperature, pH, carbon sources, and nitrogen sources to create an environment that promotes high enzyme yields. By fine-tuning these conditions, researchers aim to achieve optimal cellulase production from the selected bacterial isolates. This optimization process requires careful experimentation and analysis to identify the most favorable conditions for cellulase production.

Characterization of cellulase enzymes:

Once the cellulase-producing bacterial isolates have been optimized, the next step is to characterize the cellulase enzymes they produce. This involves determining the specific properties and behaviors of the enzymes, such as their molecular weight, pH and temperature optima, substrate specificity, and reaction kinetics. Characterization allows researchers to gain a deeper understanding of the cellulase enzymes, which can aid in their application in various industries.

One important aspect of cellulase characterization is determining the specific types of cellulases present in the bacterial isolates. Cellulases can be classified into different categories based on their mode of action on cellulose. These categories include endoglucanases, exoglucanases, and β-glucosidases. Each type of cellulase plays a distinct role in the degradation of cellulose, and understanding their presence and activity in the bacterial isolates is crucial for further research and applications.

Another aspect of cellulase characterization is studying the stability and durability of the enzymes under different conditions. This involves evaluating the enzyme's resistance to factors such as temperature, pH, and

inhibitors. By understanding the stability of cellulase enzymes, researchers can determine their suitability for various applications, such as textile waste management or biofuel production, where the enzymes might be subjected to harsh conditions.

Determination of cotton Degrading property of cellulase:

The ultimate goal of cellulase production is to utilize the enzymes for the degradation of cotton fibers. Cotton is a widely used natural fiber in textile production, but it is also a major contributor to textile waste. By harnessing the power of cellulase enzymes, researchers aim to develop efficient methods for cotton degradation, leading to reduced waste and improved resource utilization.

To determine the cotton degrading property of cellulase, various experiments are conducted. One common approach is to treat cotton fibers with cellulase enzymes and observe the extent of degradation. This can be done by measuring the weight loss of the cotton fibers or analyzing the changes in their physical and chemical properties. By quantifying the degradation, researchers can assess the effectiveness of the cellulase enzymes in breaking down cotton fibers.

Factors such as enzyme concentration, incubation time, temperature, and pH can all influence the cotton degrading property of cellulase. Researchers carefully control these variables to optimize the degradation process and maximize the efficiency of the enzymes. By understanding the factors that affect cellulase activity on cotton, scientists can develop strategies to enhance the degradation process and improve the overall efficiency of textile waste management.

Materials and Methodology

Cellulase from isolates CL002 and CL005 were used for the Degradation assessment in general biomedical waste.

The samples included general wastes of health care[a] Cotton swabs (CS) [b] Dressings and bandages (DB) [c]Plaster casts (PC)(d) Discarded gloves (DG) [e] Tissue and bits of papers (TP). Thesamples were collected in polythene bags and cut into small pieces, and aliquoted for different experimental set up.

Crude enzyme was dissolved in 50 mM sodium acetate/NaOH buffer (pH 4.5) to reach concentration of 3.0 U/ml for both isolates and then mixed with 25 mg (Initial weight-W1) of waste materials and incubated at 50°C for 2 h.

After incubation the tubes were centrifuged for 15 min using and the supernatants were transferred into clean test tubes with the concentration of the produced sugars determined by the DNS method. All absorbance readings were taken using the Double beam UV Vis spectrophotometer. For the concentration determination, the glucose standards was used as in mentioned in earlier report.

The precipitate of papers collected after centrifugation was rinsed with distilled water, oven dried and weighed (Final weight-W2).

Degradation efficiency was calculated using formula

$$\text{Degradation Efficiency (\%)} = (1-W2/W1)*100$$

where W2 and W1 are the weight of sample after reaction and the initial weight of the waste in experimental set up.

Test Sample: CL002 and CL005 ; Cotton swabs (CS), Dressings and bandages (DB), Plaster casts (PC), Discarded gloves (DG), Tissue and bits of papers (TP)

Test Methods: Spectro photometric assay (PC Based UV-Vis Spectrophotometer Systronic 2202)

Result and Observation

1. % Degradation efficiency of Crude cellulase enzyme on general biomedical waste material

Table 1: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable temperature

Parameter	Sample	CL002			CL005		
Temperature		Initial wt. (mg)	final wt. (mg)	%Degradation	Initial wt. (mg)	final wt. (mg)	%Degradation
		30					
	CS	25	18.6	25.6	25	20.6	17.6

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	DB	25	23.4	6.4	25	22.4	10.4
	PC	25	23.9	4.4	25	21.9	12.4
	DG	25	25	0	25	25	0
	TP	25	11	56	25	13.41	46.36
		35					
	CS	25	17.3	30.8	25	19.81	20.76
	DB	25	21.5	14	25	19.3	22.8
	PC	25	20.7	17.2	25	21.5	14
	DG	25	25	0	25	25	0
	TP	25	13.6	45.6	25	13.3	46.8
		40					
	CS	25	6.4	74.4	25	11.56	53.76
	DB	25	17.4	30.4	25	15.4	38.4
	PC	25	13.2	47.2	25	11.11	55.56
	DG	25	25	0	25	25	0
	TP	25	9.6	61.6	25	8.6	65.6
		45					
	CS	25	15.5	38	25	16.41	34.36
	DB	25	16.7	33.2	25	16	36
	PC	25	19	24	25	20.41	18.36
	DG	25	25	0	25	25	0
	TP	25	11.3	54.8	25	14.1	43.6
		50					
	CS	25	17.3	30.8	25	18.2	27.2
	DB	25	18.6	25.6	25	18.5	26
	PC	25	19.3	22.8	25	21.4	14.4
	DG	25	25	0	25	25	0
	TP	25	22.3	10.8	25	19.3	22.8

*****Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers - TP

Table 2: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable pH

Parameter	**Sample**	**CL002**			**CL005**		
pH							
		Initial wt. (mg)	**final wt. (mg)**	**%Degradation**	**Initial wt. (mg)**	**final wt. (mg)**	**%Degradation**
		3					
	CS	25	17.8	28.8	25	14.3	42.8
	DB	25	23.5	6	25	21.4	14.4
	PC	25	14.7	41.2	25	17.64	29.44
	DG	25	25	0	25	25	0

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	TP	25	14.9	40.4	25	14.9	40.4
		5					
	CS	25	17.3	30.8	25	18.6	25.6
	DB	25	16.3	34.8	25	14.3	42.8
	PC	25	20	20	25	18.33	26.68
	DG	25	25	0	25	25	0
	TP	25	16.7	33.2	25	15.8	36.8
		6					
	CS	25	24.7	1.2	25	12.9	48.4
	DB	25	23.3	6.8	25	17.1	31.6
	PC	25	20.9	16.4	25	11.7	53.2
	DG	25	25	0	25	25	0
	TP	25	17.9	28.4	25	10.3	58.8
		7					
	CS	25	20.6	17.6	25	21.1	15.6
	DB	25	18.1	27.6	25	24.6	1.6
	PC	25	21.09	15.64	25	24	4
	DG	25	25	0	25	25	0
	TP	25	17.7	29.2	25	20.5	18
		8					
	CS	25	19	24	25	19.33	22.68
	DB	25	19.7	21.2	25	18.5	26
	PC	25	18.5	26	25	18.5	26
	DG	25	25	0	25	25	0
	TP	25	18.7	25.2	25	17.2	31.2

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers - TP

Table 3: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable agitation speed:

Parameters	Sample	Initial wt. (mg)	final wt. (mg)	%Degradation	Initial wt. (mg)	final wt. (mg)	%Degradation
Agitation speed	100						
	CS	25	23.3	6.8	25	20.13	19.48
	DB	25	21.56	13.76	25	20.41	18.36
	PC	25	22.5	10	25	20.02	19.92
	DG	25	25	0	25	25	0
	TP	25	23.8	4.8	25	19.8	20.8
	150						
	CS	25	12.4	50.4	25	13.5	46
	DB	25	13.7	45.2	25	12.7	49.2
	PC	25	16	36	25	15.21	39.16
	DG	25	25	0	25	25	0
	TP	25	9.07	63.72	25	12.08	51.68

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	200						
	CS	25	17.9	28.4	25	19.45	22.2
	DB	25	17.9	28.4	25	21.4	14.4
	PC	25	15.4	38.4	25	16.33	34.68
	DG	25	25	0	25	25	0
	TP	25	16.3	34.8	25	18.4	26.4
	250						
	CS	25	21.05	15.8	25	22.5	10
	DB	25	23.8	4.8	25	22	12
	PC	25	23.1	7.6	25	23.5	6
	DG	25	25	0	25	25	0
	TP	25	24.5	2	25	24.8	0.8
	300						
	CS	25	21.3	14.8	25	20.4	18.4
	DB	25	23	8	25	21.4	14.4
	PC	25	23.4	6.4	25	22.5	10
	DG	25	25	0	25	25	0
	TP	25	23.7	5.2	25	21.6	13.6

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers – TP

Table 4: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable proportion of crude enzyme:

Parameters	Sample	Initial wt. (mg)	final wt. (mg)	%Degradation	Initial wt. (mg)	final wt. (mg)	%Degradation
Concentration		2					
	CS	25	19.6	21.6	25	20.5	18
	DB	25	18.33	26.68	25	14.8	40.8
	PC	25	21.5	14	25	17.5	30
	DG	25	25	0	25	25	0
	TP	25	17	32	25	15.9	36.4
		4					
	CS	25	18.75	25	25	24.3	2.8
	DB	25	18.1	27.6	25	21.5	14
	PC	25	21.26	14.96	25	20.3	18.8
	DG	25	25	0	25	25	0
	TP	25	15.3	38.8	25	19.7	21.2
		6					
	CS	25	13.4	46.4	25	15.5	38
	DB	25	11.84	52.64	25	13.56	45.76
	PC	25	11.36	54.56	25	17.2	31.2
	DG	25	25	0	25	25	0

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	TP	25	10.41	58.36	25	11.71	53.16
		8					
	CS	25	14.7	41.2	25	17.9	28.4
	DB	25	15.03	39.88	25	15.01	39.96
	PC	25	15.9	36.4	25	18.3	26.8
	DG	25	25	0	25	25	0
	TP	25	12.2	51.2	25	14.5	42
		10					
	CS	25	23.65	5.4	25	20.35	18.6
	DB	25	21.42	14.32	25	20.1	19.6
	PC	25	21.5	14	25	21.5	14
	DG	25	25	0	25	25	0
	TP	25	21.9	12.4	25	19.9	20.4

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers - TP

Table 5: Sugar estimation in degradation reaction from crude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable temperature:

Parameter	**Sample**	**CL002**		**CL005**	
		Absorbance	**Concentration (mg/ml)**	**Absorbance**	**Concentration (mg/ml)**
Temperature		**30°C**			
	CS	0.910	1.810	1.180	2.363
	DB	0.070	0.089	0.170	0.294
	PC	0.040	0.027	0.960	1.913
	DG	0.030	0.007	0.040	0.027
	TP	1.210	2.425	1.040	2.076
		35°C			
	CS	0.940	1.872	0.760	1.503
	DB	0.160	0.273	1.180	2.363
	PC	0.190	0.335	0.810	1.605
	DG	0.030	0.007	0.030	0.007
	TP	1.150	2.302	0.810	1.605
		40°C			
	CS	1.240	2.486	1.120	2.240
	DB	0.860	1.708	1.040	2.076
	PC	1.250	2.507	1.280	2.568
	DG	0.040	0.027	0.030	0.007
	TP	1.390	2.794	1.350	2.712
		45°C			

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	CS	1.040	2.076	0.170	0.294
	DB	0.880	1.749	0.050	0.048
	PC	0.840	1.667	0.090	0.130
	DG	0.030	0.007	0.030	0.007
	TP	1.190	2.384	0.200	0.355
		50°C			
	CS	0.950	1.892	0.230	0.417
	DB	0.910	1.810	0.210	0.376
	PC	0.750	1.482	0.210	0.376
	DG	0.030	0.007	0.740	1.462
	TP	0.120	0.191	0.090	0.130

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers – TP

Table 6: Sugar estimation in degradationreaction fromcrude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable pH:

Parameter	Sample	CL002		CL005	
		Absorbance	Concentration (mg/ml)	Absorbance	Concentration (mg/ml)
pH		**3.000**			
	CS	0.940	1.872	1.180	2.363
	DB	0.050	0.048	0.170	0.294
	PC	1.120	2.240	0.960	1.913
	DG	0.030	0.007	0.040	0.027
	TP	1.040	2.076	1.040	2.076
		5.000			
	CS	0.930	1.851	0.760	1.503
	DB	0.910	1.810	1.180	2.363
	PC	0.630	1.236	0.810	1.605
	DG	0.030	0.007	0.030	0.007
	TP	0.870	1.728	0.810	1.605
		6.000			
	CS	0.040	0.027	1.120	2.240
	DB	0.080	0.109	1.040	2.076
	PC	0.170	0.294	1.280	2.568
	DG	0.030	0.007	0.030	0.007
	TP	0.940	1.872	1.350	2.712
		7.000			
	CS	0.200	0.355	0.170	0.294
	DB	0.940	1.872	0.050	0.048
	PC	0.160	0.273	0.090	0.130
	DG	0.030	0.007	0.030	0.007
	TP	0.910	1.810	0.200	0.355
		8.000			
	CS	0.870	1.728	0.230	0.417

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	DB	0.720	1.421	0.210	0.376
	PC	0.760	1.503	0.210	0.376
	DG	0.030	0.007	0.740	1.462
	TP	0.890	1.769	0.090	0.130

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers - TP

Table 7: Sugar estimation in degradation reaction from crude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable agitation speed:

Parameter	**Sample**	**CL002**		**CL005**	
		Absorbance	**Concentration (mg/ml)**	**Absorbance**	**Concentration (mg/ml)**
Agitation speed (rpm)		**100rpm**			
	CS	0.08	0.109	0.63	1.236
	DB	0.14	0.232	0.57	1.113
	PC	0.11	0.171	0.75	1.482
	DG	0.03	0.007	0.04	0.027
	TP	0.05	0.048	0.78	1.543
		150rpm			
	CS	1.290	2.589	1.24	2.486
	DB	1.130	2.261	1.28	2.568
	PC	0.940	1.872	0.98	1.953
	DG	0.030	0.007	0.03	0.0067
	TP	1.340	2.691	1.27	2.548
		200rpm			
	CS	0.940	1.872	0.86	1.707
	DB	0.950	1.892	0.47	0.908
	PC	0.970	1.933	0.67	1.318
	DG	0.030	0.007	0.04	0.0272
	TP	0.910	1.810	0.92	1.830
		250rpm			
	CS	0.150	0.253	0.11	0.170
	DB	0.060	0.068	0.14	0.232
	PC	0.120	0.191	0.06	0.068
	DG	0.030	0.007	0.04	0.027
	TP	0.080	0.109	0.04	0.027
		300rpm			
	CS	0.130	0.212	0.77	1.523
	DB	0.140	0.232	0.84	1.666
	PC	0.110	0.171	0.89	1.769
	DG	0.030	0.007	0.89	1.769
	TP	0.120	0.191	0.85	1.687

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers – TP

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Table 8: Sugar estimation in degradationreaction fromcrude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable proportion of crude enzyme

Parameter	Sample	CL002		CL005	
		Absorbance	Concentration (mg/ml)	Absorbance	Concentration (mg/ml)
Concentration (%)		2 %			
	CS	0.790	1.564	0.58	1.133
	DB	0.810	1.605	0.93	1.851
	PC	0.110	0.171	0.66	1.298
	DG	0.030	0.007	0.04	0.027
	TP	0.870	1.728	0.69	1.359
		4.0 %			
	CS	0.740	1.462	0.09	0.129
	DB	0.930	1.851	0.17	0.293
	PC	0.140	0.232	0.6	1.175
	DG	0.030	0.007	0.03	0.0067
	TP	0.970	1.933	0.85	1.687
		6.0 %			
	CS	1.240	2.486	0.71	1.400
	DB	1.330	2.671	0.23	0.416
	PC	1.370	2.753	0.68	1.339
	DG	0.030	0.007	0.03	0.0067
	TP	1.340	2.691	1.29	2.588
		8.0 %			
	CS	1.170	2.343	0.95	1.892
	DB	1.150	2.302	0.97	1.933
	PC	0.960	1.913	0.87	1.728
	DG	0.030	0.007	0.03	0.0067
	TP	1.300	2.609	0.95	1.892
		10.0 %			
	CS	0.060	0.068	0.88	1.748
	DB	0.110	0.171	0.74	1.461
	PC	0.100	0.150	0.81	1.605
	DG	0.030	0.007	0.9	1.789
	TP	0.090	0.130	0.74	1.461

***** Cotton swabs -CS; Dressings and bandages – DB; Plaster casts – PC; Discarded gloves – DG; Tissue and bits of papers – TP

Conclusion

The results obtained determined the variable degradation of different Cellulose content containing materials by the CL002 and CL005 bacterial isolates under various observance as per various Physiological conditions i.e Temperature, pH, Agitation speed. This concludes a good Cellulase content properties which could be further analyzed by Molecular Charcterization and sequencing of the isolates.

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