Optimization of Conditions and Assessment of Cellulolytic Activity from the Fungi Isolated from Garden Soil

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Abstract. This study focused on the evaluation of the cellulolytic fungi isolated from different garden soil samples collected from selected sites of Palakkad district (10.7867° N, 76.6548° E). Eight different fungal isolates were isolated and also identified. Then the carboxymethyl cellulose(CMC) screening was carried out of the eight isolates which were earlier labelled as I1,I2,I3,I4,I5,I6,I7,I8, from which four isolates showed increased zone of clearance:I1,I2,I3,I6. Primarily the estimation of protein was carried out followed by estimation of glucose solution by DNS method was done. = 6.18U/ mL, I6 = 7.90U/ mL showed aggrandized enzyme activity. After the screening the optimizations of the conditions (p^H and temperature) were recorded for 20 days with 5days of intervals. The I3 and I6 isolates at the fifteenth day at p^H 6 and temperature 50°C were observed to be more efficient. Maximum activity was found to be for the two isolates I3: p^H 6 -0.59OD, Temperature -0.57OD, I6: p^H 6-0.66OD, Temperature-0.75OD) and the I3, I6 isolate, were further assessed for the cellulolytic activity. These findings suggest that among I3, I6 isolates: I6(estimated as *Aspergillus niger*) isolate showed potential cellulolytic activity.

Key words: fungi, garden soil, cellulolytic activity, carboxymethyl cellulose(CMC), screening

Introduction

Fungi constitute the group of microorganisms that are widely distributed in environment especially in soil (Boer et al., 2005: 795-811). Since they produce broad variety of hydrolytic enzymes and hence exist in nature in the saprophytic mode (Ng, 2004: 1055-1073; Onion, 1981). Fungi in soil is a major occupant (Sreeremya and Shobana, 2018: 104-106). Fungi are one of the dominant groups present in the soil which strongly influence ecosystem structure and functioning and thus playing a pivotal role in many ecological services (Orgiazzi et al., 2012: e34847). Therefore, there is a growing interest in mainly assessing soil biodiversity and its biological functioning (Barrios, 2007). At the ecosystem scale, extracellular enzyme activity is influenced by the organic matter abundance and the composition (Sinsabaugh et al., 2008: 490-512).

Cellulose is the world's most abundant natural biopolymer and a biopotentially important source for the production of industrially useful materials such as the fuels and chemicals. Degradation of the cellulosic materials is mainly achieved chemically, enzymatically or by the combination of both the chemical and enzymatic methods (Bailey and Poutanen, 1987: 5-10; Christov, 1999; Christov et al., 1999: 511-517; Spreint, 1990: 511-518; Daniel, 2004: 199-204).

These microorganisms mainly influence above ground ecosystems by contributing to soil structure and the fertility among other roles (O'Dounell et al., 2001). Soil microorganisms are a valuable source of the natural products garnering important antibiotics for pharmaceuticals, enzymes and bioactive compounds for the industries (Strohl, 2000: 39-41). Since soil being a good habitat for the growth of the many number of microorganisms, majorly observed microorganisms are mainly bacteria: *Bacillus sp, Klebsiellasp, Pseudomonas sp, Serratia sp, Xanthomonas sp etc.* Many fungal species are also obtained from the higher termite soil, which encompass *Aspergillus sp, Phoma sp, Neurospora sp, Trichoderma sp, Penicillium sp.* The major actinomycetes species

observed are the *Streptomyces* sp,*Geosmin sp, Nocardia sp.* Cellulase consists of the three different types of enzymes named as endoglucanases, exoglucanses and the cellobiases. Several novel enzymes capable of degrading cellulose into sugars have insights from this discovery to create the high performance enzyme cocktail for processing the plant biomass into biofuel. (Abo Khatwa, 1977: 447-467). Fungal cellulases have proved to be a much better candidate than other microbial cellulases, with their secreted free cellulose complexes comprising all three components of cellulose. Cellulose is an bioenginnering tool which can have enormous uses (Sreeremya et al., 2016).

Material and Methods

Sample Collection

The garden soil sample was collected from three different regions of Palakkad district (10.7867^{O}N,76.6548^{O}E), Kerala. (Nelliyampati 10.5354° N, 76.6936° E, Kongad 10.8588° N, 76.5170° E, Malampuzha, 10.8281° N, 76.7368° E). The samples were collected, serially diluted and was spread plated**.**

Isolation of Fungi from Garden Soil

The garden soil samples were serially diluted and was also spread plated, from the three soil samples, the colonies with the visually distinguishable morphologies were selected and pure cultured on the PDA agar plates were labeled as I1,I2,I3,I4,I5$_{1}$,I6,I7,I8.

Morphological Examination

Eight isolates isolated from the garden soil were pure cultured and the morphological characterization were also observed.

Screening

The six isolates were inoculated by the spot inoculation and incubated at 24-48 hrs.1% of congo red solution was added to the spot inoculated plates and then excess stains were removed by using 1M NaCl and zone of clearance were observed (Sreeremya, 2017: 2-3).

Protein Assay

The culture broth after incubation was filtered through Whatmann no:1 filter and centrifuged at 7000 rpm for 5 min. About 500 µl of culture supernatant in each clean test tubeswere subjected to protein assay. Released fungal extracellular protein concentration were determined by Lowry ‘s Method (Lowry, 1951: 265-275).

Optimization of Physiological Conditions

Optimization of Temperature

Fungal isolates were inoculated in the basal media and 25°C for 48 hrs. The culture broth was keenly filtered through Whaatman no:1 filter paper then centrifuged at 7000 rpm for 5 min followed by the enzyme assay was carried out at different temperature from 20°C, 30°C ,40°C, 50°C, and 60°C, for 20 days. Enzyme activity was measured on fifth day of incubation. Enzyme activity was determined by the addition of CMC citrate buffer at p^{H} 5, the culture supernatant and after incubation for 15 min the dinitro salicylic acid is added to terminate the reaction. This assay was performed record at which temperature the enzyme activity was produced at a higher rate.

Optimization of p^{H}

Fungal isolates which was specifically inoculated at 25°C for 48 hrs in basal media. After incubation the culture was filtered and then centrifuged for 5-10minutes. The effect of p^{H} on enzyme activity was specifically determined by addition of the CMC, citrate buffer at p^{H} 5, culture filtrate, culture supernatant and after the incubation for around 15 min the

dinitro salicylic acid is added to terminate the reaction. Enzyme assay was performed in different p^H from p^H 3, p^H 4, p^H 5, p^H 6, p^H 7, p^H 8. This assay was carried to record at which temperature the enzyme activity was produced in an increased rate (Singh et al., 1988: 761-765).

Result and Discussion

Three different regions of Palakkad district (10.7867^{O}N,76.6548^{O}E), Kerala. (Nelliyampati, Kongad, Malampuzha). The soil samples were granular, humid and also appeared brownish to dark brownish in color (Fig. 2). The samples were collected, and was serially diluted and was spread plated**.** different soil samples were collected and maintained specifically at 4°C.

Spread plate technique was availed for the separation of mixed population of microorganisms so that individual colonies can be speciifcally isolated. For fungi 10^{-2} , 10^{-3} ,10^{-4} ,10^{-5}, dilution tubes, 1ml of suspension were transferred to petriplate and the inoculum was mixed by gentle rotation availing L rod. After 4 days of incubation at 25°C mixed population of microorganisms were obtained. Thus single colony of the microorganisms was formed, unique distinguishable fungal colonies were obtained from the soil collected from Malampuzha then by inoculating a loopfull of each specific colony of fungi to each potato dextrose agar plate. Incubated at for 6-7 days at 25°C. Eight different pure culture of fungus were isolated from three different soil samples from different parts of Kerala and were labeled as I1,I2,I3,I4,I5,I6,I7,I8 (Table1,2).The isolates inoculated on the PDA plates were analyzed for their colony morphology and the specific observations were tabulated in Table 1.

The Carboxy methyl cellulose assay (preliminary screening) to mainly asses the cellulose degrading efficiency, among the eight isolates mainly four isolates I1,I2,I3,I6 showed higher cellulose degrading ability (Fig. 1). This result is in accordance with the findings reported by Sreeremya (2017: 2-3). The protein content was estimated followed by assessing the enzyme activity(Enzyme activity = amount of glucose liberated(µg) / µg protein/ 30 mints).Enzyme activity of I1 = 4U/mL, I2 = 2.6U/mL, I3= 6.18U/ mL, I6 = 7.90U/ mL (Fig. 2 and Fig. 3).In the optimization of physiological conditions (temp, p^H studies among the four isolates two isolates with efficient cellulose degrading ability was taken I3 and I6 at p^H 6,15 thday,50 ^{O}C showed high cellulose degrading ability, I3 and I6 (Tables 3-10, Figs. 4-11) (Table 11, 12). Previously reported by Sreeremya et al. (2016), that the cellulose degrading ability and enzyme activity was assessed from lower termite soil.

Table 1. Cultural Characteristics

Sample	Colony Colour	Texture
I1	White	Glossy
I2	Brown	Spreaded
I3	Pink	Smooth
I4	White	Spreaded
I5	Yellow	Smooth
I6	Dark brown	Smooth
I7	White	Raised
I8	Creamish	Glossy

LCB mount observations of the isolated fungi were recorded in the Table 2.

Table 2. Microscopic Observation of Isolated Fungi

Sample	Spore Arrangement	Estimated genus as (Thrane et al., 2001)
I1	Intercalary chlamidiospores	*Candida sp*
I2	Bushyhead conidiospores	*Alternaria sp*
I3	Intercalary chlamidiospores	*Fusarium sp*
I4	Sporangiospores	*Rhizopus sp*
I5	Conidiospores	*Aspergillus sp (Aspergilllus flavus)*
I6	Conidiospores	*Aspergillus sp (Aspergillus niger)*
I7	Ascospores	*Phoma sp*
I8	Blastospores	*Trichospsoron sp*

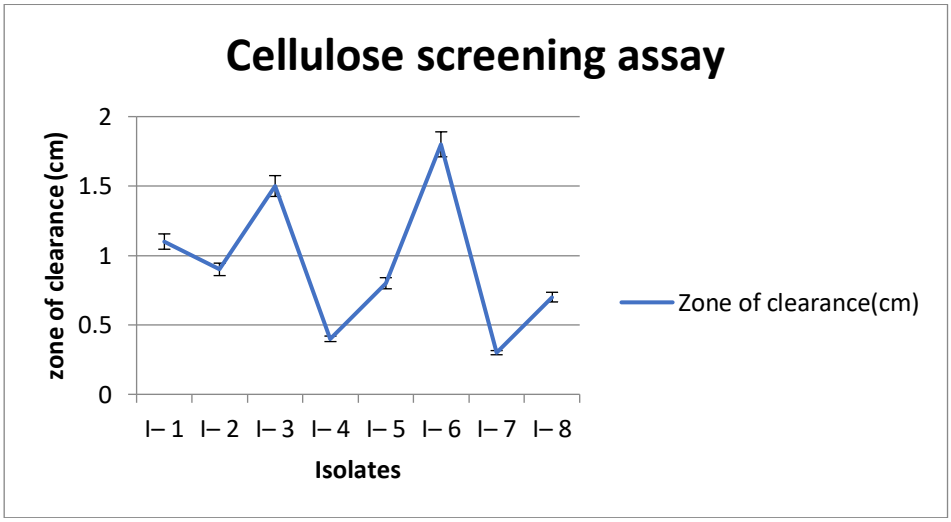

Fig. 1. Cellulose Screening

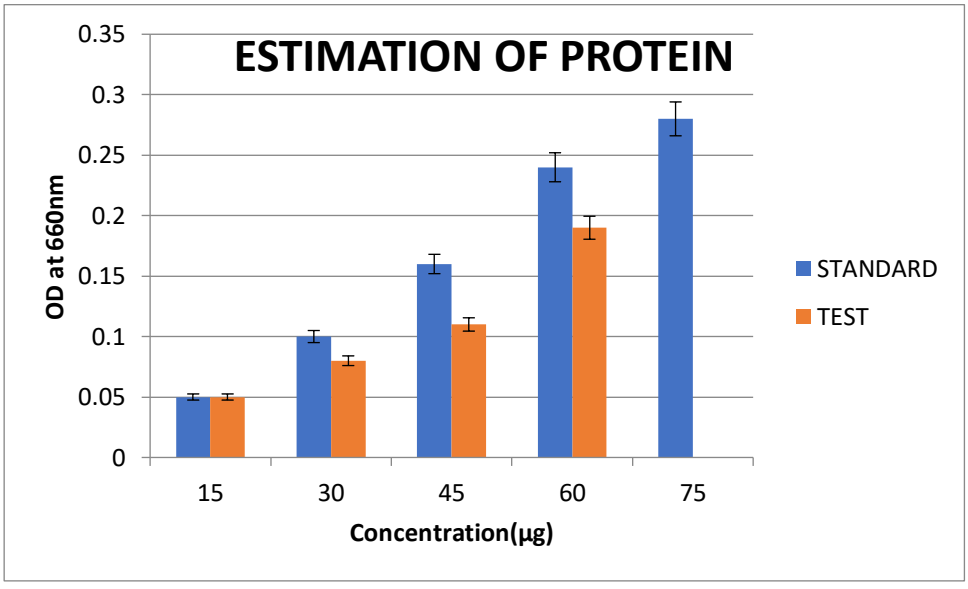

Fig. 2. Estimation of Protein

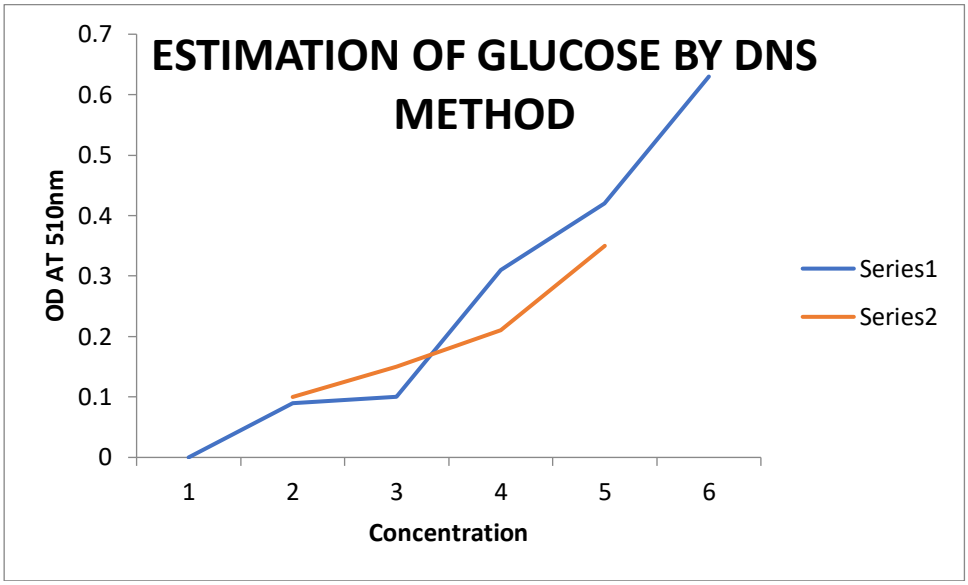

Fig. 3. Estimation of Glucose by DNS Method

Enzyme activity was assessed

(Enzyme activity = amount of glucose liberated(µg) / µg protein/ 30 mints).Enzyme activity of I1 = 4U/mL, I2 = 2.6U/mL, I3= 6.18U/ mL, I6 = 7.90U/ mL

Optimization of Phsiological Conditions

Table 3. Temperature -5th Day

SL NO:	Isolate	30°C	40°C	50°C	60°C	70°C	80°C
1	I1	0.06	0.20	0.36	0.29	0.33	0.31
2	I2	0.04	0.14	0.31	0.24	0.27	0.26
3	I3	0.08	0.26	0.42	0.39	0.35	0.37
4	I6	0.09	0.32	0.45	0.32	0.31	0.30

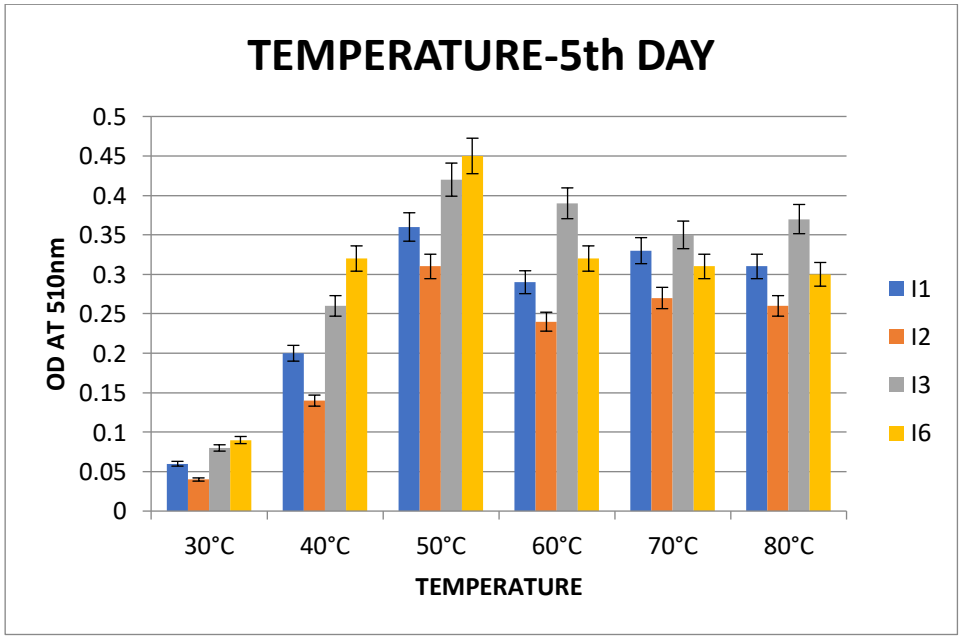

Fig. 4. Temperature -5th Day

Table 4. Temperature -10th Day

SL NO:	Isolate	30°C	40°C	50°C	60°C	70°C	80°C
1	I1	0.25	0.29	0.47	0.44	0.41	0.39
2	I2	0.10	0.14	0.30	0.29	0.22	0.24
3	I3	0.33	0.34	0.49	0.47	0.42	0.39
4	I6	0.35	0.38	0.56	0.50	0.54	0.57

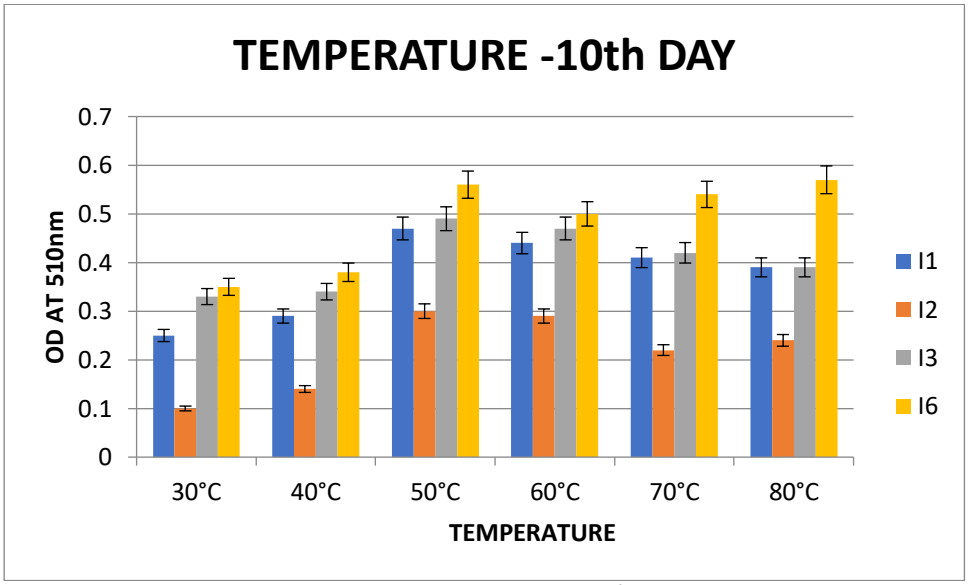

Fig. 5. Temperature -10th Day

Table 5. Temperature -15th Day

SL NO:	Isolate	30°C	40°C	50°C	60°C	70°C	80°C
1	I1	0.27	0.30	0.33	0.31	0.29	0.28
2	I2	0.13	0.16	0.31	0.28	0.22	0.20
3	I3	0.49	0.50	0.57	0.54	0.51	0.49
4	I6	0.51	0.52	0.66	0.63	0.60	0.57

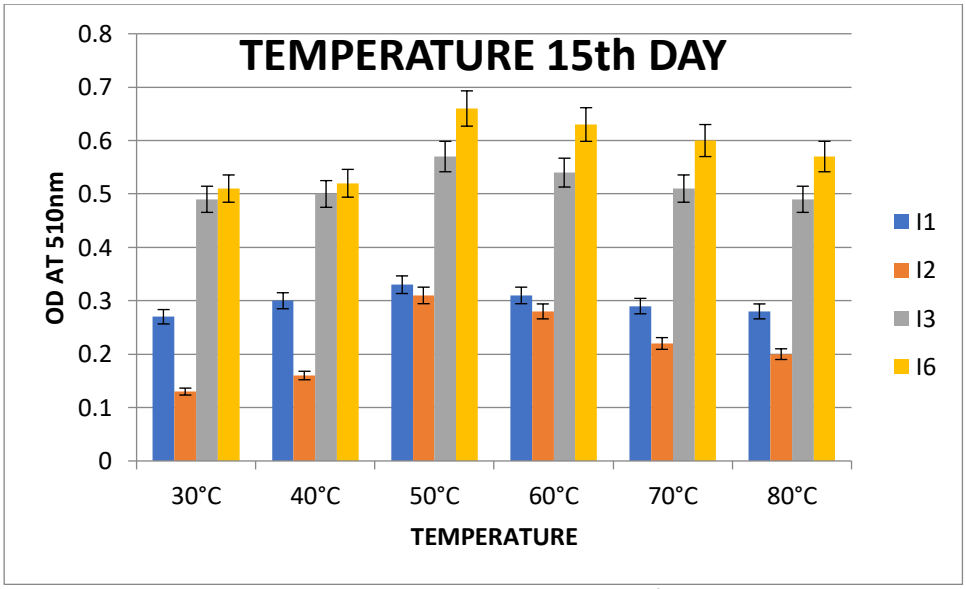

Fig. 6. Temperature -15th Day

Table 6. Temperature-20th Day (OD at 510nm)

SLNO:	Isolate	30°C	40°C	50°C	60°C	70°C	80°C
1	I1	0.24	0.26	0.30	0.27	0.22	0.19
2	I2	0.12	0.14	0.28	0.26	0.21	0.16
3	I3	0.39	0.49	0.54	0.52	0.47	0.41
4	I6	0.40	0.50	0.60	0.58	0.51	0.42

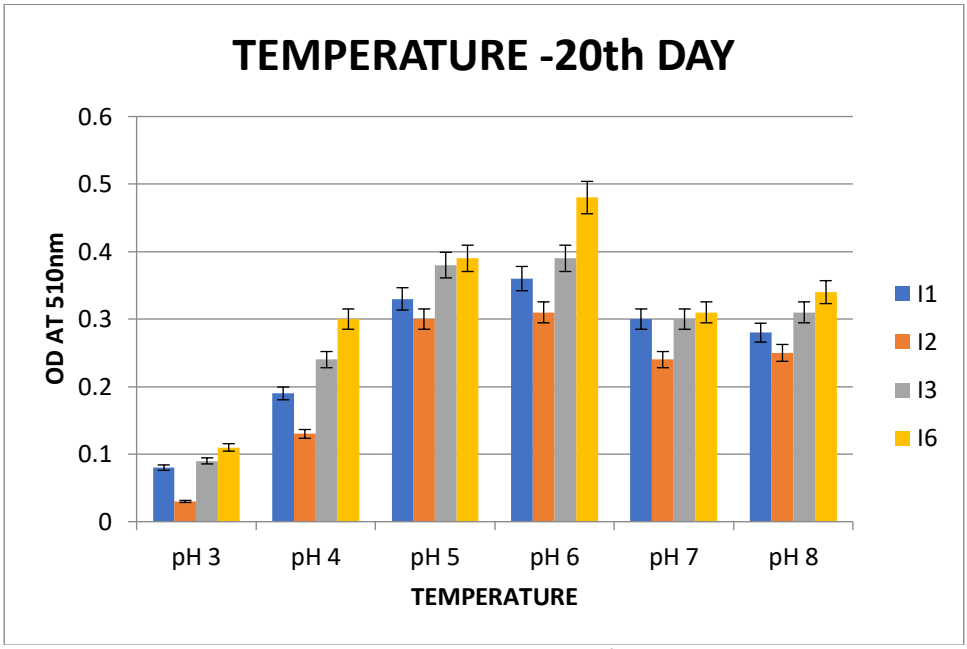

Fig 7. Temperature-20th Day

pH Data

Table 7. PH 5th Day (OD at 510nm)

SLNO:	Isolate	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
1	I1	0.08	0.19	0.33	0.36	0.30	0.28
2	I2	0.03	0.13	0.30	0.31	0.24	0.25
3	I3	0.09	0.24	0.38	0.39	0.30	0.31
4	I6	0.11	0.30	0.39	0.48	0.31	0.34

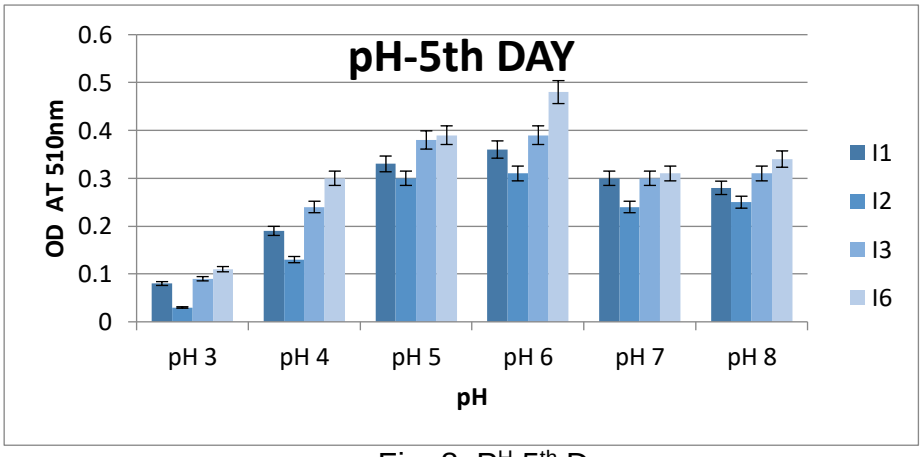

Fig. 8. PH 5th Day

Table 8. P^H 10 th Day (OD at 510nm)

SLNO:	Isolate	p^H 3	p^H 4	p^H 5	p^H 6	p^H 7	p^H 8
1	I1	0.22	0.23	0.33	0.47	0.40	0.34
2	I2	0.11	0.16	0.27	0.30	0.19	0.18
3	I3	0.31	0.37	0.38	0.54	0.44	0.39
4	I6	0.37	0.39	0.41	0.59	0.56	0.51

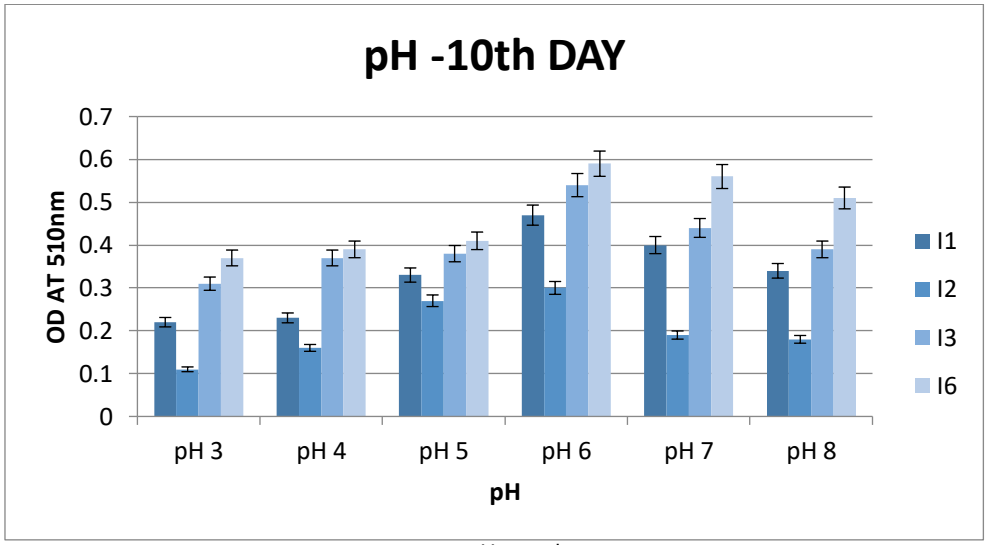

Fig. 9. P^H 10 th Day

Table 9. P^H 15th Day (OD at 510nm)

SLNO:	Isolate	p^H 3	p^H 4	p^H 5	p^H 6	p^H 7	p^H 8
1	I1	0.29	0.32	0.31	0.34	0.26	0.21
2	I2	0.18	0.19	0.23	0.25	0.17	0.19
3	I3	0.48	0.51	0.55	0.59	0.49	0.45
4	I6	0.56	0.53	0.63	0.75	0.66	0.60

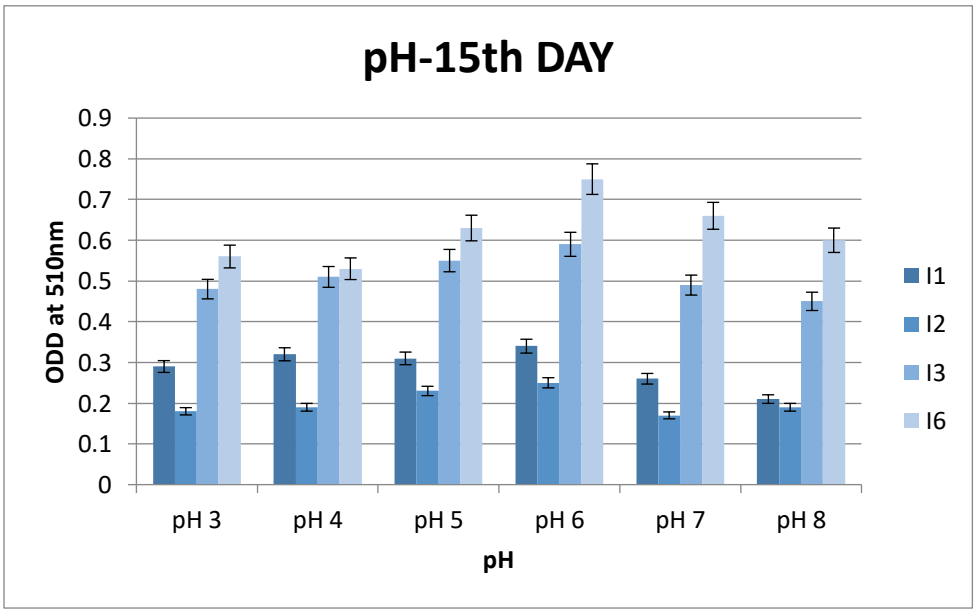

Fig. 10. P^H 15th Day

Table 10. P^H 20th Day (OD at 510nm)

SLNO:	ISOLATE	p^H 3	p^H 4	p^H 5	p^H 6	p^H 7	p^H 8
1	I1	0.21	0.22	0.26	0.29	0.24	0.23
2	I2	0.10	0.13	0.18	0.20	0.17	0.14
3	I3	0.34	0.44	0.39	0.49	0.43	0.40
4	I6	0.39	0.49	0.52	0.63	0.55	0.51

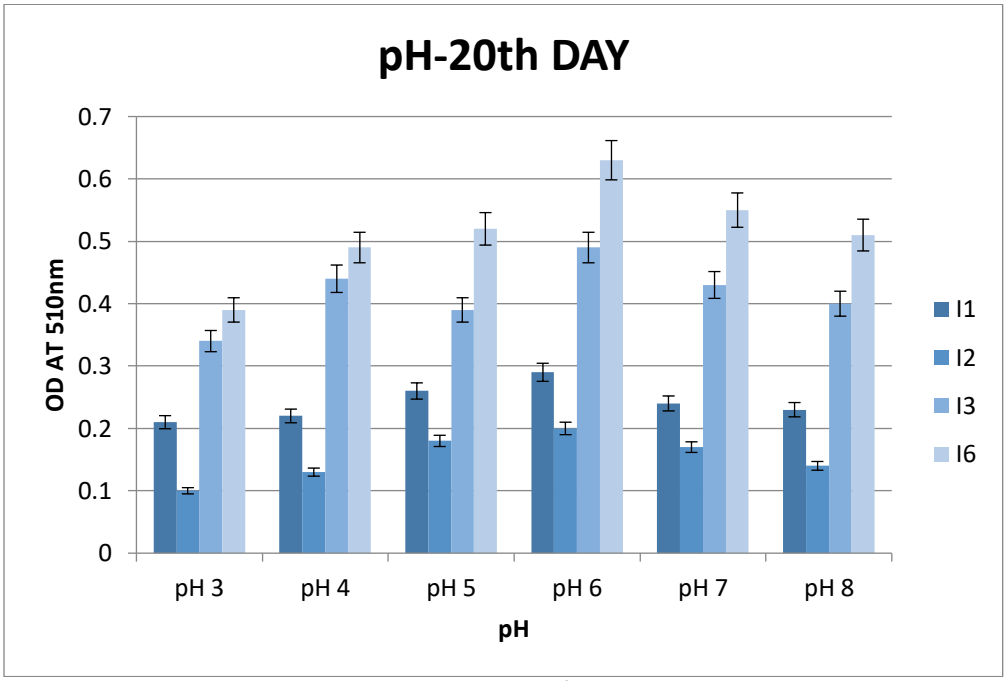

Fig 11. P^H 20th Day

Conclusion

These findings suggest that among I3, I6 isolates: I6 isolate showed potential cellulolytic activity. The biopotential I6 isolate was found to be *Aspergillus niger*. These fungi have tremendous application in the field of environmental biotechnology. It has the ability to degrade major lignocellulosic waste substrates like sugar cane baggase, corn cob, newspaper even some categories of biomedical waste too.

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