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# SCREENING OF CELLULASE PRODUCING MICROORGANISMS FROM LAKE AREA CONTAINING WATER HYACINTH FOR ENZYMATIC HYDROLYSIS OF CELLULOSE

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**ABSTRACT**

Microorganisms are capable to hydrolysis of cellulose, only a few of these produce significant quantities of cell free enzyme capable of completely hydrolyzing crystalline cellulose *in-vitro*. Fungi are the main cellulase producing microorganisms, through a few bacteria and actinomycetes have also been reported to yield cellulase activity like *Trichoderma Sp.*, *Aspergillus Sp., Pseudomonas Sp.*, *Cellulomonas Sp.*, *Bacillus Sp.,* and *Micrococcus Sp.* Most of the aquatic systems are infested with fast growing rate and high capacity for survival *Eichhornia crassipes* (water hyacinth). Plants (water hyacinth), water and soil collected from Coimbatore lakes and isolated thirty six species of microorganisms have been isolated, which were used for the enzyme (cellulase, xylanase) production. Ten fungi (F), eight bacteria (B), twelve yeasts (Y) and six actinomycetes (A) were used for degradation of cellulose and xylan for bio-fuel production. The cellulose and xylan activity of the organisms was measured diameter of a clear zone around the colony and hydrolytic value on cellulose Congo red agar media. Growth of organisms is very effective at temperature 28 ± 2 °C and pH 6.0 ± 0.4 in aerobic condition and 2 to 5 days of the incubation period.

**Keywords:** Cellulose, Xylan, Bio-fuel, Renewable Energy, Water hyacinth, Enzymatic hydrolysis

## 1. INTRODUCTION

Cellulose found in nature exclusively in plant cell walls and produces by some animals like tunicates and few bacteria. An inexhaustible supply of perpetual renewable source such ligno-cellulose ensures *via* the process of photosynthesis [1]. Cellulose is a linear, unbranched homo-polysaccharide of glucose subunit joined *via* β 1-4 glycosidic linkages and totally insoluble in water. Single molecule of Cellulose (polymer) usually arranged in bundles or fibrils and varies widely in length and can occur in crystalline or paracrystalline (amorphous) structures [2-5]. Lignocellulose is most abundant bio-energy source available for soil microorganisms. All microorganisms do not have same ability to produce cellulase and xylanase enzyme and breaking down lignocelluloses to simple reducing sugar. The ability to produce cellulase enzyme is widespread among microorganisms and this has become the subject of extensive investigation [6]. Cellulolytic fungi and bacteria play an important role in nature's biodegradation of plant lignocellulosic material are efficiently degraded by cellulolytic enzyme [7]. The enzyme or multienzyme-complex involved in the hydrolysis of cellulose name as cellulases [1, 2]. Generally, three major types of cellulolytic enzyme: endoglucanase, exoglucanase and cellobiohydrolase produce by microorganisms which are inductive in nature and extracellular [7, 8, 9]. Basic enzymatic hydrolysis process of cellulose: (I) hydrolyses internal β-1, 4 glucan chain of cellulose at random by Endoglucanase (EG or CX), which display low hydrolytic activity toward crystalline cellulose within amorphous regions; (II) cellobiohydrolases (CBH), removes non-reducing end of cello-oligosaccharide and of crystalline, amorphous and acid or alkali treated cellulose from cellobiose by Exoglucanase; (III) complete depolymerization of cellobiose by hydrolysis of cellulose to glucose molecule by Cellobiase or β-glucosidase (BGL) [2,4,10, 11,12]. In industrial applications cellulases have enormous potential which produce glucose from ligno-cellulosic substrate and subsequent fermentation of glucose yield to valuable end products. Cellulase enzyme have found novel application in industries like foods and manufactured goods such as butanol, methane, ethanol, single-cell protein, amino acid, paper, rayon, cellophane, production and processing of chemicals and extensively utilized for extraction of valuable components from plant cells, preparation of plant protoplasts in genetic research and improvement of nutritional values of animal feed [7, 13]. Cellulases have also used for several years in feed preparation, food processing, detergent formulation, textile production, production of wine, beer and fruit juice, waste-water treatment and in other areas [2, 4, 14]. Considering the importance and application of the cellulases and xylanase, this study aimed to screen mainly fungi, yeast,

actinomycetes, and bacteria isolated from the various locations of Coimbatore city and source like root soil, water, and plant.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection

Sampling sites was chosen that have the large number of ligno-cellulolytic and xylenolytic resident microbial population. Water hyacinth plant, water, and soil samples were collected from different areas of Coimbatore ponds. The water and soil sample were collected from the surface of water hyacinth plant.

Samples were collected from the fresh water lake in the city of Coimbatore, Tamil Nadu, India, in the rainy months (August) of the year 2012. Two fresh water ponds namely, Big lake (Periya Kulam Lake) (Latitude (10.979065) and Longitude (76.959969) and Valan Kulam Lake (Latitute (10.993052) and Longitude (79.969796) were selected in a random manner in Coimbatore, Tamil Nadu, India, for cellulase and xylanase producing organisms from water hyacinth plant, surrounded water and soil. These lakes were contaminated with water hyacinth plant. The details of the sampling sites are given in Table 1.

***Table 1. Occurrence of water hyacinth plant in fresh water ponds in Coimbatore city.***

| S. No. | Sample place | Sample Name | Sample No. | Type of sample | Amount of sample |
|---|---|---|---|---|---|
| 1. | Big lake (Periya Kulam lake), fresh water lake, Coimbatore. | L1S1 | P1 | Plant | 100ml (water), 1 (plant), 10g (soil) from each sampling site |
| | | | W1 | Water | |
| | | | S1 | Root Soil | |
| | | L1S2 | P2 | Plant | |
| | | | W2 | Water | |
| | | | S2 | Root Soil | |
| 2. | Valan Kulam, fresh Water Lake, Coimbatore. | L2S3 | P3 | Plant | |
| | | | W3 | Water | |
| | | | S3 | Root Soil | |
| | | L2S4 | P4 | Plant | |
| | | | W4 | Water | |
| | | | S4 | Root Soil | |

### 2.2. Serial dilution and spread plating methods

One g of plant material was cut into small pieces using a sterile scalpel blade, 1ml of water sample and 1g of soil sample suspended in 10mL of sterile saline water (0.85% NaCl) and vortexed it. A series of dilutions from $10^{-1}$ to $10^{-7}$ were made, these dilutions were used in the spread plate method [7]. The resulting serial dilution of plant material, soil and water (0.1 mL) was pipetted on different agar media in triplicates according to microorganisms. Dilutions of $10^{-3}$ to $10^{-5}$ were used for fungi on potato dextrose agar (PDA) (M096, HiMEDIA, India), dilutions of $10^{-4}$ to $10^{-6}$ were used for yeast, actinomycetes on yeast malt agar (YMA) (M425, HiMEDIA, India) and starch casein agar (SCA) (M801, HiMEDIA, India) respectively, and dilutions of $10^{-5}$ to $10^{-7}$ were used for bacteria on nutrient agar (NA) (M001, HiMEDIA, India). All culture media supplemented with 1% CMC (carboxymethyl cellulose). All agar plates were incubated at 30° C and different incubation time for each microorganism. Fungi were incubated for 6-7 days, yeast incubated for 2-3 days, actinomycetes and bacteria incubated for 1-2 days.

### 2.3. Microscopy

Labomed – iVU3000 microscope used for microscopy. LPCB (lacto phenol cotton blue) stain used to visualize the morphology of the filamentous fungal spores.

### 2.4. Screening of Cellulase and Xylanase producing microorganisms

#### *2.4.1. Primary screening*

*2.4.1.1. Congo red test*

Preliminary qualitative analysis conducted by using Congo red dye for cellulolytic microorganisms. Microorganisms were grown on CMC/xylan agar containing NaCl 0.5, $KH_2PO_4$ 1.0, $MgSO_4.7H_2O$ 0.5, $MnSO_4.H_2O$ 0.01, $NH_4NO_3$ 0.3, $FeSO_4.7H_2O$ 0.01, CMC/xylan 10.0, Agar 12.0 (g/L) at 7.0 pH. For the secretion of cellulase enzyme CMC agar plates were incubated at 30±2°C for 7-8 days. After incubation culture plates are flooded with 0.1% Congo red solution for 15 minutes and Congo red solution poured off and further plates washed by flooding with 1M NaCl for 10 minutes. A clear zone formation around the microbial colonies

indicates the hydrolysis of cellulose or CMC. The highest cellulase activity producer select by measured the ratio of the colony diameter to clear zone diameter. The highest activity assumed by the largest clear zone ratio [15, 16].

*2.4.1.2 Well diffusion method*

The modified agar well diffusion method employed to measure cellulase activity of crude enzyme. Sterile agar contains 1% CMC poured in sterile petri plates and after solidified agar punched with a six millimeters diameter wells. Wells filled with 100 µL of pasteurized crude enzyme and blanks (sterile distilled water). Crude enzyme exposed to a temperature of about 60° C for 30 min and after 30 minutes and kept at 4° C. The test was carried out by triplicate. The plaques were incubated at 30 ± 2°C for 24 h. After incubation culture plates were flooded with 0.1% Congo red (30025G25, SDFCL, India) solution for 15 minutes and Congo red solution poured off and further plates were washed by flooding with 1M NaCl for 10 minutes. A clear zone formation around the microbial colonies indicates the hydrolysis of cellulose or CMC. The highest cellulase activity showing colonies were selected by measuring the ratio of the colony diameter to clear zone diameter. The highest activity assumed by the largest clear zone ratio [17].

*2.4.1.3. Test tube assay*

Primary screening was made by minimal basal salt media supplementing with cellulose and xylan. The uniform distribution of cellulose and xylan agar tubes was allowed to solidify. Isolated microbial cultures were inoculated on agar slant and tubes were incubated at 30±2°C for 7 days and measured the vertical zone of clearance [2].

***2.4.2 Secondary screening***

Large clearing zones presenting potential microbes in Congo red test used in enzyme production for secondary screening. Minimal basal salt medium supplemented $(NH_4)2SO_4$, 1.4 g; urea, 0.3 g; $KH_2PO_4$, 2.0 g; $MgSO_4$-$7H_2O$, 0.3 g; $CaCl_2$, 0.3 g is used in g/L with 1% cellulose/xylan at 5.3 pH adjusted with 1N NaOH and 1N HCl used for enzyme production. Trace elements like $FeSO_4$ $7H_2O$, $ZnCl_2$, $MnSO_4$ $H_2O$, $CoCl_2$ $6H_2O$, $CuSO_4$ $.5H_2O$, and $20MO_3$. $2H_3PO_4$. $48H_2O$ are added with the concentration for cellulase and xylanase production is Fe, 1.0; Zn, 0.8; Mn, 0.5; and Co, 0.5 ppm. 250 mL Erlenmeyer flask filled with 100 mL of the above medium and used shake flask technique. After autoclaving 5 mL of 6-day-old specific culture broth of potential microbes were inoculated in flask. Triplicate Cultures are incubated at 28 ± 2° C for 15 days and determined pH, cellulose, xylose, reducing sugar concentration and enzyme activity by the anthrone and dinitrosalicylic acid method at regular interval [16, 18, 19].

## 3. RESULTS

### 3.1. Serial dilution and Spread plating methods

The numbers of microorganisms isolated from fresh water ponds. A total of 36 isolates were isolated; of these, 8 fungi (F) (identified by LPCB method), 8 bacteria (B) (identified by biochemical tests), 14 yeasts (Y) (not identified) and 6 actinomycetes (A) (not identified) were used for degradation of cellulose and xylan for bio-fuel production. These were FF-4 (*Basidiobolus ranarum*), FF-6 (*Aspergillus fumigates*) FF-8 (*Arthroderma benhamiae*), FF-9 (*Aspergillus niger*), FF-10 (*Penicillium marneffei*), FF-11 (*Penicillium chrysogenum*), FF-15 (*Alternaria alternate*), FF-18 (*Fusarium solani*), BB-3 (*Bacillus Sp.*), BB-6 (*E. Coli*), BB-7 (*Staphylococcus Sp.*), BB-8 (*Proteus Sp.*), BB-9 (*Klebsiella Sp.*), BF-3 (*Staphylococcus Sp.*), BF-5 (*Pseudomonas Sp.*), BF-17 (*Bacillus Sp.*), YB-1, YB-2, YB-4, YB-10, YB-11, YF-1, YF-2, YF-7, YF-14, YF-19, YF-20, YF-21, YF-22, YAN-1, AB-4E, AB-5, AF-12, AF-13, AF-17, AF-23. All the microbes were grown in different culture media supplemented with CMC and shown in Table 2. The most frequent fungi were *Alternaria alternate*, *Penicillium marneffei*, *Aspergillus niger*, *Penicillium chrysogenum* and bacteria were B-1, B-9, A-4E, and A-5.Most of the above isolates have been reported as cellulase producers [16].

***Table 2. Isolates isolated from water hyacinth plant and surrounded water and soil grown at different location of Coimbatore city***

| S. No. | Sample | Dilution | Microorganisms | CFUs (Colony Forming Units) |
|---|---|---|---|---|
| 1. | S1 | $10^{-3}$ | Y-1, Y-2, F-4 | $3.56 \times 10^6$ |
| | | $10^{-4}$ | Y-3, Y-5 | $2.69 \times 10^7$ |
| | | $10^{-5}$ | B-1, B-3, B-4 | $1.60 \times 10^8$ |
| | | $10^{-6}$ | B-2 | $9.20 \times 10^8$ |
| 2. | S2 | $10^{-3}$ | F-6, F-8, F-9 | $1.54 \times 10^6$ |
| | | $10^{-4}$ | Y-7, B-6, A-4E | $8.90 \times 10^6$ |
| | | $10^{-5}$ | B-5, A1 | $1.60 \times 10^7$ |
| 3. | S3 | $10^{-3}$ | F-10, F-12, F-13 | $2.12 \times 10^6$ |
| | | $10^{-4}$ | F-11, Y-14 | $1.34 \times 10^7$ |
| | | $10^{-6}$ | B-7, B-8, B-9 | $2.50 \times 10^8$ |
| 4. | S4 | $10^{-3}$ | F-15, F-16, Y-19, Y-20 | $1.95 \times 10^6$ |
| | | $10^{-4}$ | Y-21, Y-22, A-23 | $1.15 \times 10^7$ |
| | | $10^{-5}$ | F-17 | $4.10 \times 10^7$ |
| | | $10^{-6}$ | B-10, B-11, F-18 | $9.00 \times 10^7$ |

### 3.2. Primary screening (Congo red test, well diffusion method test and Test tube assay)

The results of Preliminary qualitative analysis showed that hydrolysis of cellulose by testing isolates differ from organism to organism. Among the 36 isolated microorganisms, only 25 were producing extracellular enzyme production after 4 days of incubations and showed clear zone in Congo red test. Extracellular enzyme activity of microorganisms on Congo red and well diffusion agar plate maximum 45 mm zone of clearance was observed shown in Table 3 and 4 indicating them to be cellulose and xylan hydrolysis. In the 36 isolates, 27 different isolates were tested for the production of cellulolytic and xylanolytic enzyme (secondary screening). The diameter of the yellow halo varied from organism to organism showed the presence or absence of the cellulase production (Table 4).

***Table 3. CMC and xylan Congo red microbial clear zone diameter (mm)***

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fungi** | Culture Name | FF-4 | FF-6 | FF-8 | FF-9 | FF-10 | FF-11 | FF-15 | FF-18 |
| | CMC supplemented agar | 8mm | 12mm | 15mm | 16mm | 30mm | 41mm | 20mm | 3mm |
| | Xylan supplemented agar | 4mm | 6mm | 16mm | 11mm | 20mm | 14mm | 5mm | 8mm |
| **Yeast** | Culture Name | YB-1 | YB-2 | YB-4 | YB-10 | YB-11 | YF-1 | YF-2 | YF-7 |
| | CMC supplemented agar | 27mm | 0mm | 12mm | 20mm | 0mm | 5mm | 4mm | 4mm |
| | Xylan supplemented agar | 35mm | 14mm | 14mm | 0mm | 9mm | 0mm | 14mm | 0mm |
| | Culture Name | YF-14 | YF-19 | YF-20 | YF-21 | YF-22 | YAN-1 | | |
| | CMC supplemented agar | 10mm | 0mm | 0mm | 7mm | 9mm | 0mm | | |
| | Xylan supplemented agar | 5mm | 15mm | 0mm | 4mm | 14mm | 0mm | | |
| **Bacteria** | Culture Name | BB-3 | BB-6 | BB-7 | BB-8 | BB-9 | BF-3 | BF-5 | BF-16 |
| | CMC supplemented agar | 0mm | 0mm | 10mm | 8mm | 3mm | 0mm | 20mm | 8mm |
| | Xylan supplemented agar | 0mm | 0mm | 12mm | 0mm | 25mm | 10mm | 14mm | 0mm |
| **Actinomycetes** | Culture Name | AB-4E | AB-5 | AF-12 | AF-13 | AF-17 | AF-23 | | |
| | CMC supplemented agar | 15mm | 15mm | 0mm | 0mm | 10mm | 10mm | | |
| | Xylan supplemented agar | 22mm | 12mm | 0mm | 0mm | 14mm | 0mm | | |

### 3.3. Secondary Screening

It is evident from the results that maximum cellulases and xylanase producing microorganisms shown in Table 5. Cellulase and xylanase activity was observed after the 7th day of incubation at 30° C in FF-6 (1.1 mg/mL), FF-9 (1.9 mg/mL), FF-10 (2.1 mg/mL), and FF-11 (2.1 mg/mL). Out of the 25 cellulase and xylanase producing isolates in five isolates (FF-6, FF-9, FF-10, and FF-11) have maximum enzyme activity was observed but FF-11 and FF-10 found good cellulase producers. In the present investigation, FF-11 was a potential strain in the secondary screening and used further utilization of water hyacinth for production of value-added product.

### 3.4. Microorganism Identification

Microorganisms identified based on colony characterization, biochemical test, microscopic methods and molecular method. Fungus identified based on colony characterization and microscopic methods, FF-4 (*Basidiobolus ranarum*), FF-6 (*Aspergillus fumigates*) FF-8 (*Arthroderma benhamiae*), FF-9 (*Aspergillus niger*), FF-10 (*Penicillium marneffei*), FF-11 (*Penicillium chrysogenum*), F-15 (*Alternaria alternate*), F-18 (*Fusarium solani*). Bacteria identified based on characterization, biochemical test, microscopic methods, BB-3 (*Bacillus Sp.*), BB-6 (*Staphylococcus Sp.*), BB-7 (*E. Coli*), BB-8 (*Proteus Sp.*), BB-9 (*Klebsiella Sp.*), BF-3 (*Staphylococcus Sp.*), BF-5 (*Pseudomonas Sp.*), BF-16 (*Bacillus Sp.*). Yeast and actinomycetes are not identified. All the colony characterization, biochemical test and microscopic methods were shown in Table 6 and Figure 1.

*Table 4. CMC and xylan Congo red clear zone diameter (mm) by well diffusion method*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fungi** | Culture Name | FF-4 | FF-6 | FF-8 | FF-9 | FF-10 | FF-11 | FF-15 | FF-18 |
| | CMC supplemented agar | 4mm | 5mm | 9mm | 20mm | 34mm | 45mm | 18mm | 0mm |
| | Xylan supplemented agar | 2mm | 2mm | 4mm | 15mm | 22mm | 18mm | 8mm | 3mm |
| **Yeast** | Culture Name | YB-1 | YB-2 | YB-4 | YB-10 | YB-11 | YF-1 | YF-2 | YF-7 |
| | CMC supplemented agar | 20mm | 0mm | 6mm | 9mm | 0mm | 2mm | 2mm | 5mm |
| | Xylan supplemented agar | 25mm | 9mm | 8mm | 0mm | 5mm | 0mm | 8mm | 0mm |
| | Culture Name | YF-14 | YF-19 | YF-20 | YF-21 | YF-22 | YAN-1 | | |
| | CMC supplemented agar | 14mm | 0mm | 0mm | 8mm | 5mm | 0mm | | |
| | Xylan supplemented agar | 8mm | 8mm | 0mm | 5mm | 9mm | 0mm | | |
| **Bacteria** | Culture Name | BB-3 | BB-6 | BB-7 | BB-8 | BB-9 | BF-3 | BF-5 | BF-16 |
| | CMC supplemented agar | 0mm | 0mm | 12mm | 9mm | 5mm | 0mm | 22mm | 10mm |
| | Xylan supplemented agar | 0mm | 0mm | 11mm | 0mm | 20mm | 11mm | 12mm | 0mm |
| **Actinomycetes** | Culture Name | AB-4E | AB-5 | AF-12 | AF-13 | AF-17 | AF-23 | | |
| | CMC supplemented agar | 0mm | 12mm | 0mm | 0mm | 9mm | 12mm | | |
| | Xylan supplemented agar | 0mm | 9mm | 0mm | 0mm | 15mm | 0mm | | |

*Table 5. Cellulase and xylanase producing microorganisms*

| | | | | | | |
|---|---|---|---|---|---|---|
| Cellulase producing | Fungi | FF-4 | FF-6 | FF-8 | FF-9 | FF-10 |
| | | FF-11 | FF-15 | | | |
| | Yeast | YB-1 | YB-4 | YB-10 | YF-1 | YF-2 |
| | | YF-7 | YF-14 | YF-21 | YF-22 | |
| | Bacteria | BB-7 | BB-8 | BB-9 | BF-5 | BF-16 |
| | Actinomycetes | AB-4E | AB-5 | AF-17 | AF-23 | |
| Non Cellulase producing | Fungi | FF-18 | | | | |
| | Yeast | YB-11 | YF-19 | YF-20 | YAN-1 | |
| | Bacteria | BB-3 | BB-6 | BF-3 | | |
| | Actinomycetes | AF-12 | AF-13 | | | |
| Xylanase producing | Fungi | FF-4 | FF-6 | FF-8 | FF-9 | FF-10 |
| | | FF-11 | FF-15 | FF-18 | | |
| | Yeast | YB-1 | YB-2 | YB-4 | YB-11 | YF-2 |
| | | YF-14 | YF-19 | YF-21 | YF-22 | |
| | Bacteria | BB-7 | BB-9 | BF-3 | BF-5 | |
| | Actinomycetes | AB-4E | AB-5 | AF-17 | | |
| Non Xylanase producing | Fungi | | | | | |
| | Yeast | YB-10 | YF-1 | YF-7 | YF-20 | YAN-1 |
| | Bacteria | BB-3 | BB-6 | BB-8 | BF-16 | |
| | Actinomycetes | AF-12 | AF-13 | AF-23 | | |

*Table 6. Biochemical test for bacterial isolates*

| Organism | Gram's Stain | Shape | Motility | IMVIC | | | | TSI | Catalase | Urease Test | Gelatin Test | Starch Hydrolysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Indole | MR | VP | Citrate | | | | | |
| BB-3 | (+) | Bacilli | (+) | (-) | (-) | (+) | (-) | (-) | (-) | (-) | (+) | (+) |
| BB-6 | (+) | Diplo- Cocci | (-) | (-) | (+) | (-) | (-) | (+) | (+) | (-) | (+) | (-) |
| BB-7 | (-) | Bacilli | (+) | (+) | (+) | (-) | (-) | (+) | (+) | (-) | (-) | (-) |
| BB-8 | (-) | Bacilli | (+) | (+) | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (-) |
| BB-9 | (-) | Bacilli | (+) | (-) | (-) | (+) | (+) | (-) | (+) | (+) | (-) | (-) |
| BF-3 | (+) | Cocci | (-) | (-) | (+) | (-) | (-) | (+) | (+) | (-) | (+) | (-) |
| BF-5 | (-) | Bacilli | (+) | (-) | (-) | (-) | (+) | (-) | (+) | (-) | (+) | (-) |
| BF-16 | (-) | Bacilli | (+) | (-) | (-) | (+) | (-) | (-) | (-) | (-) | (+) | (+) |

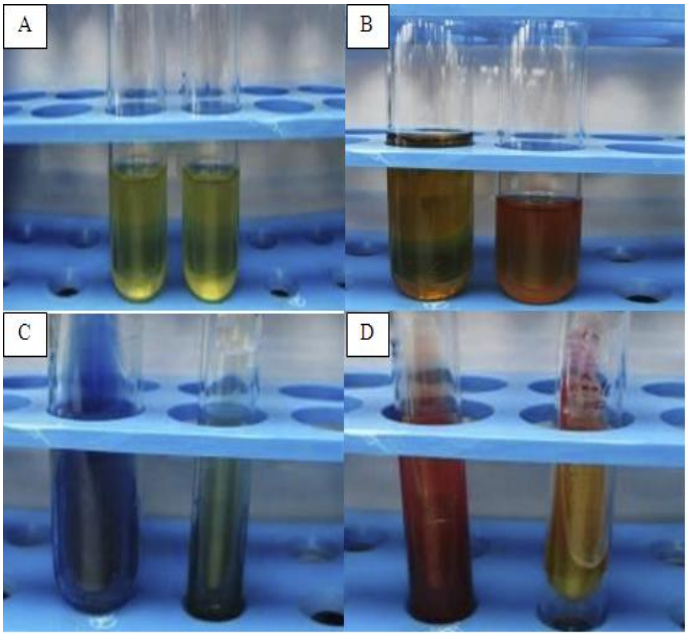


***Figure 1. Biochemical Tests Indole test (A); MRVP test (B); Citrate test (C); TSI (D).***

## 4. DISCUSSION

The most abundant organic substance in world is cellulose and comprises from photosynthetic storage of glucose which is a major component of biomass energy [20]. In the biological cycle decomposition of lignocelluloses has a special significance because a large proportion of vegetation added to soil is lignocelluloses [21]. These enzymes have novel application found in industries such as production and processing of food, chemicals, improvement of nutritional values of animal feed, extraction of valuable components from plants, and manufactured rayon, paper etc. [22]. Fungi are well known decomposer of organic matter such as cellulose, hemicelluloses etc. substrate reported by researchers [1].

Fungi have various active roles in soils and water, they serve as decomposers and symbionts such as the degradation of dead plant material due to the activity of different types of enzymes, or inactive roles where propagates are present in the soil as resting states. Therefore, soils and some water bodies have been preferred for the isolation of cellulase producing fungi [1]. Exactly identification of fungi which are present in a soil and water sample is no easy task; one of the major jobs being the fastidious nature of the great majority of species. Water hyacinth has 40-60% cellulose and hemicellulose and the characteristics of these plants, it was assumed that cellulase producing water fungi would be highly diverse having enzymes with interesting activities. The soil and water dilution plate method was used for the enumeration and isolation of fast growing cellulolytic fungi on a basal medium supplemented with CMC. 36 types of microorganisms are may be saprophytic fungi, bacteria, yeast and actinomycetes in different types of pond water, soils and plant. All isolated fungi identified by LPCB (Lacto-Phenol Cotton Blue) stain and microscopic method and compare results with Medical Mycology Research Center (MMRC), Chiba University, Japan.

F-4 (*Basidiobolus Sp.*), F-6 (*Aspergillus Sp.*) F-8 (*Arthroderma Sp.*), F-9 (*Aspergillus Sp.*), F-10 (*Penicillium Sp.*), F-11 (*Penicillium chrysogenum Sp.*), F-12 (*Geotrichum Sp.*), F-13 (*Arthrographis Sp.*), F-15 (*Alternaria Sp.*), F-18 (*Fusarium solani Sp.*). Due to the selection procedure final selected strains producing high cellulase levels were members of the *Penicilli*, *Aspergilli* and *Alternaria* groups since they are fast growing and highly sporulating fungi [23]. Out of thirty six different microorganisms' isolates that grew on the CMC and xylan supplemented medium, 11 were finally selected by using the CMC and Congo red plate clearing assay. In these 4 fungal *Sp.* selected for enzyme production that have greater clear zone. This assay gives a fairly good correlation with production assays and useful for the rapid screening of fungal colonies under several conditions. The glucose concentration during enzyme

production, which shows a glucose level increase up to third day and after the third day rapid decrease in glucose level. In batch culture microorganisms producing cellulase and xylanase enzyme, which hydrolyses cellulose and xylan and increase glues level in culture media and utilize that glucose for growth [23]. The cellulose concentration in batch culture, which shows a cellulose concentration decrease slowly up to third day then it was decrease faster rate because after third day microbial concentration increase and that produce large amounts of cellulase and hydrolyses cellulose to glucose for growth. In this sense, three strains (*Aspergillus Sp.* FF-6, and FF-9, *Penicillium Sp.* FF-10, and FF-11) have shown to be suitable for cellulase production under batch conditions as they also have higher specific productivities. Optimal cellulase yield and time is depending on species to species and also depend on substrate. Time is shorten when incubated on pure cellulose compare to lignocellulosic materials. In the present study FF-6, FF-9, FF-10, and FF-11 were best organisms for cellulase production using pure cellulose as a carbon source. On the other hand FF-4, FF-8, FF-15, and FF-18 gave the least cellulase activity compare to other four fungi [24].

Among the 36 isolates 8 fungal strains were able to isolate few strains of *Basidiobolus Sp.*, *Aspergillus Sp.*, *Arthroderma Sp.*, *Aspergillus niger Sp.*, *Penicillium Sp.*, *Penicillium Sp.*, *Geotrichum Sp.*, *Arthrographis Sp.*, *Alternaria Sp.*, *Fusarium Sp.* which utilize basal media supplemented with cellulose as carbon source. Among the 8 fungal isolates we were able to isolate only few strains of *Aspergillus Sp.*, *Penicillium Sp.*, which is the most extensively studied cellulase producer. Majority of *Fusarium, Aspergillus*, *Penicillum*, *Alternaria*, *Rhizopus*, and *Trichoderma* isolates were found to possess cellulytic activity.

A wide range of enzymes produces by *Aspergillus Sp.*that capable of degrading plant cell wall polysaccharide. A wide range of *Aspergillus Sp.* has been reported to produce all component of cellulase enzyme system which is part of present study [25]. The most common and most potent cellulase producers are *Trichoderma koningii, T. ressei, Aspergillus Sp.*, *Fusarium Sp.*, and *Penicillium Sp.* [26]. Results observed during this study suggests that cellulase activity of *Aspergillus Sp.*, *Penicillum Sp.* were found relatively high compare to *Basidiobolus ranarum*, *Arthroderma benhamiae*, *Geotrichum candidum*, *Arthrographis kalrae*, *Alternaria alternate*, *Fusarium solani*. In addition, all the experiments *Aspergillus Sp.* and *Penicillium Sp.* showed comparatively good endoglucanase activity. This result is in par with Sone *et al* [27].

## 5. CONCLUSION

The data presented in the study represents that three strains (*Aspergillus Sp.* FF-6, and FF-9, *Penicillium Sp.* FF-10, and FF-11) have shown to be suitable for cellulase production under batch conditions as they also have higher specific productivities. In conclusion, the three strains selected in this work may be further studied to develop processes for alkaline cellulase production that is demanded by the modern textile industry. Furthermore, it could be helpful in bio pretreatment of lignocellulosic biomass for bioethanol production. Soil, water, plant from Coimbatore fresh water ponds are good sources of fungi with particular characteristics.

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