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Isolation and biochemical identification in an Anaerobic Baffled Reactor for the treatment of Textile Wastewater

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Abstract: Textile wastewater containing toxic elements which are discharged from the industries and they extremely affect soil fertility, water resources, aquatic organism and also eco system. To ensure the safety of environment, proper technologies need to be used for the treatment of wastewater before discharge into land or water course. Treatment of wastewater by the method of Biodegradation can produce less hazardous. The microorganisms were initially isolated into pure culture and characteristics were identified based on morphological and staining method. The aim of this study is to isolate and biochemical identification of organism from textile effluent. The results revealed that *Alcaligenes faecalis* (Gram negative bacteria) was found in higher concentrations in textile wastewater. The species are isolated through 16S rRNA, biochemical and colony morphology methods.
Keywords: *Alcaligenes faecalis*, Anaerobic Baffled Reactor, Biodegradation, Morphological Characterization, Textile Wastewater.

Introduction

Textile industry is one of the oldest industries in India. Taking into account the volume and composition of effluent, the textile wastewater is rated as the most polluting one among all the industrial sectors [1&2]. In general, the wastewater from a typical textile industry is characterized by high values of BOD, COD, colour and pH [3&4]. It is a complex and highly variable mixture of many polluting substances ranging from inorganic compounds and elements to polymers and organic products[5]. In-complete use and the washing operations give the textile wastewater a considerable amount of dyes[6]. The untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources due to its high BOD value. The effluents with high levels of BOD and COD values are highly toxic to biological life. The high alkalinity and traces of chromium which is employed in dyes adversely affect the aquatic life and also interfer with the biological treatment processes[7]. It induces persistent colour coupled with organic load leading to disruption of the total ecological/symbiotic balance of the receiving water stream[8]. Dyes with striking visibility in recipients may lead to reduced light penetration in aquatic environment which will significantly affect the photosynthetic activity.

The genus *Alcaligenes* is formed by some common, apperantly saprophytic bacteria. They seem to be ubiquitous and have been found in soil and water, dairy products, rotten eggs and the intestinal tract of vertebrates where they probably live saprophytically[9]. When *A.faecalis* was described as the type species of the genus Alcaligenes no reference was made to the strong fruity odor. Later a Gram negative organism was

isolated from human faeces and called "Bacterium alkali-aromaticum". These bacteria were motile by peritrichous flagella and grew in broth with intense fruity odor which disappeared after a week, to be replaced by a cheesy odor. An awareness of environmental problems and potential hazards caused by industrial wastewater has prompted many countries to limit the discharge of polluting effluents into receiving [10,11&12].

Currently, much research has been focused on the biodegradation of the industrial effluents [13&14]. It mainly shows interest towards the pollution control using bacteria, fungi in combination with physico-chemical methods [15&16]. The conventional biological processes are not effective because the dye content in the textile effluent is toxic to the microorganisms used[17&18]. Although a number of workers described microbial degradation of textile effluent, limited literature is available on bioremediation of textile effluent using immobilized bacterial cells. The aim of the present research is to isolate and biochemical identification of *Alcaligenes faecalis* microorganism from textile wastewater.

Materials and Methods

Reactor Configuration

In the present study an experimental model of Anaerobic baffled Reactor was fabricated to conduct experiment for real time waste streams of textile industry to evaluate the treatment efficiency under varying experimental conditions. The experimental laboratory model was made up of Plexiglass. The size of the anaerobic baffled reactor was: length 50cm, width 24cm, depth 30cm and working volume of the reactor was 36 liters. A proper construction of the baffles allowed wastewater to flow through the sludge bed from bottom up. The model have five compartments and the distance of the upper edge of baffles between the ascending and descending compartments from the water level was 3cm above the reactor's base at a 45° angle to direct the flow evenly through the up-corners. The liquid flow is alternatively upwards and downwards between compartment partitions. This produced effective mixing and contact between the wastewater and biosolids at the base of each up-corners. Sampling ports were used for drawing biological sludge and liquid samples. A variable speed Peristaltic Pump (PP -30) was used to control flow rate. The schematic of the experimental setup is shown in **Figure 1.1**

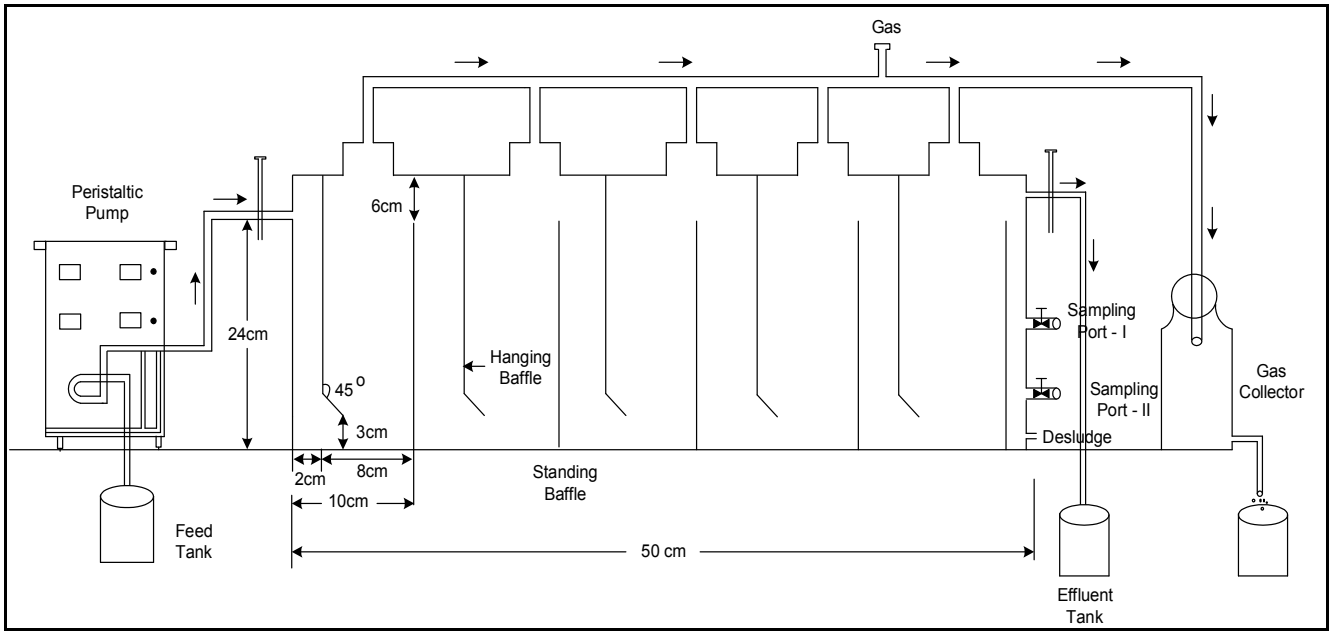

Fig. 1.1 Schematic of Anaerobic Baffled Reactor

The treatment process for acclimation was achieved by operating the plant with screened seed sludge drawn from the treatment facilities of Annamalai University. The textile effluent was collected from M R S Dyeing private limited, Avinashi road, Tirupur, Tamil Nadu.

Analysis of effluent

The samples were analyzed and characterized as per the Standard Procedure [19] which are presented in **Table 2.**The textile wastewater was allowed to the reactor in gradual addition of 20%, 40%, 60%, 80% and 100%. After allowing 100% textile wastewater to the reactor, the COD removal efficiency was monitored.

Table 2 Characteristics of wastewater parameters from Textile industry

Sl. No.	Parameters	Sample-I	Sample-II	Sample-III	Desirable limit of IS 10500
1	pH	8.9	8.6	9.2	6.5 to 8.5
2	Total solids, mg/l	2670	2680	2850	500
3	Total suspended solids, mg/l	600	550	650	100
4	Total dissolved solids, mg/l	2070	2130	2200	500
7	BOD_5@ 20°C, mg/l	1750	1206	1658	30
8	COD, mg/l	4400	3880	4160	250
9	Ammonical Nitrogen, mg/l	73.00	85.60	84.20	50
10	Chlorides, mg/l	4320	4005	4125	250
11	Turbidity(NTU) , mg/l	14.8	16.6	12.5	1
12	Temperature, °C	28	29	28	< 40
13	Sulphates, mg/l	2250	2050	2180	200
14	Phosphate, mg/l	68.8	72.8	85	NA
15	Hardness, mg/l	1800	1900	1750	200
16	Sodium, mg/l	2833	2900	2710	200
17	Potassium, mg/l	2524.5	2685	2490	NA
18	Calcium, mg/l	2378.5	2321.7	2185	75
19	Lithium, mg/l	83.6	75.5	68	2.5

Preparation of Mass Cultures

To enhance the degradation of effluent, mass cultures of the isolated organisms were prepared from the pure cultures.

Identification of Bacterial Isolates

The bacterial isolates were subjected to various tests from the study of their morphology and growth pattern on nutrient agar media, micro biological identification tests such as gram staining and motility tests followed by biochemical identification tests like catalase, citrate utilization, urease, indole production, hydrogen sulphide production, nitrate and nitrite reduction, methyl red, Voges Proskeur and sugar fermentation tests. Molecular identification was also done as per Standard protocol.

Molecular Identification

Genomic DNA was extracted by BLAST protocol. In brief, bacterial cells were collected by centrifugation at 8000 g for 10 min and Iysed with 5.5% sodium dodesyl sulphate/ 0.125 mg ml -1 proteinase K solution. Bacterial nucleic acids were extracted by a phenol/ chloroform/ isoamyl alcohol (25:24:1 v/v/v) mixture and chloroform-isoamly alchol (24:1 v/v mixture. The nucleic acids were precipitated by adding two volumes of ethanol in the presence of 0.3M sodium acetate.

Bacterial 16S rRNA was amplified by using the universal bacterial 16S rRNA primers,27f:(5'-AGAGTTTGATCCTGGCTCAG-3') and 1522r:(5'- AAGGAGGTGATCC ANCCRCA – 3'). The PCR (50ml) contained 0.5ml of each forward and reverse primer, 1.5mM of $Mgcl_2$, 1U of Taq DNA polymerase (MBI Fermentas) and 10 μ L of 10xTaq polymerase buffer. PCR conditions were as follows: denaturation at 95°C for 5 min,35 cycles of denaturation at 94°C for 45 sec, annealing at 56°C for 45sec, extension at 72°C for 1 min, and a final extension step at 72°C for 5 min using a thermocycler (iCycler; Bio-Rad Laboratories, CA). The

PCR products obtained from DNA extracted from the samples were first analyzed by electrophoresis in 1% agarose gel and was stained with ethidium bromide and visualized under short-wavelength UV light.

Result and Discussions

Isolation and Identification of Microbes from Effluent

Isolated organism was *faecalis* species and the organism have maximum efficiency of degradation was identified as *Alcaligenes faecalis* by using biochemical and 16S rRNA gene sequencing. The organism was observed as gram negative bacteria observed

as pink red rods colony on nutrient plate was shown in the Fig 1.2.

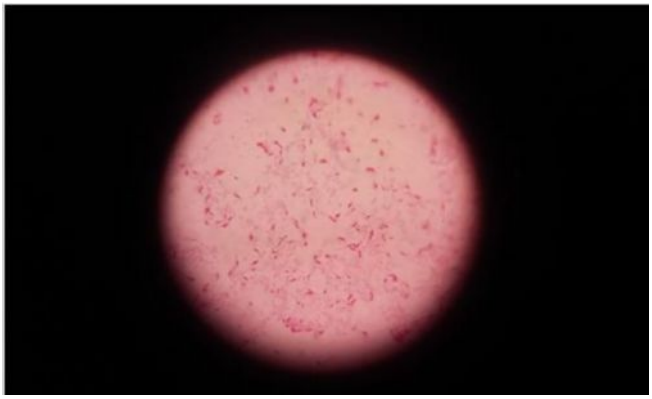

Fig 1.2 Microscopic image of Gram Negative bacteria (Pink red rods)

Morphology and Cell Structure

Alcaligenes faecalis is a motile gram negative, rod –shaped non-sporulating bacterium. In agar, colonies vary from non-pigmented to grayish-white, and can grow up to 2mm was shown in the Fig 1.3.

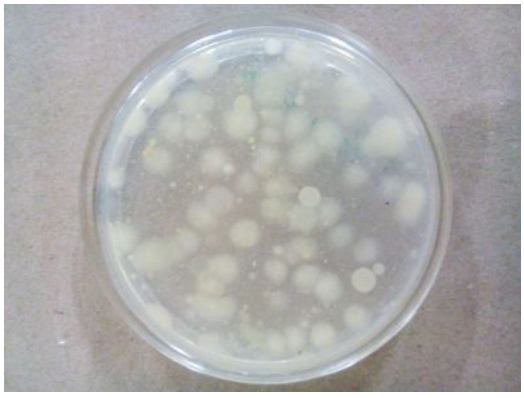

Fig 1.3 Isolated Colonies on Nutrient agar plate

Growth and Metabolism

Optimal growth of *Alcaligenes faecalis* (Fig 1.3) occurs between 20^0 C to 37^0 C. It grows best on soy agar and in soy broth, but can grow on any nutrient agar. Growth of Alcaligenes faecalis is usually characterized by its green with block colour fungal media grown on Potato Dextrose Agar was shown in the Fig 1.4.

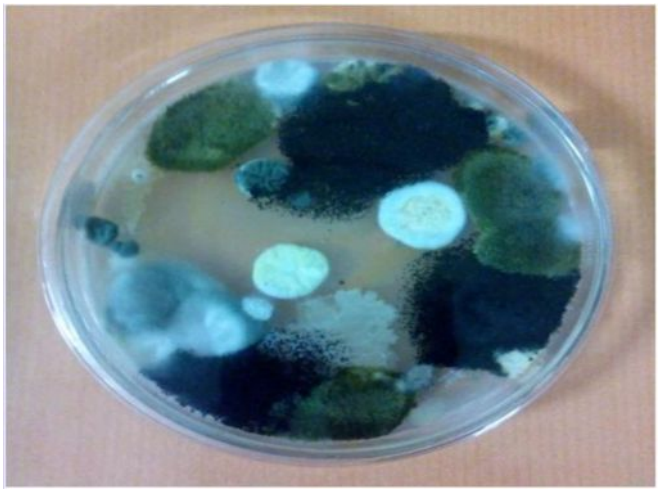

Fig 1.4 Microscopic image of fungas media growing on Potato Dextrose Agar

Most strains of *Alcaligenes faecalis* produce a characteristic aromatic fruity odor and form colonies with a thin spreading irregular edge. Carbohydrates are not utilized as the sole carbon source. It is oxidase, citrate and catalase positive. It is non nitrate reducing or lactose-fermenting. It has ability to convert the most toxic varieties of arsenic acid, and arsenite to its less dangerous form-arsenate during growth in media containing these chemicals and it's capable of degrading urea and producing ammonia which increases the pH of the environment.[20]. The organism is found in aqueous environmental sources [21].

Scanning Electron Microscopy (SEM)

The sample were observed under various magnifications in a Scanning Electron Microscopy (Fig 1.5). Most of the studies were focused on the microbial population distribution in the ABR, the results showed partly disparity of microbial population distribution under different experimental conditions[22]. In this study, the sludge was light brown and was taken in the compartment. On the 210 days of the ABR start-up, the seed sludge and the anaerobic sludge in the compartments were taken for SEM examination.

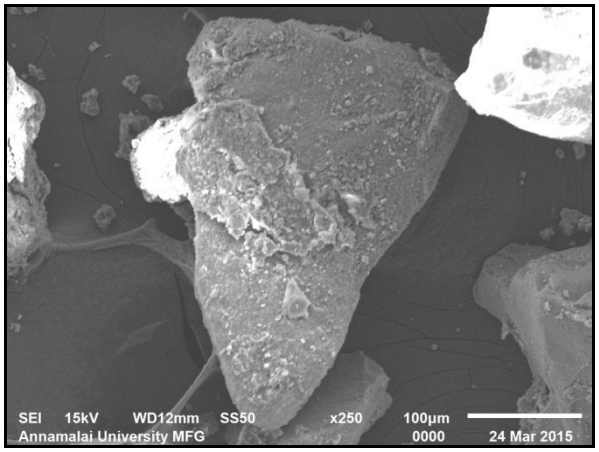

Fig. 1.5 SEM Image of Sludge Granule

Sequence Analysis of PCR Products

Bacterial species was isolated from ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, USA). DNA of the bacteria was extracted and precipitated. DNA amplification was done at 1 KB in a PCR. The amplified DNA fragments were purified sequenced and analyse (Fig. 1.6).

Phylogenetic Analysis

The sequence of these 16S rRNA gene was compared against the sequences available from GeneBank using the BLASTN program and were aligned using CLUSTAL W software. Distances were calculated according to Kimura's two-parameter correction[23]. Phylogenetic trees were constructed using the neighboring-joining method[24] Bootstrap analysis was done on 1000 replications. The MEGA4 package[25] was used for all analysis (Fig. 1.7).

Genomic DNA of Selected Bacterial Isolate

Figure 1.6 Genomic DNA of selected bacterial isolate

Lane 1(Sample 1)

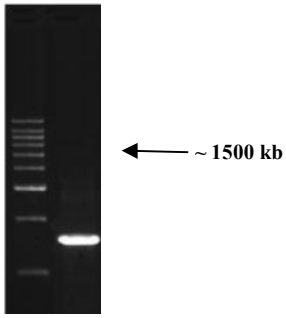

Figure1.7 PCR amplification profile of selected bacterial isolate

Conditions: 1% agarose gel electrophoresis (Lane M: 1 KB DNA Ladder; 1: Sample)

1 KB DNA Ladder (bp):1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000

Sequences of the Sample

GAGTTTGATCCTGGCTCAGATTGAACGCTAGCGGGATGCTTTACACATGCAAGTCGAACGG
CAGCACGAGAGAGCTTGCTCTCTTGGTGGCGAGTGGCGGACGGGTGAGTAATATATCGGAACGTG
CCCAGTAGCGGGGGATAACTACTCGAAAGAGTGGCTAATACCGCATACGCCCTACGGGGGAAAG
GGGGGGATTCTTCGGAACCTCTCACTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGT
AAAGGCTCACCAAGGCAACGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGA
TCCAGCCATCCCGCGTGTATGATGAAGGCCTTCGGGTTGTAAAGTACTTTTGGCAGAGAAGAAAA
GGTATCTCCTAATACGAGATACTGCTGACGGTATCTGCAGAATAAGCACCGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGTGTAG
GCGGTTCGGAAAGAAAGATGTGAAATCCCAGGGCTCAACCTTGGAACTGCATTTTTAACTGCCGA
GCTAGAGTATGTCAGAGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGATATGTGGAGG
AATACCGATGGCGAAGGCAGCCCCCTGGGATAATACTGACGCTCAGACACGAAAGCGTGGGGAG
CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTT
AGGCCTTAGTAGCGCAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAA
CTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGA
AAAACCTTACCTACCCTTGACATGTCTGGAATGCCGAAGAGATTTGGCAGTGCTCGCAAGAGAAC

CGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGTCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACA
ATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGGGAGCCAATCTCAGAAACCCGATCGTAGTC
CGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGTC
GCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTTCACCAG
AAGTAGGTAGCCTAACCGTAAGGAGGGCGCTTACCACGGTGGGATTCATGACTGGGGCGAAGTCG
GAACAAGGTATTCCGACG

Further the above sequences of these 16S rRNA was deposited in a gene bank in NCBI and was numbered. On comparing the sequences with the available sequence, phylogenetic trees were constructed and based on the phylogeny analysis it was clearly revealed that the given sample is belong to the taxa *Alcaligenes Faecalis* (NR043445) was shown in the Fig 1.8.

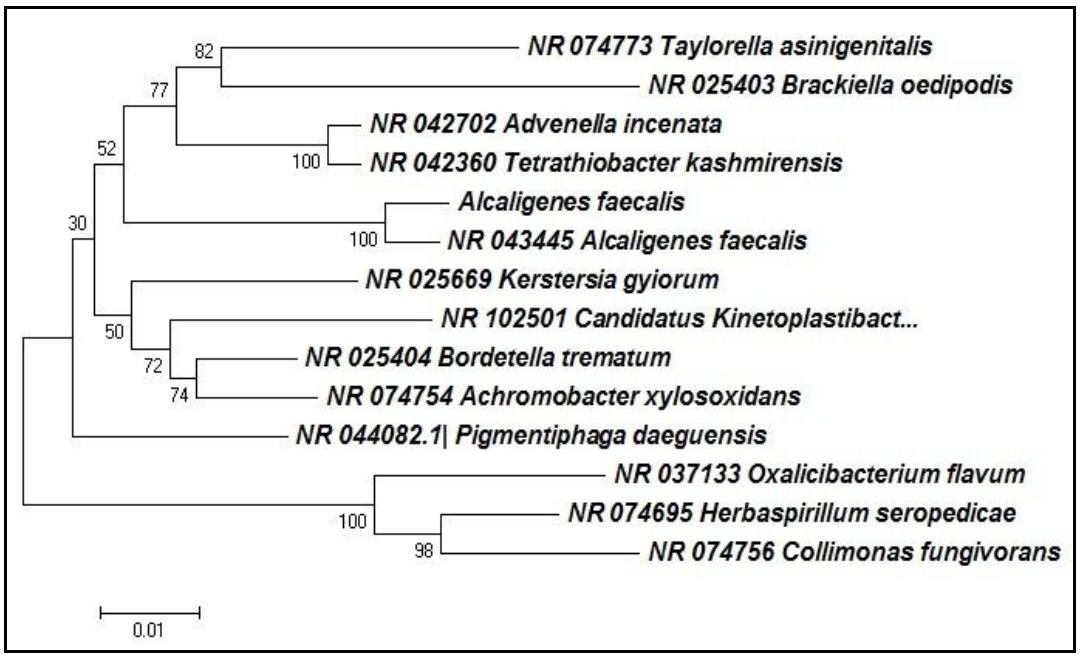

Fig. 1.8 Phylogeny Tree Analysis of the Sample

Conclusions

The gram negative rod shaped Bacilli bacterium, such as *Alcaligenes faecalis* by 16S rRNA sequencing and NCBI Acc. No: NR 043445 was identified. It can produce more enzyme activity in the presence of soil and water enchances its chance of contamination to the environment. Present study was based on the toxic nature of metals against bacterial growth which can be used as inhibitor against the pathogenic culture of bacteria and can be used to improve the effect of microbial bio-fertilizer especially in the fields where metal concentrations is more than permitted limit. The identified culture is reported as *Alcaligenes faecalis* which is morphologically gram negative rod and is able to ferment Glucose, Sucrose and Fructose.

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