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Identification of Potentially active Methanogenic Population for Degradation of Organic Substances in UASFFR for Treating Sago Wastewater

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Abstract*: *Anaerobic digestion process is the best method for treating industrial wastewater with dynamic relationship between microorganisms. The objective of this research work is to assess the microbial population that coordinates with anaerobic codigestion of biomass cultivated on sago wastewater. An upflow anaerobic sludge fixed film reactor was used to achieve high rates of microbial decomposition. The bacterial colonies were counted and predominant culture was streaked in newer sterile nutrient agar plate. The predominant culture was subjected to molecular identification using 16S rRNA gene sequencing and Gram's staining. Isolated organism chryseomicrobium species and the organism are identified as Chryseomicrobium palamuruense by using the biochemical and 16S rRNA gene sequencing.

Keywords* : *characterization, isolation, Up-flow Anaerobic Sludge Fixed Film Reactor, sago wastewater.

I. INTRODUCTION

Even though the development of industries is an advantage for the society, the environmental pollution is extremely harmful to the environment which should be controlled. The industrial wastes are carried away through rivers and oceans. The water contaminant such as pesticides, fertilizers, pathogens etc., leads to chronic water borne diseases. We have so many methods for the treatment of industrial waste water. But the industries need cost effective waste water treatment methods affordable to them. Sago bark, sago wastewater and sago smith are the three wastes generated from sago industry. Appropriate method of reutilizing these waste to form a value added product found mandatory.

In India there are three states namely Kerala, Tamilnadu and Andhrapradesh, the chief producers of tapioca. About 7 million tonnes of tapioca are produced for every year from above three states. Yield of about 200 kg/tonne was processed. This is a water consuming industry about 8000 litres of water is required to process one tonne of tapioca. About 25,000 to 35,000 litres of effluent is generated per tonne of sago production. The effluents are organic in nature with high polluting effects on environment. In the last 50 years these industries in Tamil Nadu showed an extraordinary growth.

Anaerobic digestion (AD), being a dynamically changing microbiological process, has long been manipulated only at the level of reactor design and physicochemical maintenance. Manipulation on the level of microorganisms in the system is more recent as evidenced by the rising number of studies investigating key bacterial players in AD [B. St-Pierre and A.-D. G. Wright, (2014), F. A. Shah, Q. Mahmood et al., (2014) , D. Rivière, V. Desvignes, E. Pelletier et al., (2009), A. Schlüter, T. Bekel, N. N. Diaz et al., (2008), S. S. Patil, M. S. Kumar, and A. S. Ball (2010)]. The process of Methanogenesis in anaerobic condition is a multifaceted reaction. Variety of anaerobic micro-organisms subjected to symbiotic effect converts the organic substances to methane and carbon dioxide(Naik SN, Goud VV, Rout PK, Dalai AK(2010)). Commonly, this method consists of liquefaction and hydrolysis of insoluble compounds and gasification midway. Which is accompanied by a partial or complete mineralization and humification of organic substance (Lyberatos G, Skiadas I (1999))? The production of biogas and methane through anaerobic digestion is a fuel used to produce environmental-friendly energy (Boone DR et al., (1993) and Mata-Alvarez J, Macé S, Llabrés P(2000)). In this regard anaerobic digestion was followed in industries for a long period. The digestion of organic wastes results in production of biogas and methane. Maize plants are grown in abundant in European countries for the production of feed for animals as well as in the production of sugar. European nation has adopted procedures to diminish monoculture of maize for energy production (de Graaf D, Fendler R (2010) and Weiland P (2010).

This study emphasis the performance of Upflow Anaerobic Sludge Fixed Film Reactor (UASFFR) in treating sago effluent and the microbial communities occupied in the process for their identification.

II. MATERIALS AND METHODS

Materials that been used is sago wastewater obtained from traditional sago processing industry in Salem District, Tamilnadu. Fresh sago wastewaters were collected and stored in sterilized bottled. Sampled was brought to laboratory for further analysis.

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Samples were stored to fermentation for 1, 3 and 7 days in room temperature

A. Isolation of bacteria from Sago Sample

1 ml of sago effluent sample was diluted and 6th&7th dilutions were spread plated sterile nutrient agar plate.

After 24-48 hours of incubation period the bacterial growth was pragmatic. The bacterial colonies were then counted and the resulted predominant culture was splashed in a fresh sterile nutrient agar plate. The molecular identification was done by means of 16S rRNA gene sequencing and Gram's staining.

B. Genomic DNA isolation and PCR analysis

Genomic DNA was haul out during the night time using QIAGEN DNA separation equipment, suspended in elution buffer and then OD was measured by 260 nanometer. PCR augmentation was carried out by means of a 50 µL reaction fusion. The progressions of 16S rRNA primers used were as follows.

C. Phylogenetic analysis

BLASTn program was used to check the similarity of 16S rRNA genes with GenBank. After the similarity check alignment was carried out by means software named CLUSTAL W. evolutionary distances were evaluated by means of Kimura's 2 parameter approach. Phylogenetic analysis was carried out using Molecular Evolutionary Genetics Analysis (MEGA4) software (Kumar et al., 2007).

Fig.1. Genomic DNA of given bacterial isolate sago waste

D. Sequences of the sago bacteria

>Seq_Sago Isolate
GGCTCAGGACGAACGCTGGCGGCGTGCCTAATACCTGCAA
GTCGAGAGGATGACGAAGGA
GCTTGCTCCTTCTGATTCAGCGTCGGACGGGTGAGTATCA
CGTGGGCAACCTGCCCTGTA
GATTGGGATAACTTTGGGATTTCGGGGCTAATACCGAATA
TTCCATCGGACCTCATGGTC
TGATGTTGAAAGACGGTTTCGGCTGTCACTACAGGATGGG
CCCGCGGCGCATTAGCTAGT
TGGTGAGGTAAAGGCTCACCAAGGCGACGATGCGTAGCCG
ACCTGCGAGGGTGATCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCAC
AATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAA
GAAGGTTCTCGGATGGTAAA
ACTCTGTTGTGAGGGAAGAACAAGTATCGGAGTAACTGCC
GGTACCTTGACGGTGGCTCA
TTAGAAAGCCACGCCTAACTACGTGCCAGCAGCCGCGGTA
ATACGTAGGTGGCAAGCGTT
GTCCGGAATCATTGGGCGTAAAGCGCGCGCAGGCGGTCCC
TTAAATCTGATGTGAAAGCC
CACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACT
TGAGTGCAGAAGAGGAAAGC
GGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGA
GGAACACCAGTGGCGAAGGC
GGCTTTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGT
GGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG
TTAGGGGGTTTCCGCCCCTT
AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGT
ACGGTCGCAAGACTGAAACT
CAAAGGAATTACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGC
GAAGAACCTTACCAGGTCTTGACATCCCGCCGACCGCCCA
GGAGACTGGGCCTTCCCTTC
GGGGACGGCGGTGACAGGTGGTGCATGGTTGTCGTCAGCT
CGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCA
GCATTCAGTTGGGCACTCTA
AGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA
CGTCAAATCATCATGCCCCT
TATGACCTGGGCTACACACGTGCTACAATGGATGGTACAA
AGGGCTGCGAACCCGCGAGG
GGGAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTA
GGCTGCAACTCGCCTACATG
AAGCTGGAATCGCTAGTAATCGTGGATCAGCATGCCACGG
TGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCC
GAAGTCGGTGGGGTAACCTT
TATGGAGCCAGCCGCCGAAGGTGGGACTAGATGACTTGG

III. RESULTS AND DISCUSSION

A. Isolation and Identification of Microbes from Effluents

Isolated organism *chryseomicrobium* species and the organism are identified as *Chryseomicrobium palamuruense* by using the biochemical and 16S rRNA gene sequencing. The organism was observed as gram-positive and rod shaped bacteria as shown in Fig.2.

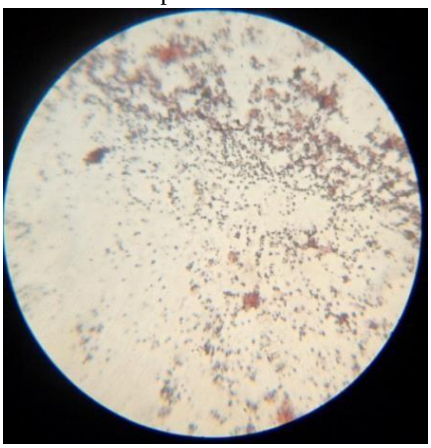

Fig.2 Microscopic image of Gram-positive bacteria

B. Morphology and Cell structure

Chryseomicrobium palamuruense is a motile gram-positive, rod-shaped bacterium. In agar, the colonies vary from non-pigmented to grayish-white, which is shown in the Fig.3.

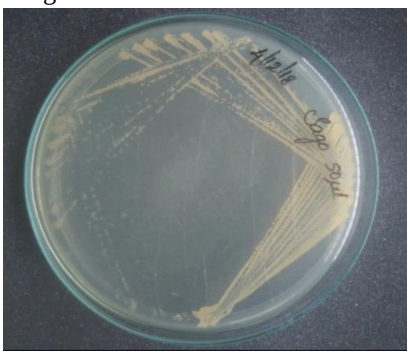

Fig.3 Isolated sago bacteria on Nutrient agar plate

C. Phylogeny tree analysis of the sago bacteria

The Neighbor-Joining method suggested by Saitou and Nei, (1987) was used in evolutionary history. The branch length of 0.1854 was found in the optimal tree. In bootstrap test the coupled taxa was thousand replicates. The phylogenetic tree was drawn to scale with the computed evolutionary distances by means of the Kimura approach. The position of Codon comprises first three with noncoding. All positions are done by means of pair wise deletion preference. In the ultimate dataset a total of 1486 positions are found. MEGA4 was used for Phylogenetic analyses (Tamura et al., 2007). BLAST analysis revealed that the sample belongs to taxa is *Chryseomicrobium palamuruen.*

IV. CONCLUSION

This digestion processes convert biomass to energy, used the organic wastes in the upflow anaerobic sludge bed fixed film reactor. In this study the sieve sludge was screened for the bacteria from the sago wastewater having extreme concentration of organic pollutants. The isolated organism *chryseomicrobium* species and the organism are identified as *Chryseomicrobium palamuruense* by using the biochemical and 16S rRNA gene sequencing. The organism was observed as gram-positive and rod shaped bacteria. BLAST analysis make known that the sample belongs to taxa is Chryseomicrobium palamuruen.

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