

**ENHANCEMENT OF ANAEROBIC DIGESTION
POTENTIAL IN WASTE ACTIVATED SLUDGE
BY BIOLOGICAL PRETREATMENT**

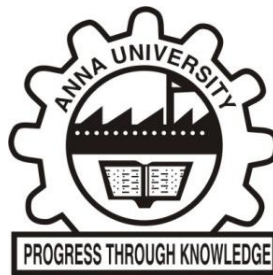
A THESIS

Submitted by

GOPIKUMAR S

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY



FACULTY OF CIVIL ENGINEERING

ANNA UNIVERSITY

CHENNAI 600 025

MARCH 2015



CENTRE FOR RESEARCH
ANNA UNIVERSITY, CHENNAI – 600 025



CERTIFICATE

This is to certify that all corrections and suggestions pointed out by the Indian /Foreign Examiner(s) are incorporated in the Thesis titled “Enhancement of Anaerobic Digestion Potential in Waste Activated Sludge by Biological Pretreatment” submitted by Mr./Ms S. Gopikumar.

- NA -

Signature of the Joint Supervisor
(if applicable).

J. P. Jeeva
Signature of the Supervisor
17/3/15

Place: Tirunelveli

Date: 17.03.2015



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PROCEEDINGS OF THE Ph.D. VIVA-VOCE EXAMINATION OF Mr./Ms. S.GOPIKUMAR HELD AT 10.30 A.M./P.M. ON 17.03.2015 IN DEPARTMENT OF CIVIL ENGINEERING, REGIONAL OFFICE, ANNA UNIVERSITY, TIRUNELVELI REGION

The Ph.D. Viva-Voce Examination of Mr./Ms. S. Gopikumar (Reg. No. 2011110104) on his/her Ph.D. Thesis Entitled "Enhancement of Anaerobic Digestion Potential in Waste Activated Sludge by Biological Pretreatment" was conducted on 17.03.2015 at 10.30 A.M./P.M. in the Department of Civil Engineering, Regional Office, Anna University, Tirunelveli Region,

The following Members of the Oral Examination Board were present:

- | | | |
|----|-----------------------|---------------------------|
| 1. | Dr. K. N. Yogalakshmi | Indian Examiner |
| 2. | Dr. P. Sivashanmugam | Subject Expert |
| 3. | Dr. J. Rajesh Banu | Supervisor & Convener |
| 4. | NA | Joint Supervisor (If any) |

The research scholar, Mr./Ms. S. Gopikumar presented the salient features of his/her Ph.D. work. This was followed by questions from the board members. The questions raised by the Foreign and Indian Examiners were also put to the scholar. The scholar answered the questions to the full satisfaction of the board members.

The corrections suggested by the Indian/Foreign examiner have been carried out and incorporated in the Thesis before the Oral examination.

Based on the scholar's research work, his/her presentation and also the clarifications and answers by the scholar to the questions, the board recommends that Mr./Ms. S. Gopikumar be awarded Ph.D. degree in the Faculty of Civil Engineering.

- | | | |
|----|--------------------------------------|---------------------------|
| 1. | Dr. Yogalakshmi k.n. | Indian Examiner |
| 2. | Dr. P. Sivashanmugam | Subject Expert |
| 3. | Prof - NIT, Tiruchirappalli - 620015 | Supervisor & Convener |
| 4. | Dr. J. Rajesh Banu | Joint Supervisor (if any) |
| | | -NA- |


ANNA UNIVERSITY
CHENNAI 600 025


CERTIFICATE

The research work embodied in the present Thesis entitled **“ENHANCEMENT OF ANAEROBIC DIGESTION POTENTIAL IN WASTE ACTIVATED SLUDGE BY BIOLOGICAL PRETREATMENT”** has been carried out in the Department of Civil Engineering, Regional Centre of Anna University Tirunelveli. The work herein is original and does not form part of any other thesis or dissertation on the basis of which a degree or award was conferred on an earlier occasion or to any other scholar.

I understand the University’s policy on plagiarism and declare that the thesis and publications are my own work, except where specifically acknowledged and has not been copied from other sources or been previously submitted for award or assessment.

Counter Signed by


GOPIKUMAR S.
Date: 17.03.2015


Dr. J. RAJESH BANU
SUPERVISOR
Assistant Professor
Department of Civil Engineering
Regional Centre of Anna University
Tirunelveli - 627007

ABSTRACT

The objective of this study is to isolate thermophilic protease secreting bacteria from waste activated sludge. The waste activated sludge was collected from secondary clarifier in a municipal wastewater treatment plant in Trivandrum, Kerala in a sterilized container. Towards this end, six bacterial strains were isolated from the waste activated sludge and acclimatized to a thermophilic condition (55°C). The strains causing hydrolysis on skim milk agar medium led to the formation of clear halo zone. Based on the highest relative halo size percentage the strain B1 was used for further studies. B1 in the skim milk agar medium was white in colour, filamentous in shape with a lobate margin, raised elevation, wrinkled surface and opaque. Bacterial growth parameters were optimized using response surface methodology. A central composite design (CCD) made with Design-Expert software and version was employed to investigate the simultaneous effect of three independent variables: pH, temperature and time. The central point offered maximum dry cell weight 1.45 g/L, which corresponded to pH 6.5, at a temperature of 55°C and an incubation time of 24 h.

Optimization of bacterial pretreatment with strain B1 on sludge solubilisation potential was measured in terms of soluble chemical oxygen demand and mixed liquor suspended solids. The COD Solubilisation was found to be 8%, 26.7% for control and B1 respectively. Thus the protease secreting bacteria B1 enhanced the COD Solubilisation by 221% as compared to the control. Suspended solids reduction was found to be 7% and 22.8% for control and B1 respectively. The strains B1 enhanced the SS degradation by 218% as compared to the control. It was also observed that MLSS reduced rapidly when more SCOD was released, thereby increasing sludge solubilisation. The strain B1 was subjected to identification and was used for

further studies. Using 16s rRNA gene technique the sequence obtained was blasted in NCBI and identified that the bacterial strain B1 displayed highest similarity with *Bacillus licheniformis* (97%). Cation binding agents such as Citric acid (CA), Ethylenediaminetetraacetate (EDTA) and Sodium tripolyphosphate (STPP) were added to accelerate the sludge solubilisation potential of *Bacillus licheniformis*. Sludge solubilization of CA-B1 (63%), EDTA-B1 (41%) and STPP-B1 (36%) was observed to be greater than the solubilisation of control C-B1 (26%). Similarly suspended solids reduction of CA-B1 (46%), EDTA-B1 (33%) and STPP-B1 (30%) was found to be greater than control C-B1 (21%). Finally, it is evident that Citric acid-*Bacillus licheniformis* (CA-B1) was more effective in solids reduction than EDTA and STPP.

Following pretreatment, the anaerobic degradability of sludge was carried out in semi-continuous anaerobic reactors with the working volume of 3.5 L. The solids content of the sludge fed to the anaerobic digester was in the range of 5-15 g/L with the optimized dosage of citric acid i.e., 50 mmol/L along with 1.45 g dry cell weight/L of *Bacillus licheniformis*. Among four solids retention time (SRT) and eight organic loading rate (OLR), an OLR of 1.0 g SS m⁻³ reactor d⁻¹ operated at 15 days SRT was preferably the pertinent OLR for the efficient digestion. Citric acid-*Bacillus licheniformis* pretreatment with anaerobic digestion led to suspended solids reduction in the range of 11-43% and volatile solids reduction in the range of 16-48% respectively, with the total biogas production in the range of 48-201 mL/g VS added.