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STUDY ON REMOVAL OF ORGANIC POLLUTANTS USING THE EXPANDED GRANULAR SLUDGE BED REACTOR

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Abstract – Slaughterhouse wastewater contains various and high amounts of organic matter, which contributes paunch, feces, grease, fat, and lard, undigested foods, blood, suspended material, urine, loose meat, soluble proteins, excrement, manure, grit and colloidal particles. The purpose of this research work is to investigate the feasibility study of EGSBR for treating slaughter house wastewater with the impact of HRT with respect to the organic removal efficiency. The experimental model was run with six average different ranges of influent COD of 1752, 1744, 1820, 2264, 2768, and 3176 mg/L. The maximum efficient organic removal was attained at 90.17% with a HRT of 4 days at an OLR of 0.081Kg COD/m^3.day. The study was run at mesophilic range of temperature for efficient growth of organism. The slaughter house wastewater was observed under various magnifications in a scanning microscopy. The organism responsible for digestion was identified as Bacillus haikouensis, which is a gram-positive and rod shaped bacteria.

INTRODUCTION

Wastewater discharged from the slaughterhouse is a combination of the processing water from both the slaughtering line and the guts cleaning, which causes a large variation in organic matter concentration. The organic load sources to these effluents are paunch, feces, fat and lard, grease, undigested food, blood, suspended material, urine, loose meat, soluble proteins, excrement, manure, grit and colloidal particles (Asselin *et al.* (2008), Tezcan *et al*., (2009). There are about 4,000 licensed slaughter houses with the local authorities in India and more than 25,000 unauthorized premises, where animals are slaughtered to meet domestic consumers demand. Approximately 45-50 percent of the animals can be converted into edible items (MEAT). Around 15 percent of waste and the remaining 40-45 percent of the animal is transformed into products such as Leather, Soaps, Candles (tallow) and adhesives. The organic load contributors to these effluents are paunch, feces, grease, fat, and lard, undigested foods, blood, suspended material, urine, loose meat, soluble proteins, excrement, manure, grit and colloidal particles (Bazrafshan *et al*., 2007). Slaughter house wastewater are typically assessed using bulk parameters because of various pollutants loads derived from the type and the number of animals slaughtered that fluctuate amid the meat industry (Bustillo-Lecompte and Mehrvar, 2015).

The treatment of slaughterhouse wastewater by various methods such as aerobic and anaerobic biological systems (Masse L and Masse DI (2005); Torkian *et. al*. (2003), Manjunath *et al*. (2000); Palatsi *et al*. (2011)) and hybrid systems (Tezcan *et al*. (2009) have been intensively studied. Sugito *et al.* (2016) determined the role of BOD concentrate influencing the removal of pollutant load in chicken slaughterhouse effluent. Stets *et al.*, (2016) studied that Microbial community and performance of slaughterhouse wastewater treatment filters. In this study, the efficiency of a slaughterhouse effluent treatment using three AFs containing different support media was tested, and the microbial diversity was investigated by amplified ribosomal DNA restriction analysis and 16S rRNA gene sequencing. Dipti Giri *et al*. (2015) studied that the slaughterhouse wastewater treatment by anaerobic fixed film fixed bed reactor packed with special media. Gajender C Sunder *et al.,* (2013) achieved a maximum efficiency for treating Slaughterhouse

Wastewater by using Anaerobic Hybrid Reactor Packed with Special Floating Media.

It is also reported that a serious disadvantage of anaerobic therapy is sensitive to high organic loading rates (Cuetos *et al.,* 2008, Torkian *et al.,* 2003). While biological processes are efficient and cost effective, both biological processes require long hydraulic retention time and large volumes of reactor, high concentrations of biomass and sludge loss control to prevent sludge washing out. Dissolved air flotation (DAF) and coagulation–flocculation systems are commonly used in physico-chemical processes to extract the total suspended solids (TSS), colloids, and fats from slaughterhouse wastewaters (Asselin *et al.,* 2008). In order to achieve a stable operation, Sludge retention Time should usually be used two to three minutes above the bacterial doubling time (Syutsubo *et al*., 1997; de la Rubia *et al.,* 2006). Inoculation of granular sludge was found to be effective in shortening the reactor start-up time needed to maintain a adequate sludge retention time (Syutsubo *et al.,* 1997; Syutsubo *et al.,* 1998; Syutsubo *et al.,* 2008). In this present study, an attempt was made to explore the output efficiency of EGSBR technology for withdrawing soluble organic matter from slaughterhouse wastewater and also identifying the microbial populations.

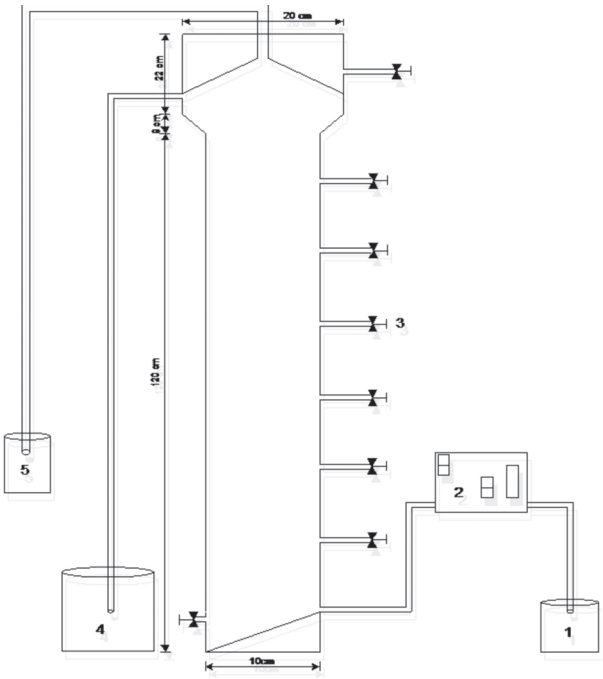
MATERIALS AND METHODS

The current experimental work has been conducted to evaluate the reliability of anaerobic expanded granular sludge bed reactor to eliminate organics in the wastewater slaughter. Figure 1 displays a schematic outline for an EGSB reactor. The plexiglass material was used to develop the laboratory model. The reactor consists of a 9.54 Litres column part, and a 4.93 liter gas-solid separator (GSS). The reactor's operating volume is 14.47 litres, including GSS. The fraction of the cylinder column is 121.5 cm long and 10 cm in diameter inside. Table 1 displays the physical characteristic of the experimental setup.

RESULTS AND DISCUSSION

The start-up cycle is regarded as the amount of time taken to achieve a stable activity of the microorganism. The real time slaughterhouse wastewater was used during the experimental study period. The AEGSB reactor was pumped with six set of average COD loading with 1752, 1744, 1820, 2264, 2768, and 3176mg/L with the flow rates of 0.930, 0.744, 0.558, 0.372 and 0.186 Kg COD/ m^3.day continuously using peristaltic pump. The reactor attained a steady state from 54 to 61th day with a maximum COD removal efficiency of 91.14% during start-up stage with a biogas conversion of 0.0034 m^3 of biogas per kg COD removed. Mesophilic range temperature influenced the bacterial degradation kinetics, allowing an increase in the percentage of organic matter removal. The HRT quantified in this study was 2.0, 2.5, 3.0, 4.0, and 8.0 days.

After achieving a steady state, 100%of slaughter house wastewater was allowed to determine the



1. Influent tank
2. Peristaltic Pump
3. Sampling Ports
4. Effluent tank
5. Bio gas collecting jar

Fig. 1. Schematic of Anaerobic Expanded Granular sludge bed reactor

Table 1. Physical features and process parameters of experimental model

Specification	Dimensions
Total height of the reactor	152.5cm
Column portion	121.5cm
Diameter of the cylinder column	10cm
Triangle portion	9cm
Total liquid volume	14.47liters
Peristaltic pump	PP – 10 model
Free board	11cm

reactor efficiency of organic pollutant removal. The performance characteristics of HRT in days with respect to percentage COD removal efficiency is shown in Figure.2. The overall performance of the EGSB reactor was carried out between the temperatures from 24 °C to 37 °C. In these experiment three sets of analysis was carried out without addition of co-substrate in a real time slaughter house wastewater with an average influent COD of 1752, 1744, 1820mg/L. The maximum COD removal was achieved in the without addition of co-substrate was 84.32% with a HRT of 8 days. The removal efficiency was not up to the level so planned to add Potato Dextrose Aga as a co-substrate to improve the removal efficiency for another three sets of experiment in an average influent COD of 2264, 2768, 3176mg/L. By increasing the HRT, the COD removal efficiency was also steadily increased from the HRTs of 2.0, 2.5days and the removal was slightly fluctuating at 3.0 days of HRT and attained a maximum COD removal was achieved at 90.17% with a HRT of 4 days with an influent COD of 3176 mg/L with the addition of 3g/L of co-substrate. The biogas generation was estimated by using the method of water displacement. The Bio gas conversion with respect to OLR is shown in Figure.3 focused the maximum conversion was obtained 905 mL/minute at an OLR of 0.040 Kg COD/m^3.day with a HRT of 8

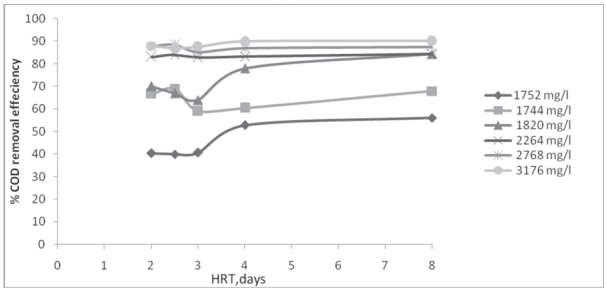


Fig. 2. Profile of %COD removal efficiency in an EGSBR using slaughter house wastewater

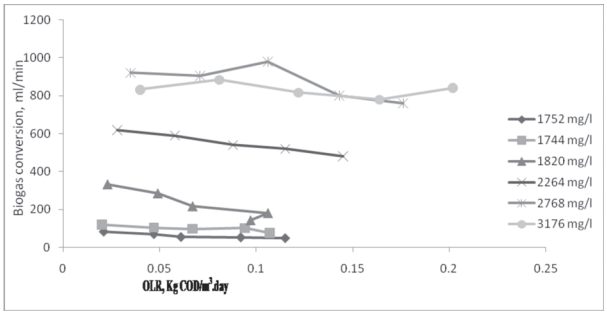


Fig. 3. Profile of Biogas collection in an EGSBR using slaughter house wastewater

days. The conversion was initially fluctuating due to the starvation of the organisms and also the pH and environmental conditions within the reactor.

Identification of Microbial population in the reactor

Isolation of bacteria from SLAUGHTER Sample

About 1 mL of slaughter effluent sample was serially diluted and 6 and 7th dilutions were spread plated in the sterile nutrient agar plate. After 24-48 h incubation at bacteriological incubator sample was observed for the bacterial growth. 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.

Genomic DNA isolation and PCR analysis

Using QIAGEN DNA isolation kit (Qiagen), immersed in 100 µL of elution buffer (10 mM/L Tris-HCl, pH 8.5) and computed by assessing OD at 260 nm, Genomic DNA was isolated from identified bacterial isolates over night. PCR amplification was performed using a 50 µL reaction mixture containing 100 ng of template DNA, 20 µmol of 16S rRNA primers, 200 µM of dNTPs, 1.5 mM of $MgCl_2$, 1U of *Taq* DNA polymerase (MBI Fermentas) and 10 ìL of 10x *Taq* polymerase buffer. The sequences of 16S rRNA primers used were as follows.

27f: (5'-AGAGTTTGATCCTGGCTCAG-3')

1522r: (52-AAGGAGGTGATCCANCCRCA-3')

Amplification was carried out with an initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 sec, annealing at 56 °C for 45 sec, extension at 72 °C for 1 min and final extension at 72 °C for 5 min using a thermocycler (iCycler; Bio-Rad Laboratories, CA).PCR products were analyzed on 1% agarose gel for 16S rRNA amplicons in 1x TBE buffer at 100 V. The amplified product was sequenced using ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

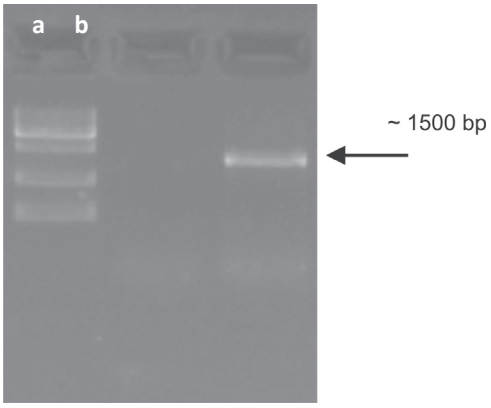
Phylogenetic analysis

The sequences of these 16S rRNA genes were compared against the sequences available from GenBank using the BLASTN program (Stephan Altschul *et al.*, 1990) and were aligned using CLUSTAL W software (Thompson *et al.*, 1994). Distances were calculated according to Kimura's two-parameter correction (Kimura, 1980).

Phylogenetic trees were constructed using the neighbour-joining method (Saitou and Nei, 1987). Bootstrap analysis was done based on 1000 replications. The MEGA4 package (Kumar *et al.*, 1993) was used for all analyses. The Genomic DNA of the bacterial population was isolated from slaughter wastewater is shown in Figure.4.

(Arrow indicates the Genomic DNA)

Fig. 4. Genomic DNA of given bacterial isolate from slaughter



(Lane a: 1kb DNA Ladder; b: Sample)

Fig. 5. PCR amplification profile of given bacterial isolate from slaughter

Conditions: 1.5% agarose gel electrophoresis
1 KB DNA Ladder (bp):5000, 4000, 3000, 2000, 1000

RESULTS

Sequences of the slaughter bacteria

>Seq_Slaughter
ACGAACGCTGGCGGCGTGCCTAATACATGCA
AGTCGAGCGGATCGATGGGAGCTTGCTCC
CTGAGATCAGTGGCGGACGGGTGAGTAA
CACGTTTGTAACCTGCCTGTAAGACTGGGATAACT
CCGGGAAACCGGGGCTAATACCGGATTA
TTTAGTTCCTCGCATGTGGAACTGTTGAAA
GGTGGCTTCGGCTACCACTTACAGATGG ACC
CGCGGCGCTTTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCAACGATGCGTAGCC
GACCTGAGAGGGTGATCGGCCACACTGGG
ACTTAGACACGGCCCAGACTCCTACGGGAGG
CAGCAGTAGGGAATCTTCCGCAATGGAC
GAAAGTCT GACG GAGCAACGCCGCGTGAG
TGAAGAAGGTTTTCGGATCGTAAAACTCT
GTTGTTAGGGAAGAACAAGTGCCGTTCGA
ATAGGGCGGCACCTTGACGGTACCTAACC
AGAAAGCCACGGCTCACTACGTGCCAGCA GC
CGCGGTAATACGTAGGTGGCAAGCGTTGTCC
GGAATTATTCCCCGTAAAGCGCGCGCAGGTGG
TTTCTTAAGTCTGATGTGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACTGGGGA
ACTTGAGTGCAGAAGAGGAAAGTGGA
ATTCCAAGTGTAGCGGTGAAATGCGTAGAT
AGGTGG AGGAACACCAGTGGCGAAGGCGA
CTTTCTGGTCTGTAACTGACACTGAGGCGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGAGTGCTAA
GTGTTAGAGGGTTTCGGCCCTTTAGTGCTGCAGCTA
ACGCATTAAGCACTCCGCCTGGGGAGTAC
GGTCGCAA GACTGAAACTCA AAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATG
TGGTTTAATTCGAAGCAACGCGAAGAACC
TTACCAGGTCTTGACATCCTCTGACAACCC
TAGAGATAGGGCTTTCCCCTTCGGGGGACA
GAGTGACAGGTGGTGCATGGTTGTCG
TCAGCTCGTGTCGTGAGATGT TGGGTTA AGTC
CCGCAACGAGCGCAACCCTTGATCTTAGTT
GCCAGCATTCAGTTGGGCACTCTA AGATGAC
TG CCGGTGACAAACCGGAGGAA GGTGGGGA
TGACGTCAAATCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGACGGTACA
AAGGGCAGCGAGACCGCGAGGTTTAGCCAA
TCCCATAAAACCGTTCTCAGTTCGGATTGCAG
GCTGCAACTCGCCTGCATGAAGCTGGAATCG
CTAGTAATCGCGGATCAGCATGCCGCGGTGA
ATACGTTCCCGGTACCTTGTACACACCGCCCG
TCACACCACGAGAGTTCGTAACACCCGAA
GTCGGTGAGGTAACCTTTTGGAGCCAGCCGC
CTAAGGTGGGACTAGCATGACTTGGCGG AGA

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.18542627 is shown. The percentage of replicate

trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The PCR amplification profile of the isolation of bacterial population from slaughter house wastewater is presented in Figure. 5. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The Phylogeny tree analysis of the slaughter bacteria is shown in Figure.6. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd + 3rd+ Noncoding. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 1486 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007). Based on the BLAST analysis in the NCBI, RDB taxonomy analysis and phylogeny tree clearly revealed that that the given sample was belog to the taxa is *Bacillus haikouensis*.

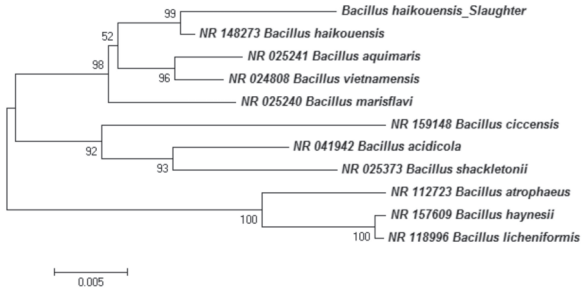


Fig. 6. Phylogeny tree analysis of the slaughter bacteria

SEM Image of the slaughter effluent

The sample was observed under various magnifications in a scanning Electron Microscopy (Figure 7). Most of the studies were focused on the microbial population distribution in the ABR, and the results showed partly disparity of microbial population distribution under different experimental conditions (Sallis and Uyanik, 2003). In this study, the sludge was taken for SEM examination. The image of SEM analysis is shown in Figure 6.

Isolation and Identification of Microbes from Effluents

Isolated organism *Bacillus* species and the organism is identified as *Bacillus haikouensis* by using the biochemical and 16S rRNA gene sequencing. The organisms were observed as gram-positive and rod shaped bacteria. The microbial image of gram positive bacteria is presented in Figure 6.

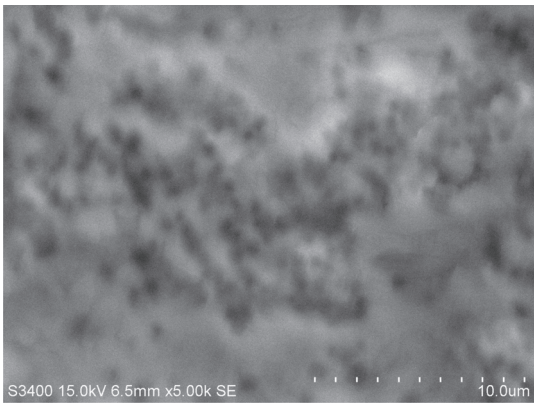


Fig. 7. SEM Image of the slaughter effluent

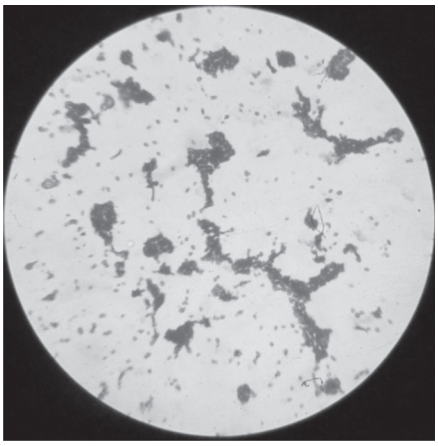


Fig. 8. Microscopic image of Gram-positive bacteria

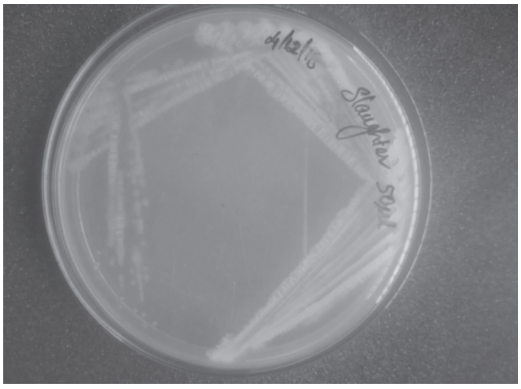


Fig. 9. Isolated slaughter bacteria on Nutrient agar plate

Morphology and Cell structure

*Bacillus haikouensis*is a motile gram-positive,rod-

shaped endospore forming bacterium. In agar,the colonies vary from non-pigmented to grayish-white, which is shown in the figure.9.

CONCLUSION

The Expanded granular sludge bed reactor was operated to treat the slaughterhouse wastewater with high efficiency in a mesophilic range. The maximum COD removal efficiency was achieved at a maximum of 90.17% with a HRT of 4 days with an influent COD of 3176 mg/L at an OLR of 0.081Kg COD/ m^3. Days with addition of co-substrate. The expanded granular sludge bed bioreactor showed the most popular technology because it operates using a fluidized bed, which allows increasing in organic load and in cell retention times, generating higher treatment efficiencies and renewable energy. Finally according to the result of this study, the AEGSB reactor seeding with granular sludge exhibits good process performance for the treatment of slaughter house wastewater at mesophilic range. The temperature was the determinant factor for achieving high efficiency. The microorganism responsible for decomposition of organic matter was identifies *Bacillus haikouensis.* The results achieved in this study were comparable with other process.

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