© 2018 IJSRST | Volume 4 | Issue 5 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Development of an Effective Microbial Consortium for Greywater Treatment

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ABSTRACT

Greywater consists of used water from showers, baths, wash basins, washing machines and kitchen sinks and excludes black water (urine and sewage flush water). About 60-70% of the total domestic wastewater generated comprises greywater. In modern times with an exploding population and limited water resources, effective water management is a challenge for civic authorities and greywater recycling can substitute drinking water usage for purposes like toilet flush, gardening etc. Microorganisms have the potential to degrade organic and inorganic pollutants and are used for such purposes in STP's and CETP's globally. The present study deals with the development of a microbial consortium capable of treating kitchen sink greywater collected from institutional canteen with the objective of development of a novel and economic system for its treatment. For this purpose 13 isolates were tested for their potential of treating such water and 6 isolates were developed into a consortium referred to as Bacterial Seed (BS). The treatment process consisted of 50 mg/L BS and 1% KMnO4 with aeration. These levels were subsequently brought down to 5mg/L BS and 0.01% KMnO4. Treated water was tested for BOD and total suspended solids (TSS) reduction at ……. days and achieved 92.5% and 75% reduction in these values which met the national and international guidelines for reuse. Further bacteria were characterized and 16S rRNA sequencing for one isolate was done. The developed consortium can be implemented for large scale greywater treatment with the huge potentia for non-potable reuse within communities.

Keywords : CETP, STP, BOD, TSS, Bacterial Seed

I. INTRODUCTION

India houses 16% of world's population with 4% of is water resources (Metha .P 2012). About 30% of India's population receives less than 40 lit/capita/day of water for their daily needs and face severe water scarcity & in spite of being called the land of rivers India is ranked 132 of 180 nations in terms of water availability (Jindal. A. 2016 & Metha. P. 2012). In global terms around 2.8 billion people (40%) have to manage with some form of water scarcity. In 2016, India faced the worst water crisis in a decade, with a severe water shortage spread out throughout the country the year 2016 had been very harsh on cites and many of the urban and suburban sectors in cities like Mumbai, New Mumbai, Delhi, Latur etc. had to face severe water cuts affecting thousands of citizens (Whagmare. A. 2016). In times like these the need for effective water management strategies are realized for e.g. rain water harvesting, waste water recycling etc. One such strategy for waste water management is greywater recycling.

Greywater can be defined as household wastewater generated from sources like bathrooms, washing machines and kitchen sinks as a result of day to day household activities like bathing, washing clothes, vegetables and utensils etc. Greywater essentially

comprises of all the household wastewater except that which is generated from toilets.

This represents a substantial amount of around 50-80 % of the total water consumption in a household, which can be easily captured and reused post proper treatment (Hernandez et. al., 2007). This water can be used for toilet flushing, gardening etc. thus reducing the consumption or saving, potable water which would rather have been consumed for these purposes. The fragment of Kitchen sink greywater, which has been considered for this study, comprises around 15% of the total greywater and contains impurities like Bacteria, Oxygen demand, Food particles, Oil and grease, Soaps, Suspended solids, and Turbidity (Ghatidak et. al., 2013).

Currently the most common technologies used for greywater treatment include the use of rotary biological contactors (RBC), constructed wetlands, membrane bioreactors (MBR) and sequence batch reactors (SBR) which involve microorganisms along with physical processes for treatment of greywater. Filtration methods involving filters like sand filters, activated carbon filters, polypropylene spun filters etc. (Buchanan et. al., 2004). However each of these systems have to face a different set of challenges like High Capital cost, Spatial requirements, Operating costs and requirements and reduction in efficiency or inability to remove certain impurities (Buchanan et. al 2004).

Hence there is a need of a system which can treat greywater with the same efficiency along with an attempt to overcome the limitations as far as possible.
The present study aims at the development of an effective microbial consortium which can be used for efficient treatment of greywater independently or in combination with any of the above processes.

II. MATERIALS AND METHODS

2.1 Source of Greywater:

Kitchen greywater was collected from the kitchen sink of college canteen of VES College of Arts, Science and Commerce, Sindhi society, Chembur, Mumbai. 400071.

Source of Bacterial Isolates

Bacterial isolates were primarily obtained from 3 places including Kitchen sink greywater of VES college canteen, Surface soil from Deonar dumping ground at Deonar, Mumbai and Compost sample obtained from VES college compost pit.

Table1: Different bacterial isolates as per their source of isolation

Greywater	Deonar Dump	VES Compost pit
1.GY1 2.GY2 3.GY3 4.GY4 5.GY5 6.GY6	1.DG1a 2.DG1d 3.DG2a 4.DG2c	1.Ca 2. Cb 3. Cd

Isolation of strains was done by using modified Bushnell and Hass (BH) medium (Himedia) with kitchen sink greywater replacing phenol as the carbon source. The samples were inoculated in sterile liquid medium and incubated at room temperature for 7 days. Post incubation, a loop full of the medium were streaked on Nutrient agar (Himedia) plates to isolate single colonies. These strains were maintained on slants for further characterization.

Primary Screening for Enzyme Producing Bacteria

The individual isolates were subjected to the following assays to detect the production of enzymes which would be helpful in degradation of different types of waste particles expected to be found in kitchen greywater and thus be helpful of treatment of greywater.

1) Cellulase production: The individual isolates were spot inoculated on Carboxymethyl Cellulose medium (Himedia) and incubated at 37ºC for 1 week. The plates were flood with 1% Congo red to observe the cellulolytic activity of isolated strain (Kaliwal et al., 2014).

2) Pectinase production: The individual isolates were spot inoculated on Pectinase screening agar medium (PSAM Himedia) plates and incubated at 37ºC for 24 hrs. The plates were then flooded with 50mM iodine solution and incubated for 15 min at 37ºC (Venkata et al., 2013).

3) Amylase production: The individual isolates were spot inoculated on Starch agar plates (Himedia) with starch as the only carbon source. After incubation at 37ºC for 24-48hrs, plates were flooded with Gram's iodine to produce a deep blue coloured starch-iodine complex. In the zone of degradation no blue colour forms, which is the basis of the detection and screening of an amylolytic strain (Sasmita et al. 2008).

4) Lipase production: The individual isolates were spot inoculated on Tributyrin agar base plates (Himedia) and incubated at 37ºC for 2 days. Post incubation a zone of clearance around the colony indicated lipolytic activity (Lakshmi et al., 2010).

5) Protease production: The individual isolates were spot inoculated on Skimmed milk agar plates (Himedia) and incubated at 37ºC for 48 hrs. Post incubation a zone of clearance around the colony indicated proteolytic activity (Anushka et al. 2015).

D. BOD reduction of greywater by individual isolates.

The individual isolates were tested for their ability to treat greywater considering BOD reduction as the criteria. 1 ml of 10^6 cells/ml saline suspension of individual isolates were inoculated in 100 ml of sterile kitchen sink greywater flasks. The flasks were incubated at room temperature on shaker condition for 24 hrs. Following incubation the treated water samples were subjected to BOD determination along with untreated water sample. BOD was determined as per the Indian Standards IS 3025 (Part 44):1993.

E. Development of microbial Consortium for treatment of grey water.

The isolated bacteria were grouped into 2 consortia namely Consortium 1 and Consortium 2 based on their source of isolation, enzyme production and individual BOD reduction ability. The grouped consortia were then subjected to compatibility assay tested for their potential to treat greywater considering BOD reduction and TSS reduction as the criteria. The consortium which had greater greywater treatment potential and compatibility was selected for further studies.

1). Compatibility assay

An individual strain was inoculated as a 1.5-cm-wide streak diametrically across on Nutrient agar plates and the rest of the strains to be used in forming the consortium were streaked singly at right angles to the original inoculum by using a wire loop. The plates were incubated at 27°C overnight, and inhibition was recorded where the indicator strains crossed the original inoculum. This procedure was repeated independently with each strain being streaked centrally (Amrita et. al. 2014)

2) Determination of greywater treatment potential: All the strains were grown overnight and 1 ml saline suspensions containing 10^6 cells /ml were added to Consortium 1 containing 6 strains (Table 3). From this consortium 2 ml suspension was inoculated in 200 ml kitchen greywater and incubated at room temperature for 24hrs on shaker for treatment.

Table 3. List of isolates in the 2 consortia along with their proportion.

Consortium 1	Consortium 2	Ratio
1.GY1 2.GY2 3.GY3 4.GY4 5.GY5	1.DG1a 2.DG1d 3.DG2a 4.DG2c 5.Ca	All the isolates were taken in equal proportion.

6.GY6	6.Cb 7.Cd	

Post incubation the treated and untreated water samples were subjected to BOD and TSS determination as per method mentioned earlier. The same procedure was also followed for Consortium 2. The consortium which performed better in terms of greywater treatment was selected for further studies.

F. Bacterial treatment of Greywater

The selected consortium was labeled as Bacterial Seed (BS). For treatment of greywater 5 mg/L BS along with 0.01% KMnO4 was selected. The process designed was an aerobic batch process in a 1 lit flask with a small fish tank sparger (3 L/min) for aeration and an antifoaming agent (Harmony antifoam Eco 5233). The whole set up was developed and kept for 24 hrs. Water quality parameters like BOD, TSS and pH were determined before treatment and post treatment as per standard methods described earlier.

G. 16S rRNA sequencing of isolates

Molecular characterization of these strains was done by standard method of DNA isolation, DNA purification and PCR amplification of 16SrDNA gene followed by sequencing. A similarity search for the nucleotide sequence of 16S rDNA of the isolate was carried out online at http://www.ncbi.nlm.nih.gov using the BLAST search program for the nucleotide database maintained in Gene Bank.

III. RESULTS AND DISCUSSIONS

A. Isolation of bacteria

A total of 13 different strains were obtained from the three different sources which were selected for further studies.

B. Enzyme production

All the isolates were capable of enzyme production as given in Table 4. The isolates GY2 and GY4 gave the best results producing 4 enzymes each, while all other isolates were capable of producing 2-3 enzymes out of the 5 enzymes tested. GY1 could produce only 1 of the 5 tested enzymes.

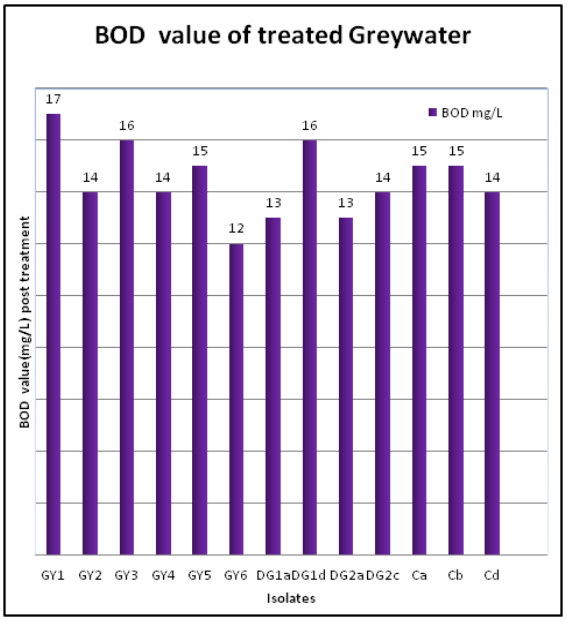
Table 4. Qualitative enzyme production by individual isolates

Isolate	Cellulase	Pectinase	Amylase	Lipase	Protease
GY1	-	-		++	-
GY2	+	-	+	++	++
GY3	-	-	+	+	-
GY4	++	-	++	++	+
GY5		-	+		++
GY6	++	-	++	+	-
DG1a	-	-	++	+	+
DG1d	-	+	++	+	-
DG2a	-	+	++	-	-
DG2c	-	-	++	++	++
Ca	-	-	++	++	++
Cb	-	+	++	-	-
Cd	+	-	++	-	-

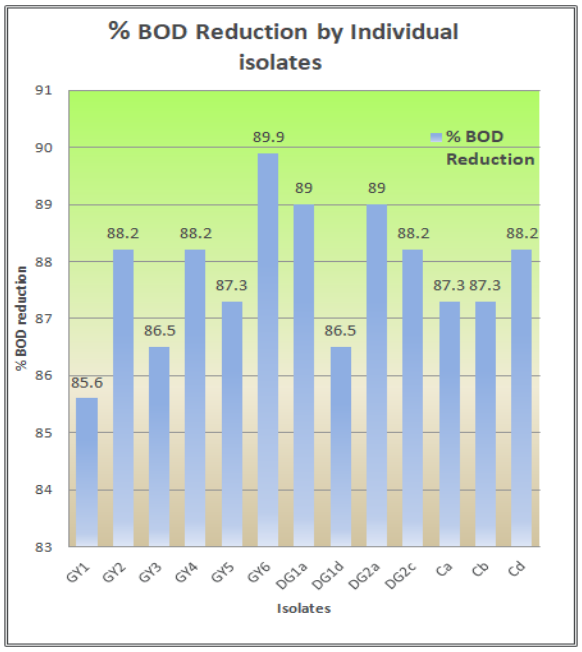
Key: + indicates enzyme production, ++ indicates larger zone compared to + which indicates better enzyme producer, - indicates no enzyme production.

C. Individual BOD reduction potential of the isolates

In order to determine the individual BOD reduction potential of the isolates they were inoculated in greywater having initial BOD 120 mg/L and it was observed for 24 hrs. It was found that there was maximum reduction of BOD to the extent of 89.9 % in the strain GY 6.



Graph 1



Graph 2. BOD values and % reduction in greywater by individual isolates

D. Development of microbial Consortium for treatment of grey water.

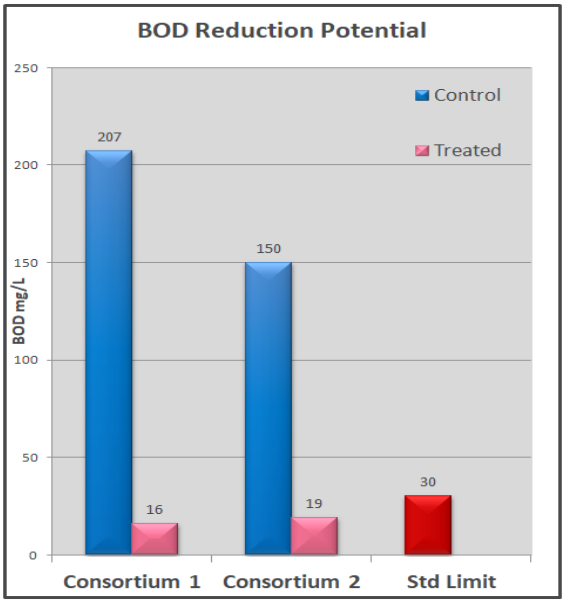
The total isolates were grouped in 2 categories on the basis of their source of isolation, enzyme production and individual BOD reduction and mixed in equal proportion to from the consortia for treatment of grey water.

3.4.1 Compatibility Assay

It was found that none of the isolates in a consortium inhibited the growth of other and showed no signs of antagonism.

E. Determination of greywater treatment potential.

The 02 consortia were compared in terms of their capacity to reduce BOD and TSS and found that consortium 1 was able to reduce BOD and TSS by 96 % and 56 % respectively while consortium 2 reduced BOD and TSS by 88 % and 45% respectively (Graph 3). It was found that Consortium 1 reduced BOD from 207 mg/L to 16 mg/L while Consortium 2 reduced it to 19 mg/l from 158 mg/l (accepted limit for the same is 30 mg/L). Thus both the limits are below acceptable limits prescribed by Environment (Protection) Rules (EPA) 1986.

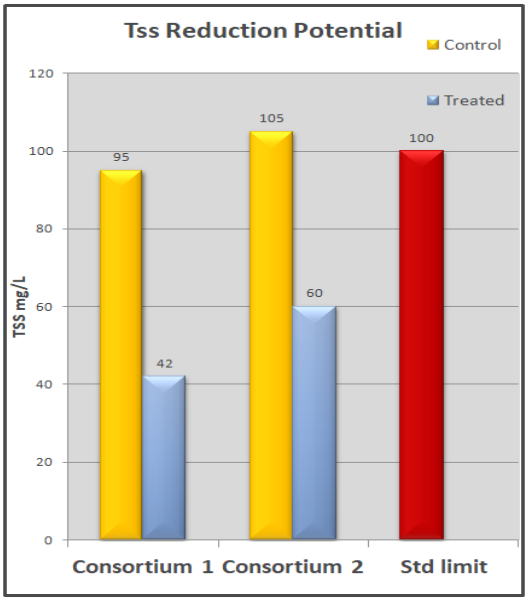


Graph 3. BOD reduction by Consortium 1 & Consortium 2.

Key: Control- BOD mg/L of untreated greywater, Std Limit- Permissible limit for discharge of treated wastewater as per Environment (Protection) Rules (EPA) 1986.

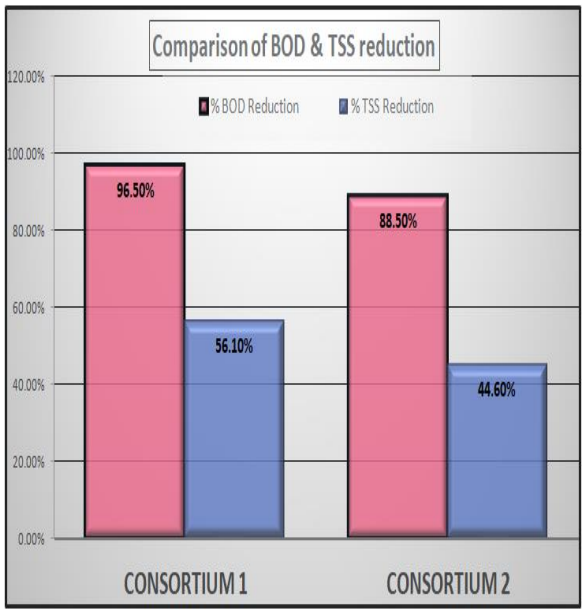
For TSS reduction, it was found that consortium 1 reduced TSS to 42 mg/l from 95 mg/l while

Consortium 2 reduced TSS to 60 mg/l from 105 mg/l (Graph 4). The accepted limit for the same is 30 mg/l.



Graph 4. TSS reduction by the two consortia.

Key: Control- BOD mg/L of untreated greywater, Std Limit- Permissible limit for discharge of treated wastewater as per Central Pollution Control Board (CPBC) 2017.



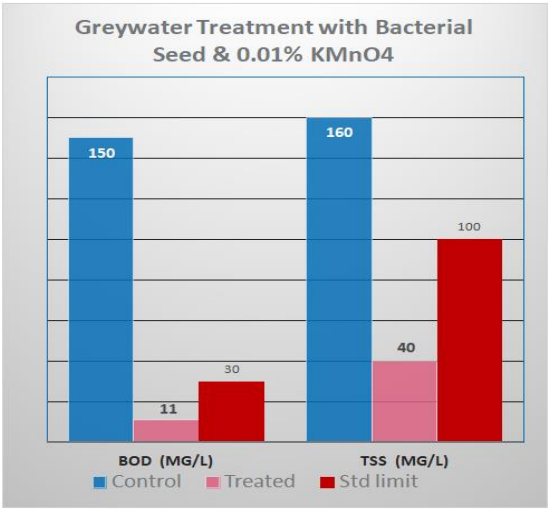
Graph 5. Comparison of % reduction in BOD and TSS levels of greywater by the two consortia.

Key: BOD & TSS reduction by Consortium 1 Vs BOD & TSS reduction by Consortium 2

Thus consortium 1 was able to reduce BOD and TSS by 96% and 56% respectively while Consortium 2 was able to reduce BOD and TSS by 88% and 45% respectively.

F. Bacterial treatment of greywater

Based on BOD & TSS reduction results consortium 1 was selected for grey water treatment. Lab scale treatment of greywater showed reduction of BOD to 11 mg/L from 150 mg/L giving 92.5 % removal efficiency. Similarly TSS levels were brought down to 40 mg/l form 160 mg/l giving 75 % removal efficiency. Both BOD and TSS levels of the treated greywater met with the standard guidelines for reuse.



Graph 6. Bacterial treatment of greywater.

G. 16S rRNA sequencing of the isolate GY3

The strain GY3 was identified on the basis of 16S rRNA sequencing by Sanger's dideoxy method. Full length sequencing was carried out and a 1400 base pair and showed more than 93% homology with Bacillus gottheilii (Singh et al. 2017).

IV. CONCLUSIONS

Grey water treatment has gained importance due to its potential to reuse water. During present study 13 strains were obtained having better ability to treat the greywater. The reduction in BOD was to the extent of 88%. Use of microbial consortium is preferred method of grey water treatment considering the fluctuations

in open environmental conditions. So consortium 1 & consortium 2 were able to reduce BOD & TSS values by well below the standard limits required for their reuse. However Consortium 1 consisting of 6 isolates (GY1- GY6) was more effective than Consortium 2 and gave 96% BOD & 56 % TSS removal was achieved for BOD & TSS removal of Greywater. This consortium along with 0.1% $KMnO_4$, gave even better results of 92% BOD & 75% TSS reduction. Thus the worked proved the potential of greywater treatment for purposes like gardening, toilet flush etc. and thus saving precious drinking water.

V. ACKNOWLEDGEMENTS

Author kindly acknowledges help and support of Dr. Sharad P. Kale, Ex Head, NABTD, Ms. Richa Shah and Dr. Mrs J. K. Phadnis, Principal and all Teaching & Non-Teaching Staff Microbiology Department, VES College, Chembur.

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