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Treatment of greywater using consortium of *Micrococcus luteus*, *Rhodococcus equi* and *Aspergillus niger*

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Greywater is a domestic wastewater excluding toilet waste. Greywater treatment and its reuse can contribute to the reduction of pressure on water resources, and lowering the demand for portable water for purposes that do not require water of high quality. The present study investigated the performance of individual bacterial strain; *M. luteus* and *R. equi*, and fungus strain; *A. niger* for treatment of greywater. In addition, consortium of these three strains has been developed, and further studied for treatment of greywater. The greywater treatment performance of individual strains and consortium was compared in terms of reduction in objectionable physical as well as chemical parameters of greywater. It has been observed that the consortium has shown much better results for treatment of greywater in comparison to individual strains.

Keywords: Greywater, fungal-bacterial consortium, wastewater, bioremediation.

Introduction

Reduction in quality and quantity of freshwater resources is shifting the way towards the use of alternative water sources[1]. Greywater, that is wastewater discharged from showers, baths, kitchen and washing basins represents an attractive alternative water source for non-potable uses[2]. Greywater generation could be up to 75% of the water volume used by households[3]. Potential savings of fresh water can be achieved from greywater recycling[4]. Even though the greywater stream is less polluted, but still it requires treatment for further use. The presence of pollutants is represented as chemical oxygen demand (COD), biological oxygen demand (BOD_5), total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP)[1,5,6]. Since it is the less polluted wastewater stream, a mild treatment is required to make it reusable[4,7].

Various treatment techniques have been studied for their applicability in treating greywater. These mainly include sedimentation, screening, aeration, filtration, floatation, degasification, chlorination, ozonation, neutralization, coagulation, sorption, ion exchange, etc.[8–12]. Several limitations of physicochemical methods including partial treatment, higher cost, generation of secondary pollutants, and use of chemical agents make the bioremediation a favorable alternative for the removal of pollutants[12]. Bioremediation is a process that uses living microorganisms or their enzymes to treat the pollutants, and is highly studied area in this context[13]. Bioremediation is a green and low-cost process which has potential to be a substitute to physical and chemical treatment. Microbial consortium (a group of microbes) is of growing attention because of their ability to simultaneously optimize multiple tasks[14]. Microbial consortia can perform complicated functions that individual population cannot because it is more robust to environmental fluctuations[15,16]. Emphasizing the importance of the bioremediation process, which contributes substantially towards a safe environment, the present work was focused on the greywater treatment through microbial consortia of bacteria and fungus. There are limited scientific studies focusing on greywater reuse, particularly addressing the performance of microbial consortium as a technological solution for the treatment of greywater[17,18]. Cultivation of microbial and fungal consortium into nutrient-rich greywater for treatment has been studied in the present work. The objective of the current study was to investigate the bioremediation capability of individual fungal and bacterial strains in comparison with artificially made consortium for recovery of greywater. It can provide a rationale and scalable solution for removal of pollutants from greywater which will be relevant in terms of recycling and reuse.

Materials and methods:

Microbial stock cultures and wastewater samples:

The microbial isolates *Micrococcus luteus (*Strain no. MTCC 4698), *Rhodococcus equi (*Strain no. MTCC 6939*)* and fungal isolate *Aspergillus niger (*Strain no. MTCC 16888) used in this study were procured from IMTECH Chandigarh, Punjab, India. Stock cultures were refrigerated at 4°C in the refrigerator. Fresh cultures from stock were prepared for degradation studies of pollutants present in greywater.

Residential wastewater (excluding toilet wastewater) was collected from household drains of Whitefield locality of Bangalore, and stored for further study. The samples of greywater collected was stored at a temperature below 4°C to avoid any physicochemical changes. Standard methods were followed for the characterization of greywater[19].

Maintenance of cultures, isolation of micro-organisms and inoculum preparation:

The bacterial and fungal strains were maintained on nutrient agar and potato dextrose agar slants, respectively at 4°C for further study. The stored bacteria and fungus strains were then inoculated on nutrient agar and potato dextrose agar plates, respectively for growth of the culture. The inoculated medium was kept in the incubator for 48 h at 32°C and 96 h at 37°C for bacteria and fungus respectively. From each plate, microbial suspension cultures were prepared in 100 ml of autoclaved inoculum media. The flasks containing cultures were kept on a shaker incubator (150 rpm) for 48 h at 32°C for bacteria, and 96 h at 37°C for fungus.

Consortium development:

For consortium development, a loop full of *M. luteus*, *R. equi* and *A. niger* pure cultures were taken in equal volume and then transferred aseptically into soybean casein agar medium, followed by 96 h incubation at 32°C. The consortium thus obtained used for further studies as a source of inoculum.

Characterization of greywater:

The greywater was characterized for pH, EC (electric conductivity), TDS (total dissolved solids), TS (total solids), TSS (total suspended solids), O and G (oil and grease), BOD (biological oxygen demand), COD (chemical oxygen demand), Mn (manganese), Fe (iron) and turbidity. The pH was measured with the help of a pH meter, while EC and TDS were determined by a meter (Eutech con 700). The turbidity was measured by Nephelometric method. Total solids (TS) and oil and grease (O and G), were estimated by the gravimetric method. Total suspended solids (TSS) were determined by subtracting total dissolved solids (TDS) from total solids (TS). BOD was estimated by preparing a required volume of dilution water with the addition of nutrients and incubation period of five days at 25°C, while chemical oxygen demand (COD) determination was based on rapid dichromate oxidation method. Total organic carbon (TOC) was measured using TOC analyzer (Shimadzu). Inductively coupled plasma mass spectrometry (ICP-MS) was used to detect heavy metals manganese (Mn) and iron (Fe).

Evaluation of consortium and individual strains for greywater treatment:

The individual strains (i.e. *M. luteus, R. equi* and *A. niger*) were used separately to treat the greywater. The bacterial strains (*M. luteus* and *R. equi*) were separately inoculated into the 100 ml of greywater contained the flasks. These inoculated flasks of greywater were then placed in an shaking incubator at 120 rpm and 32°C.

Similarly, the developed consortium (combination of *M. luteus*, *R. equi* and *A. niger*) were separately inoculated into the 100 ml of greywater contained the flasks. These inoculated flask of greywater were then placed in an shaking incubator at 120 rpm and 32°C. The performance of individual strains and consortium for treatment of greywater was then evaluated based on the reduction in pollutant parameters after 96 h.

The fungal strain (*A. niger*) was separately inoculated into the 100 ml of greywater contained the flask. The inoculated flasks of greywater was then placed in an shaking incubator at 120 rpm and 37°C.

Results and discussion

The characteristics of raw greywater are shown in Table 1 and Table 2. The pH of raw greywater highly depends on pH of the water supply and the chemicals used in several activities. The pH was slightly acidic i.e. 6.2, and turbidity

Table 1. Characteristics of *M. luteus* and *R. equi* treated greywater

Parameters	Raw greywater	Treated greywater							
		M. luteus				*R. equi*			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
pH	6.37	7.37	7.60	7.5	7.5	7.37	7.77	6.45	6.34
Colour	Milky	Milky	Milky	Less milky	Less milky	Milky	Clear	Clear	Clear
Odour	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Odorless	Odorless	Odorless
Turbidity, NTU	40.43	38.45	25.40	24.35	23.45	5.80	19.60	18.45	17.34
Electrical conductivity (μS/cm)	2048.17	1062.10	1045.40	1045.31	1044.21	1078.03	1016.07	1015.34	1014.34
Total suspended solids (mg L^{1})	680.67	510.63	425.57	424.34	424.12	609.70	466	465.32	464.89
Total dissolved solids (mg L^{-1})	1230.67	1110.40	850.77	849.76	848.21	1210.37	930.57	929.09	928.34
TOC (mg L^{-1})	385.90	249.27	136.50	135.43	134.22	246.80	129.63	128.45	128.45
Iron (μg L^{-1})	556.47	215.63	96.87	96.84	95.33	125.77	45.73	45.34	44.45
Manganese (μg L^{-1})	87.70	68.03	53	52.11	52.01	37.52	26.37	25.45	25.36

reported was 40 NTU. The color and order was milky and pungent, respectively as judged by the senses of sight and smell. The electrical conductivity was 2048 μS/cm and total solids (TS) concentration was 1910 mg L^{-1}. Initial BOD, COD, and TOC values were 540 mg L^{-1}, 1165.6 mg L^{-1} and 176.5 mg L^{-1}, respectively. Most of the organic carbon detected in greywater sample is originated in the detergents used and the clothes impurities. Total organic carbon (TOC) in greywater is due to laundry detergents and dishwashing. Greywater has a significant amount of oil and grease as it is the waste from kitchen sinks. A total of 58 mg L^{-1} of oil and grease was recorded in raw greywater. Heavy metals content is low for untreated greywater samples and conform to Drinking Water Directive 2015/1787/EC and WHO (2008) drinking water quality guidelines. The concentration of heavy metals often found in greywater is due to detergents and dishwasher. It is well known that laundry detergents are a source of many heavy metals. When the greywater in the present sample was tested for the presence of metals, iron and manganese were found 556.4 μg L^{-1} and 87.2 μg L^{-1}, respectively. Tables 1 and 2 also show the change in various parameters of the greywater when treated with the microbes. The pH of the treated greywater shifts towards neutral pH after treatment. The reduction in turbidity due to *M. luteus, R. equi, A. niger* and consortium after time period of 96 h was observed to be 23.45 NTU, 17.34 NTU, 18.50 NTU and 1.30 NTU, respectively as shown in Table 1 and Table 2. The consortium caused highest reduction in turbidity due to higher consumption of suspended solids by microorganisms as their nutrients for further growth. Consortium showed better re-

Table 2. Characteristics of *A. niger* and consortium treated greywater

Parameters	Raw greywater	Treated greywater							
		A. niger				Consortium			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
pH	6.37	7.37	7.60	7.30	8.03	7.37	7.77	7.38	7.26
Colour	Milky	Milky	Milky	Milky	Milky	Milky	Clear	Clear	Clear
Odour	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent	Odorless	Odorless	Odorless
Turbidity, NTU	40.43	40.43	38.3	22.80	18.50	35.80	19.60	2.94	1.30
Electrical conductivity (μS/cm)	2048.17	2048.17	1045.40	1078.53	1001.33	1078.03	1016.07	765.69	480.70
Total suspended solids (mg L^{1})	680.67	680.67	679.57	420.80	362.73	609.70	466	291.70	136.76
Total dissolved solids (mgL^{-1})	1230.67	1230.40	1229.77	890.53	690.93	1210.37	930.57	929.09	928.34
TOC (mg L^{-1})	385.90	385.27	136.50	171.40	96.67	246.80	129.63	118.33	630.23
Iron (μg L^{-1})	556.47	556.23	556.87	45.73	36.03	425.77	345.73	55.9	12.8
Manganese (μg L^{-1})	87.70	87.03	86.56	26.37	15.50	37.52	26.37	12.20	8.55

duction (480.70 µS/cm) in electrical conductivity (EC) than *M. luteus* , *R. equi* and *A. niger* with 1044.21 µS/cm, 1014.34 µS/cm and 1001.33 µS/cm, respectively. The reduction in EC might be due to use of ions by microorganisms for their growth and survival. After 96 h, the reduction in suspended solids by *M. luteus* , *R. equi, A. niger* and consortium was shown to be 424.12 mg L^{-1}, 464.89 mg L^{-1}, 362.73 mg L^{-1} and 136.76 mg L^{-1} , respectively. The consortium was the most efficient in TDS, TS and TOC reduction also. The organic contents along with other mineral ions of greywater could have been used by microorganisms to cause overall reduction of total solids which also proves the efficiency in removal of nutrients. It is also evident from Tables 1 and 2 that consortium was able to remove Fe and Mn at significantly higher levels as compared to those achieved by individual strains.

Three significant parameters of greywater are BOD, COD and oil and grease. Therefore, these parameters have been discussed separately in form of graphical representation. Fig. 1 shows the performance of individual strains and consortium in term of reduction in BOD of the greywater. The consortium was able remove BOD at significantly higher levels as compared to those achieved by individual strains. The result showed that the consortium accounted for the maximum removal (454.5 mg L^{-1}) of BOD in 96 h. In contrast, 245.9 mg L^{-1}, 377.2 mg L^{-1}, and 442.1 mg L^{-1} of BOD removal was observed in case of *M. luteus*, *R. equi* and *A. niger* respectively. The *A. niger* has shown minute reduction in BOD during initial 48 h because its negligible growth during this time. Fig. 2 shows the reduction in COD by individual strains and consortium. A COD removal of 915.7 mg L^{-1} was obtained using consortium, while 712.3 mg L^{-1}, 774.4 mg L^{-1} and 875.07 mg L^{-1} of COD removal was obtained by employing *M. luteus*, *R. equi* and *A. niger* respectively. Almost no reduction in COD has been shown during initial 42 h by *A. niger* because of its negligible growth in this time. Fig. 3 shows the reduction in oil and grease content by individual strains and consortium. A significant reduction of 48.09 mg L^{-1} was recorded in case of consortium whereas, less reduction can be seen for individual microbial strains: 24.2 mg L^{-1} (*M. luteus*), 42.2 mg L^{-1} (*R. equi*), 30.9 mg L^{-1} (*A. niger*).

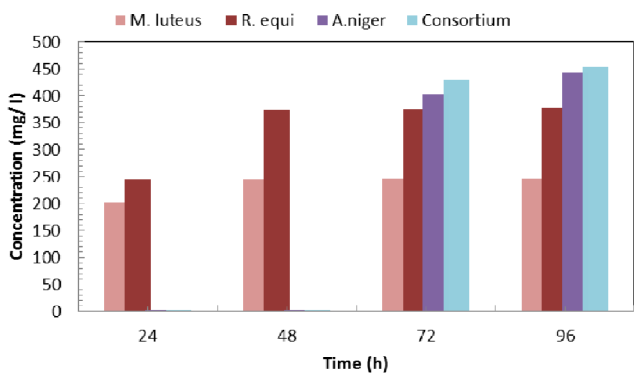

Fig. 1. Reduction in biological oxygen demand (BOD).

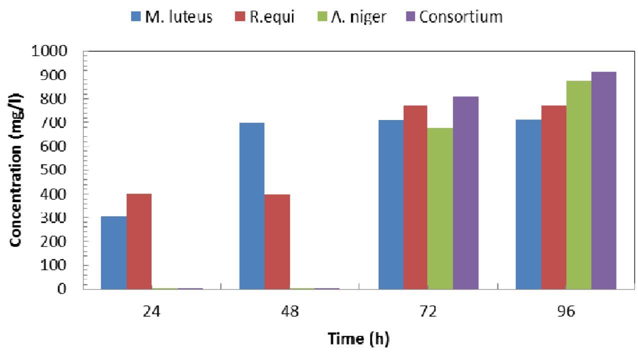

Fig. 2. Reduction in chemical oxygen demand (COD).

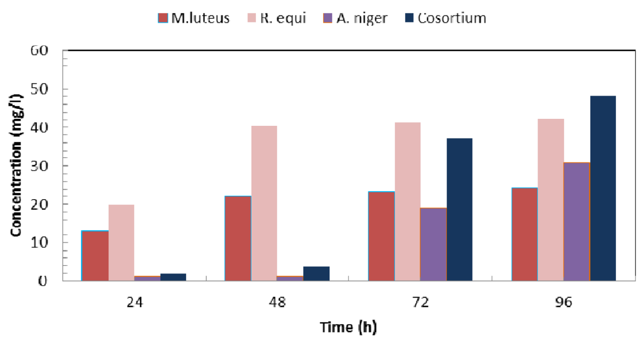

Fig. 3. Reduction in oil and grease content.

It has been observed that consortium of microbes has worked significantly better than individual strains as far as treatment of greywater is concerned. Microorganisms in a group or consortium work synergistically where growth, biological processes and enzymatic activities are conducted more effectively and efficiently than microorganisms on an individual population basis[20]. Similarly, Tuerson *et al.* (2013) has reported that the symbiotic relationship among the microorganisms in consortium provides the basis for its stability and efficiency[21]. Microorganisms in a consortium also maintain metabolic and ecological compatibility and stability.

Conclusions

The present study investigated the performance of individual bacterial and fungus strains for treatment of greywater. The performance of the individual strains is compared with the performance of consortium for treatment of greywater. It has been observed in the study that microbial consortia has shown much better capability to treat the greywater as compared to individual strains. The data and information obtained in the present study may be further used for the improvement of biodegradation of pollutant of greywater.

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