Remediation of Domestic Wastewater by Using Algal and Fungal Mixed Culture: An Experimental Study

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Abstract

The objective of this study was to assess the ability of the algal and fungal mixed culture in wastewater treatment. The algal and fungal samples were collected from River Mula, Pune. These samples were used to isolate most dominant and pollution tolerant algae such as *C. Vulgaris, S. quadricauda* and fungi such as *A. terreus, A. niger* which used for the treatment. The experiments were carried out using various combinations of algae and fungi, the following method was employed, i) Wastewater treated with *AtCv, AnCv, AtSq, AnSq, AtAn* and *CvSq* and ii) Wastewater treated without Wastewater treated with *AtCv*, *AnCv*, *AtSq*, *AnSq*, *AtAn* and *CvSq* (Control). Samples were periodically (every 5th day) analyzed for physico-chemical parameters such as pH, phosphate, nitrate, BOD and COD using standard methods. The mixed culture of *AtCv* and *AtAn* were best result of COD reduction at 15th days of treatment. The mixed culture of *CvSq* and *AtCv* were observed best result of BOD reduction at 15th days of treatment. *CvSq* have capacity removal of nitrate and phosphate was best upto 15th days of treatment. Present investigation focuses on the bioremediation of wastewater by using mixed culture of algae and fungi.

KEYWORD: bioremediation, wastewater, algae, fungi, mixed culture, physico-chemical parameters

Introduction

Bioremediation is an eco friendly approach for remediation of contaminated soil and water. Biological treatment can be accomplished in a number of ways, but the basic characteristic of the system is the use of mixed microbial culture; bacteria, fungi and / or algae, for the conversion of pollutants (Azab, 2008). Most of the studies on the use of microalgae for wastewater treatment have been based on the use of a monoculture to remove a specific nutrient. The use of microalgae for removal of nutrients from different wastes has been described by a number of authors (Benemann *et al.*, 1977; Gupta and Rao, 1980; Williams, 1981; Kunikane *et al.*,1984; Senegar and Sharma, 1987; Tam and Wong, 1989; Gantar *et al.*, 1991; De la Noue, 1992; De-Bashan *et al.*, 2002; Queiroz *et al.*, 2007; Rao *et al.*, 2011; Kshirsagar, 2013). The use of fungi for wastewater treatment has been described by many authors (Hasija, 1994; Pletsch *et al.*, 1999; Kapoor and Viraraghavan, 1995; Denizli *et al.*, 2004; Aksu, 2005; Kshirsagar, 2013). But only a few studies have been reported on the use of mixed algal cultures for wastewater treatment (Gantar *et al.*, 1991).

The main objective of this study, to reduce the COD, BOD, nitrate and phosphate level of wastewater using a biological method employing algae and fungi which was isolated from a local area that is Mula river from Pune city. Present investigation focuses on the bioremediation of wastewater by using mixed culture of algae and fungi.

Materials and Methods

Collection of wastewater

The domestic wastewater samples used in this study were collected from sewage wastewater treatment plant Bopodi from Pune city.

Analytical methods

The wastewater was analyzed for pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), phosphate and nitrate were before and after using algae for treatment using the standard techniques described by (APHA, 1998).

Microorganism selection

The algal species used in this study were isolated from water from river Mula, Pune (Kshirsagar and Gunale, 2011; Kshirsagar *et al.*, 2012). The most dominant and pollution tolerant algal species such as *Chlorella vulgaris* Beijerinck and *Scenedesmus quadricauda* (Turpin) Breb. and fungal species such as *Aspergillus terreus* Thom., *Aspergillus niger* Tiehhem were used as test organisms for the treatment processes of wastewater.

Algal and fungal medium and culture

The individual algal culture (Kshirsagar, 2013) in Bold Basal Medium and fungal cultures in Potato Dextrose Agar and Malt Extract Broth (Kshirsagar, 2013) were grows and maintained before applying for wastewater treatment.

Experimental set-up

The test species of algae, *Chlorella vulgaris* (*Cv*) and *Scenedesmus quadricauda* (*Sq*), *Aspergillus terreus* (*At*) and *Aspergillus niger* (*An*) were used for wastewater treatment. The wastewater samples collected from sewage wastewater treatment plant Bopodi from Pune city were used for determined the algal and fungal capacity for removal of pollutants. The water samples were analyzed for pH, BOD, COD, nitrate and phosphate. The wastewater was filtered using Whatman No. 1 filter paper to remove suspended solids particles.

To study the role of mix culture of algae and fungi in wastewater treatment, the following method was employed, i) Wastewater treated with *Aspergillus terreus* + *Chlorella vulgaris* (*AtCv*), *Aspergillus niger* + *Chlorella vulgaris* (*AnCv*), *Scenedesmus quadricauda* + *Aspergillus terreus* (*AtSq*), *Scenedesmus quadricauda* + *Aspergillus niger* (*AnSq*), *Aspergillus terreus* + *Aspergillus niger* (*AtAn*) and *Chlorella vulgaris* + *Scenedesmus quadricauda* (*CvSq*) and ii) Wastewater treated without *Aspergillus terreus* + *Chlorella vulgaris* (*AtCv*), *Aspergillus niger* + *Chlorella vulgaris* (*AnCv*), *Scenedesmus quadricauda* + *Aspergillus terreus* (*AtSq*), *Scenedesmus quadricauda* + *Aspergillus niger* (*AnSq*), *Aspergillus terreus* + *Aspergillus niger* (*AtAn*) and *Chlorella vulgaris* + *Scenedesmus quadricauda* (*CvSq*) (Control). Experiments were conducted in triplicates.

In this experiment, algae and fungi mixed cultures at the following ratios were used i.e. 1:1 respectively. Pollution tolerant mixed cultures at respective ratios were incubated in 250 ml capacity flask containing 200 ml wastewater at room temperature. Sample was set with untreated wastewater as a control. The experiment was conducted under controlled conditions (temperature 27 ± 2ºC) for a total duration of 20 days. Samples were periodically (every 5th day) analyzed for various physico-chemical parameters such as pH, phosphate, nitrate, BOD and COD using standard methods (APHA, 1998).

Result and Discussion

The experiments were carried out using various combinations of algae and fungi to know the removal of BOD, COD, nitrate and phosphate from wastewater. Using mix culture of *Chlorella vulgaris* (*Cv*) and *Scenedesmus quadricauda* (*Sq*), *Aspergillus terreus* (*At*) and *Aspergillus niger* (*An*) were showed significant result in COD, BOD, nitrate and phosphate reduction level.

The effect of pH of wastewater using mixed culture of algae and fungi for treatment were observed. The effect of various pH using mixed culture as mention in **(Table-1)**. The initial pH of wastewater was 7.41 ± 0.10. Wastewater treated with mix culture of *AtCv*, *AnCv*, *AtSq*, *AnSq* and *AtAn* were 7.24±0.06, 7.30±0.02, 7.16±0.04, 7.24±0.04 and 6.85±0.13 upto 10th day respectively. pH were firstly decreased after that pH were increase while wastewater treated with *CvSq* pH was increased from 5th days. Manoharan and Subramanian (1993) found a rise in pH value up to the tenth day of growth of algae in paper mills wastewater treatment.

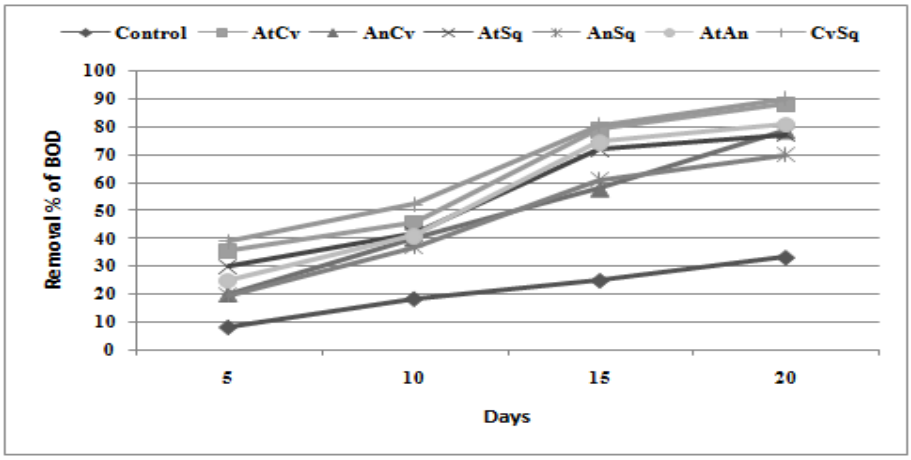
Table-1: Analysis of pH from wastewater using mixed culture of algae and fungi.

Initial pH	**7.41±0.10**						
Days	**Control**	***AtCv***	***AnCv***	***AtSq***	***AnSq***	***AtAn***	***CvSq***
5th	7.69±0.07	7.04±0.05	6.85±0.11	6.76±0.12	7.04±0.08	6.40±0.04	7.68±0.07
10th	7.08±0.06	7.24±0.06	7.30±0.02	7.16±0.04	7.24±0.04	6.85±0.13	7.98±0.04
15th	7.66±0.14	7.47±0.06	7.24±0.07	7.58±0.04	7.16±0.05	7.16±0.06	7.75±0.06
20	7.73±0.06	7.08±0.03	7.58±0.03	7.31±0.04	7.36±0.08	7.42±0.10	8.09±0.07

Values represent the mean ± SD of three replicates.

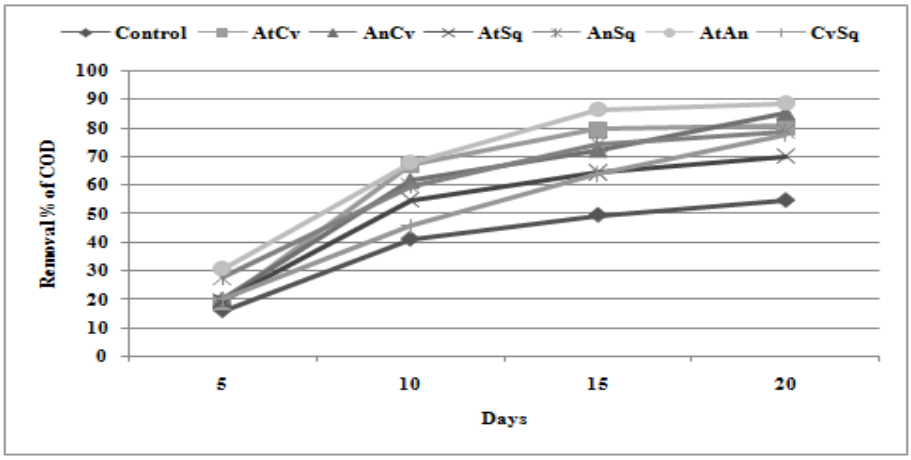
The ability of mixed culture in reducing BOD of wastewater was also conducted. The mixed culture consisted of *Aspergillus terreus* + *Chlorella vulgaris* (*AtCv*), *Aspergillus niger* + *Chlorella vulgaris* (*AnCv*), *Scenedesmus quadricauda* + *Aspergillus terreus* (*AtSq*), *Scenedesmus quadricauda* + *Aspergillus niger* (*AnSq*), *Aspergillus terreus* + *Aspergillus niger* (*AtAn*) and *Chlorella vulgaris* + *Scenedesmus quadricauda* (*CvSq*) were 78.9 %, 57.63 %, 71.85 %, 61.11 %, 74.41 % and 80.6 % respectively upto 15th days **(Fig.1)**. The mixed culture of *AtCv* and *CvSq* were showed best result of BOD reduction. Travieso *et al*., (2006) tested the effect of initial concentration of settled piggery wastewater in the range (250-1100) mg/L on mixed culture of *Chlorella vulgaris* and bacteria, they have found 88 % removal efficiencies of COD from initial concentration (250 mg/L) for 190 hrs at 20°C.

Fig. 1: Removal % of BOD of wastewater using mixed culture of algal and fungi.



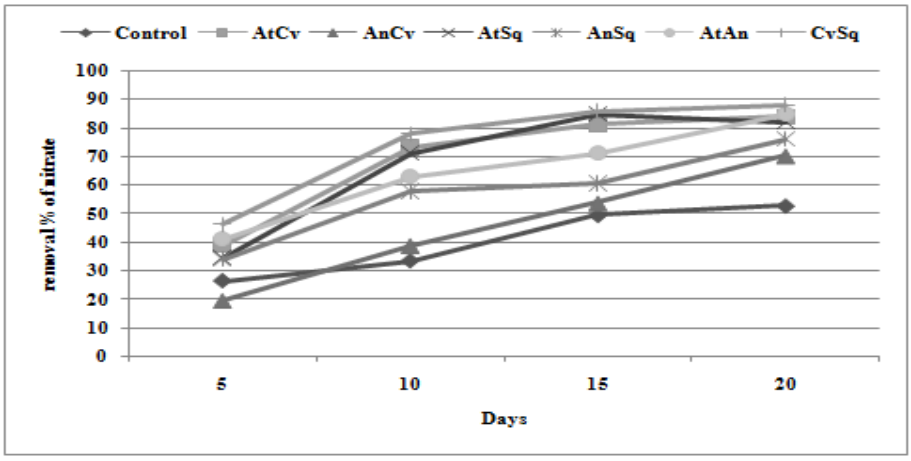
The reduction of COD increased by using the mixed culture of *Aspergillus terreus* + *Chlorella vulgaris* (*AtCv*), *Aspergillus niger* + *Chlorella vulgaris* (*AnCv*), *Scenedesmus quadricauda* + *Aspergillus terreus* (*AtSq*), *Scenedesmus quadricauda* + *Aspergillus niger* (*AnSq*), *Aspergillus terreus* + *Aspergillus niger* (*AtAn*) and *Chlorella vulgaris* + *Scenedesmus quadricauda* (*CvSq*) were 79.36 %, 72.26 %. 64.52 %, 74.19 %, 86.45 % and 63.87 % respectively upto 15th days **(Fig.2)**. The mixed culture of *AtCv* and *AtAn* were showed best result of COD reduction at 15th days of treatment. The removal percentage of COD was in order of *AtAn* > *AtCv* > *AnSq* > *AnSq* > *AnCv* >*AtSq* >*CvSq* > Control **(Fig.2)**. The mixed culture was able to reduce the COD level of textile wastewater having diverse and complex structures at faster rate than individual cultures (Kehra *et al*., 2005).

Fig. 2: Removal % of COD of wastewater using mixed culture of algal and fungi.



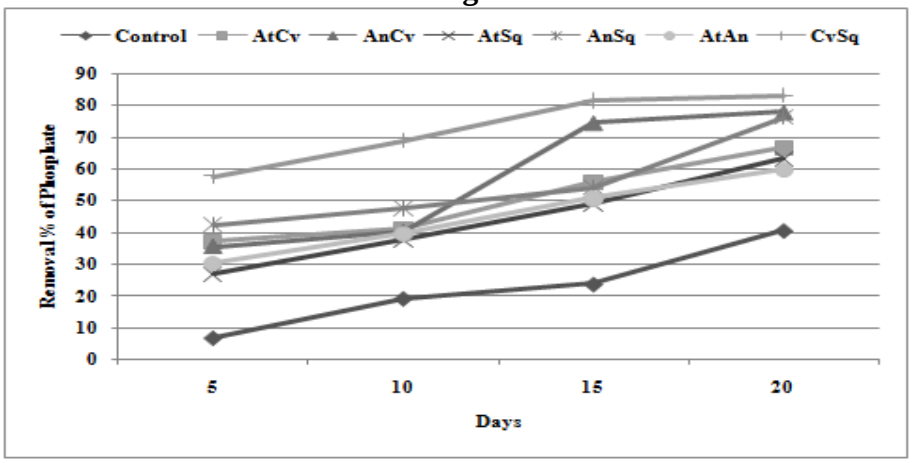
Bioremediation of nitrate from wastewater was 52.75 % in control while with *Aspergillus terreus* + *Chlorella vulgaris* (*AtCv*), *Aspergillus niger* + *Chlorella vulgaris* (*AnCv*), *Scenedesmus quadricauda* + *Aspergillus terreus* (*AtSq*), *Scenedesmus quadricauda* + *Aspergillus niger* (*AnSq*), *Aspergillus terreus* + *Aspergillus niger* (*AtAn*) and *Chlorella vulgaris* + *Scenedesmus quadricauda* (*CvSq*) were 81.31 %, 53.84 %, 84.00 %, 60.44 %, 71.14 % and 85.79 % respectively upto 15th days **(Fig-3)**. Nitrate reduction order in wastewater was *CvSq* > *AtSq* > *AtCv* > *AtAn* > *AnSq* > *AnCv*. Gonzales, (1997) found that the microalgae *Chlorella vulgaris* and *Scenedesmus* sp removed 95% of ammonium-nitrogen and 50% of phosphorus in wastewater. Good efficiency of removal of ammonia nitrogen using *Chlorella* and *Scenedesmus* mixed culture (Witt and Borchar, 1960).

Fig. 3: Removal % of nitrate of wastewater using mixed culture of algal and fungi.



The percentage of phosphate reduction through control treatment was 23.74% while *Aspergillus terreus* + *Chlorella vulgaris* (*AtCv*), *Aspergillus niger* + *Chlorella vulgaris* (*AnCv*), *Scenedesmus quadricauda* + *Aspergillus terreus* (*AtSq*), *Scenedesmus quadricauda* + *Aspergillus niger* (*AnSq*), *Aspergillus terreus* + *Aspergillus niger* (*AtAn*) and *Chlorella vulgaris* + *Scenedesmus quadricauda* (*CvSq*) were 55.92 %, 74.58 %, 49.16 %, 54.25 %, 50.83 % and 81.34 % respectively upto 15th days. *CvSq* was showed best removal capaciy of phosphate upto 15th days of treatment **(Fig-4)**. Phosphate removal of wastewater order among mixed culture of fungi and algae was as *CvSq* > *AnCv* > *AtCv* > *AnSq* > *AtAn* >*AtSq*> Control **(Fig-4)**. *Chlorella* sp. and *Scenedesmus* sp. have been widely used in wastewater treatment as they have fast growth rates and high nutrient removal capabilities (Rao *et al*., 2011). The removal percentage of phosphate by *Chlorella vulgaris* and *Scenedesmus quadricauda* was higher in initial. Freshwater unicellular microalgae, mainly *Chlorella* and *Scenedesmus*, have been used in wastewater treatment for the removal of nitrogen and phosphorus compounds (Hammouda *et al*., 1995; Gonzalez *et al*., 1997). Chaturvedia *et al*., (2006) also described that each strain in mixed culture played a major role in bioremediation of effluent.

Fig. 4: Removal % of phosphate of wastewater using mixed culture of algal and fungi.



Conclusion

COD, BOD, nitrate, phosphate of wastewater were showed reduction capacity using mixed culture of algae and fungi. The mixed culture of *AtCv* and *AtAn* were best result of COD reduction at 15th days of treatment. The mixed culture of *CvSq* and *AtCv* were observed best result of BOD reduction at 15th days of treatment. *CvSq* have capacity removal of nitrate and phosphate was best upto 15th days of treatment. Therefore, it was found that the bioremediation using algae and fungi of wastewater provides an effective and environmentally acceptable option for waste water remediation, which not only recycles valuable nutrients but also improves water quality. These experiments confirm that mix culture of algae and fungi were showed high reduction capacity of BOD, COD, phosphate and nitrate. Therefore *Chlorella vulgaris*, *Scenedesmus quadricauda Aspergillus terreus*, *Aspergillus niger* may be considered efficient nutrient removers.

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