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A Comparative Assessment on Efficiency of *Aspergillus Flavus and Aspergillus Aculeatus* in Reducing COD Concentration of Dairy Effluent and Evaluation of Phytotoxicity Studies

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ABSTRACT
Dairy is a prospering industry due to a high demand for milk & its products. The wastewaters produced by this sector contain significant amounts of nutrients, chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), organic and inorganic substances, which, if not properly handled, can create serious environmental problems. Many treatment technologies are already in use, and due to the high biodegradable nature of dairy effluent, standard biological treatment methods are acceptable. Biological method of effluent degradation is found to be efficient and cost effective involving the natural processes resulting in the efficient conversion of hazardous compounds into simpler ones. This technique requires suitable microbial strains which can undergo various physico-chemical reactions in the polluted water and during the metabolism the pollutants are degraded and removed. In the present study, an attempt has been made to study the efficiency of Aspergillus flavus and Aspergillus aculeatus in dairy effluent treatment. Results reveal that the organisms have proved their bioremediation potency in treatment of the effluent.
Keywords: *Bioremediation, Dairy effluent, Fungi, Phytotoxicity, Aspergillus flavus, Aspergillus aculeatus, Zea maize.*

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INTRODUCTION

The versatility of water as a solvent is essential to living organisms. It is necessary for agriculture, industries, power generation and domestic purposes. Water quality is being altered by anthropogenic activities and is declining due to urbanization, industrialization, modern agriculture, population growth, climate change and other factors. Water pollution is a serious issue since water bodies are contaminated by the addition of contaminants. The resulting threat will harm the well-being of earth and its population. Industrial water pollution is one of the issues being faced by the developing countries. Rapid increase in industrialization has led to environmental degradation and the waste generated has contributed to pollution (Haldar and Islam, 2016). In India, industries are the second highest consumers of water. Industrial water demand has been increasing with the rapid pace of industrial development. The growth in some of the water dependent industries has been quite significant, putting further pressure on the industrial demand for water.

Effluents discharged directly to the fresh water stream will alter the aquatic environment, thereby threatening the aquatic life. Effluents discharged into water bodies disturb the natural environment and chemicals present in it will create the problem of bioaccumulation and bio magnification across the food chain. Physical, chemical and biological alteration of water bodies will alter the aesthetic and such water is unfit for consumption.

Dairy is one of the primary businesses in India that produces organic-rich effluent, resulting in odorous and high COD-containing water. As a result of washing the milking facility after each milking session to maintain sanitary operations, the dairy business creates wash water, a high-strength waste. Cleaning products, fresh water, milk waste, and animal waste are all present in high amounts in wash water. Dairy waste is primarily organic and slightly alkaline in nature, and when released into streams without treatment, it depletes

dissolved oxygen (DO) quickly and promotes algae development, resulting in eutrophication. The waste can become unsuitable for biological treatment due to the overuse of surfactants in dairy. The characteristics of industrial wastewater differ from one industry to the next, and within sectors, quality varies based on the operations. According to reports, the dairy sector generates 6-10 litres of waste water for each litre of milk processed. As a result, the effluents must be treated before being discharged into the environment (Shivsharan *et al.,* 2013)

Physico-chemical treatment (primary treatment) processes help to remove suspended solids, colloidal particles, floating matters, colour and toxic compounds by several conventional and non-conventional methods like sedimentation, floatation, screening, adsorption, coagulation, oxidation, ozonation, electrolysis, reverse osmosis, ultrafiltration and nano filtration technologies. However, these methods are quite expensive and not feasible (Hossain and Ismail, 2015). Biological treatment (secondary treatment) allows microorganisms to utilize the pollutants as nutrients and lower the pollutant concentration under aerobic or anaerobic conditions. Bioremediation is a technique used to treat the contaminated environment using naturally occurring or genetically modified organisms (Subashini, 2015).

Bioremediation is a pollution remediation technique that utilizes biological system to catalyze the degradation or transformation of various toxic chemicals to less toxic or non-toxic. It is a technology which serves as a potential tool to remove pollutants from contaminated site by the action of bacteria, fungi and other biological agents by natural biological activity (Olawale, 2014). This technique stimulates the growth of microorganisms that utilize the pollutants as a source of food and energy. Mycoremediation is an economical alternative to extracting, transporting and storing toxic waste and is reported in many research works. Fungal bioremediation is also found to be an effective tool in remediation of effluents (Kedia and Sharma, 2015).

In the present study fungal isolates *Aspergillus flavus* & *Aspergillus aculeatus* were used to assess their bioremediation efficiency for treatment of textile dairy effluent.

MATERIAL AND METHODS

Effluent collection

Effluent for the present study was collected from a dairy located in Machenahalli, Shimoga, Karnataka, India. Sample was collected from the inlet of effluent treatment plant using a sterile container. Dry cans were used to collect samples, which were correctly labeled. The wastewater was then taken to a laboratory and preserved at 40^0 Celsius for further research.

Isolation of fungal strains

The isolated and sequencing fungal strains *Aspergillus flavus* and *Aspergillus aculeatus* were chosen. The two fungal strains deposited in the NCBI had 100% identity with accession number MZ544387 for *Aspergillus flavus* isolate KUESCCHK -1 and accession number MZ569631 for *Aspergillus aculeatus* isolate KUESCCHK-3. Microscopic examination was done for the fungal strains at 40X magnification and macroscopic observation was also done (Table 1, figure 1 & figure 2).

Experimental setup

Aspergillus flavus and *Aspergillus aculeatus* were tested for their biodegradation ability under laboratory condition. For the treatment, effluent sample was diluted to 3 different concentrations viz. 25%, 50% and 75%. The purpose of dilution was to study the degradation efficiency of the organism at different effluent concentrations. The organism was inoculated into each effluent concentration and treatment was conducted in laboratory for 5 days.

Chemical oxygen demand was analysed using the standard methods of APHA (2017).

Phytotoxicity study of treated effluent

Phytotoxicity study was conducted to assess the toxicity of effluent on the germination of *Zea maize* seeds before and after treatment with *Aspergillus flavus and Aspergillus aculeatus*. For the experiment, 10 ml of treated and untreated effluent sample was used every day. A control set was also maintained. Treatment was conducted for 10 days and results were noted in terms of seed germination index (GI), relative seed germination (RSG) and relative root elongation (RRE).

$$\text{Relative Seed Germination (\%)} = \frac{Number\ of\ seeds\ germinated\ in\ the\ sample\ extract}{Number\ of\ seeds\ germinated\ in\ the\ control} \times 100$$

$$\text{Relative Root Elongation (\%)} = \frac{Mean\ root\ elongation\ in\ the\ sample\ extract}{Mean\ root\ elongation\ in\ the\ control} \times 100$$

$$\text{Germination Index (\%)} = \frac{(\%Seed\ germination) \times (\%Root\ elongation)}{100}$$

RESULTS AND DISCUSSION

Chemical oxygen demand

The amount of a certain oxidant that reacts with the sample under controlled conditions is known as COD. The amount of oxygen equivalency consumed is used to calculate the amount eaten. The COD of wastewater was evaluated using an open reflux method and a COD reflux device. Before treatment, the effluent had a high COD (mg/L), however it was significantly reduced following treatment with the study microorganisms (Table 2 and Figure 3).

The reduction in COD using *Aspergillus flavus* and *Aspergillus aculeatus* is as follows:

Aspergillus flavus: 2330.33±0.57 to 2040.33±0.57 (raw effluent), 1681 ±1 to 1349.63±0.55 (75% concentration), 1109.67±0.57 to 680.2±0.64 (50% concentration) and 579.66±0.57 to 344.8±0.52 (25% concentration) (Table 2). The percentage reduction in COD was 20.60% (raw effluent), 29.76% (75% concentration), 36.93% (50% concentration) and 48.10% (25% concentration) (Figure 3)

Aspergillus aculeatus: 2330.33±0.57 to 1849±0.77 (raw effluent), 1681 ±1 to 1180.1±0.15 (75% concentration), 1109.67±0.57 to 699.86±0.15 (50% concentration) and 579.66±0.57 to 301.06±0.05 (25% concentration) (Table 2). The percentage reduction in COD was 12% (raw effluent), 19.64% (75% concentration), 26% (50% concentration) and 40.05 (25% concentration) (Figure 3)

After treatment, it was found that *Aspergillus flavus* was effective at reducing COD at concentrations of 50% and 25%. These findings agree with Lokeshwari *et al.*, (2013) who worked on microbial degradation and nutrient optimization of wastewater in which COD was reduced by 90.2% by fungal treatment.

Phytotoxicity study:

In the present study, wastewater was treated with two fungal strains to reduce the concentration of hazardous pollutants. After treatment, the effluent was tested for phytotoxicity on *Zea maize* seeds to determine its effect on their germination. The percentage of seed germination, shoot length and root length were studied and the results were used to determine the germination index (GI). The findings of the study are summarized and discussed below.

The percentage of seed germination in control was 100%. In effluent treated with *A. flavus* it was 80%. In effluent treated with *A. aculeatus* it was 70%. In raw effluent it was 60%. In comparison with raw effluent, seeds cultivated in treated effluent demonstrated increased root and shoot growth. Raw effluent had a low percentage of Germination Index (GI) (22.1% for *A. flavus* and 12% for *A. aculeatus*), while effluent treated with *A. flavus* had a GI of 76.48 % and effluent treated with *A. aculeatus* had a GI of 65.69 % (Table 3). Samples with a GI value less than 50% are considered very toxic, values between 50% and 80% indicate minor toxicity, and GI values greater than 80% are deemed non-toxic (Zucconi *et al.,* 1981)

Table 1. Macroscopic and microscopic characteristics of fungal strains

Isolates	Macroscopy	Microscopy	
		Nature of hyphae	Conidia shape
MZ544387 (*Aspergillus flavus)*	The upper surface of colonies was olive green with white edge, granular surface and white coloration on the reverse side	Non-septate	Rough, irregular
MZ569631 (*Aspergillus aculeatus)*	The colonies were widely spread, black, spongy surface densely packed and brown on reverse side	Non-septate	Ellipsoidal

Figure 1. ***Aspergillus flavus (A- Surface side, B-Reverse side & C-Microscopy image)***

A **B** **C**

Figure 2. ***Aspergillus aculeatus (A-Surface side, B-Reverse side & C-Microscopy image)***

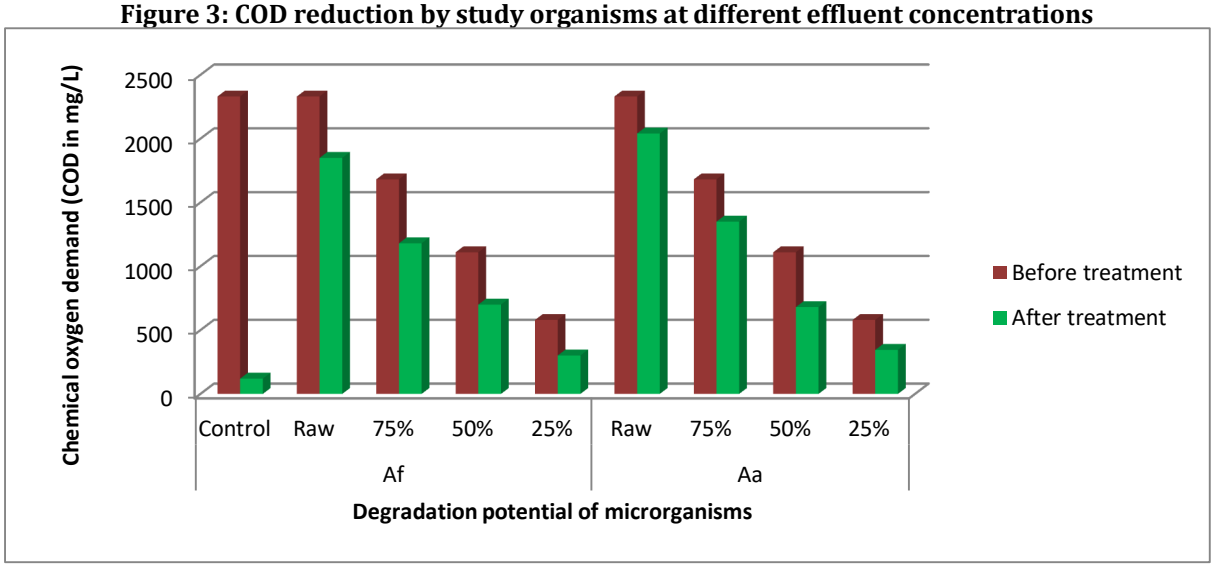
A **B** **C**

Table 2: COD (mg/L) at different concentrations before and after treatment with study organisms

Effluent Concentration	COD *Aspergillus flavus*		COD *Aspergillus aculeatus*	
	Before treatment	After treatment	Before treatment	After treatment
Control	2330.33±0.57	2210.56±0.035	2330.33±0.57	2210.56±0.035
Raw	2330.33±0.57	2040.33±0.57	2330.33±0.57	1849±0.77
75%	1681±1	1349.63±0.55	1681±1	1180.1±0.15
50%	1109.67±0.57	680.2±0.64	1109.67±0.57	699.86±0.15
25%	579.66±0.57	344.8±0.52	579.66±0.57	301.06±0.05

Values are expressed as mean ± SD (Standard deviation), Where n=3

Figure 3: COD reduction by study organisms at different effluent concentrations



Note: Af- ***Aspergillus flavus and Aa- Aspergillus aculeatus***

Table 3: Effect of effluent treated with *Aspergillus flavus* and *Aspergillus aculeatus* on the germination and growth of *Zea maize*

Sample	Shoot length (cm)		Root length (cm)		Germination (%)		GI (%)	
	Af	Aa	Af	Aa	Af	Aa	Af	Aa
Control	16.2	16.2	11.4	11.4	100	100	100	100
Raw	6	6	6.3	6.3	60	60	22.1	12
Treated	11.8	10.5	10.9	10.7	80	70	76.48	65.69

Note: Af- ***Aspergillus flavus and Aa- Aspergillus aculeatus***

CONCLUSION

The present investigation was taken up to study the efficiency of certain fungal strains in reducing COD concentration of dairy effluent. The result reveals that *Aspergillus flavus* was more effective than *Aspergillus aculeatus* at reducing COD concentration. COD concentration was lowered by 50% in *Aspergillus flavus* and 40% in *Aspergillus aculeatus*. Phytotoxicity study was conducted to determine the toxicity of effluent before

and after treatment on the germination of *Zea maize*. Reduced seed germination, root and shoot length are indicators of effluent toxicity. The germination of *Zea maize* was considerably high in 25% diluted effluent than raw effluent. From the phytotoxicity studies it was concluded that the diluted effluent has low phytotoxicity and can be utilized for crop cultivation. Hence, *Aspergillus flavus and Aspergillus aculeatus* can be recommended for bioremediation of dairy effluent.

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Conflict of Interest: Authors declared that no conflict of interest

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