A study on bioremediation potency of *Aspergillus flavus* and *Aspergillus aculeatus* in reduction of BOD concentration of dairy effluent and evaluation of phytotoxicity effect of treated effluent on *Arachis hypogaea*

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**ABSTRACT**

Water resources are of significant importance to human beings. The main sources of water contamination are toxic industrial waste, runoff from agricultural waste, and untreated home garbage discharge. During the processing of milk in dairy industry, a large amount of water is used, resulting in a large volume of effluent comprising dissolved sugars, proteins, lipids and other organic compounds. As a result, dairy effluent has a high organic matter concentration and a high BOD. Contaminants in effluents must be eliminated before they enter a water body, as they have a negative impact on aquatic ecosystems. One of the most effective strategies for treating industrial effluents is bioremediation. In the current study investigated the removal of biochemical oxygen demand in dairy effluent using fungal isolates such as *Aspergillus flavus* and *Aspergillus aculeatus*. According to a phytotoxicity test, fungal strains have ability to eliminate BOD from the effluent.

**Key words**: Dairy effluent, Biological Oxygen Demand, Phytotoxicity, Aspergillus flavus, Aspergillus aculeatus, Arachis hypogaea

**Introduction**

Rapid industrial growth has not only increased productivity, but it has also resulted in the release of harmful compounds into the environment, posing health risks. It causes a significant impact on ecosystems, vegetation and animals. The injection of contaminated effluent into water bodies reduces water’s self-cleaning capacity, causing contaminants to build up to hazardous or even harmful levels (Navitha and Kousar, 2018). Industrial effluents, which are typically released on land or into various water bodies, have received considerable attention in recent years. This is likely to contribute to environmental damage (Shivsharan and Wani, 2017).

India’s animal milk output peaked at 84.5 million tonnes in 2002 and with an annual growth rate of 4-5 percent, milk production is predicted to reach 155.2 million tonnes in 2016-17 (Mehrotra *et al.*, 2016). Water is a crucial resource in the dairy business resulting in large volumes of effluent; thus the difficulty of disposing of it cannot be ignored. Ac-
According to reports, the dairy sector generates 6–10 litres of waste water each litre of milk processed. Approximately 2% of all milk processed is estimated to be wasted in drains. Dairy wastewater comprises substantial amounts of milk ingredients such as casein, lactose and inorganic salt as well as detergents and sanitizers used in the washing process. Dairy effluents are the principal contributors to the organic load of these wastewaters, containing dissolved carbohydrates and proteins, lipids and possibly additive residues (Iqbal et al., 2020). Depending on the processes and products the composition varies. The presence of organic loading in dairy effluent has a negative influence on the environment when it is discharged into neighbouring water sources (Shete and Shinkar, 2013).

Organic chemicals, inorganic compounds, carbon, nitrogen, phosphorus, chlorides, sulphides, fats, oils, grease and other complex substances comprise the majority of dairy effluents discharged by factories. Dairy effluents deteriorate quickly, depleting the DO (dissolve oxygen) level in receiving streams and providing a breeding ground for mosquitoes and flies carrying malaria and other dangerous diseases like as dengue fever, yellow fever and chicken pox. The wastes are also characterized by strong butyric acid odor and heavy black flocculated sludge masses (Joshiba et al., 2019).

The dairy industry produces effluents that are high in fats, oils, and greases (FOGs), which can have a negative influence on waste water treatment systems by causing unpleasant aromas, pipe and sewer line blockage and other issues. The most common volatile organic components in dairy manure are volatile fatty acids (VFA), which are linked to odour nuisance. Ammonia nitrogen is present in raw milk and the presence of 50 mg/l nitrogen in the waste water stream is related to a 1% loss of milk. If nitrate is converted to nitrite, it can induce methemoglobinemia and contaminate groundwater. Another big issue is the presence of nitrogen in dairy effluent, which once transformed may contaminate ground water with nitrate (Porwal et al., 2015).

The applicability of several physicochemical approaches in wastewater treatment has been investigated like Sedimentation, screening, aeration, filtration, flotation, degassification, chlorination, ozonation, neutralisation, coagulation, sorption, ion exchange and some other processes are examples of these. Because of the limitations of physicochemical approaches such as partial treatment, greater costs, the creation of secondary pollutants, higher solids volumes and the employment of chemical agents, biological methods are a viable alternative for pollutant removal (Kousar et al., 2020).

Bioremediation is an alternate treatment method for effluent that has a broader range of applications. The use of living organisms to reduce environmental pollutants into less hazardous forms is known as bioremediation. It detoxifies toxins in the environment using naturally existing bacteria, fungus and plants. Microorganisms utilized in the treatment procedure can be native or imported from other places. The employment of fungi in the degradation of undesired materials or compounds to turn them into harmless, tolerable or beneficial products is known as mycoremediation (Kousar et al., 2021).

The bioremediation performance of fungal isolates Aspergillus flavus and Aspergillus aculeatus for treatment of dairy effluent was evaluated in this study.

Materials and Methods

Collection of effluent

The current study used effluent from a dairy in Shivamogga, Karnataka, India. Samples were collected in dark-colored dry cans that were properly labelled. Triplet samples were taken from the sampling station to ensure accuracy. The effluent was transported to the laboratory for further examination as soon as it was collected.

BOD evaluation

The BOD of wastewater was determined using the APHA’s (2017) standard dilution technique. This method consists of filling an airtight bottle with sample and incubating at 20 °C for 5 days is the process. The dissolved oxygen (DO) was measured before and after the incubation. The difference in DO was calculated as well as the BOD.

Isolation of fungal strains

The isolated and sequencing fungal strains Aspergillus flavus and Aspergillus aculeatus were chosen. With accession numbers MZ544387 for Aspergillus flavus isolate KUESCHK-1 and MZ569631 for Aspergillus aculeatus isolate KUESCCCHK-3, the two fungal strains deposited in the NCBI have 100 percent identical (Chaithra and Kousar, 2022). For the fungal strains, a 40X magnification microscopic examina-
tion was conducted and also a macroscopic observation (Table 1, Figure 1 and 2).

Setup for the experiment
Under laboratory conditions, the biodegradation capacity of Aspergillus flavus and Aspergillus aculeatus was tested. The effluent sample was diluted to varying concentrations for the treatment: 25%, 50% and 75%. Dilution was used to investigate the organism’s degrading capability at various effluent dilutions. Each effluent dilution was inoculated with both the organism and treatment was carried out in the laboratory for 5 days.

Phytotoxicity study of treated effluent
The toxicity of effluent on the germination of Arachis hypogaea seeds before and after treatment with Aspergillus flavus and Aspergillus aculeatus was investigated in a phytotoxicity study. Every day 10 ml of treated and untreated effluent sample was used in the experiment. There was also a control set. The treatment ran 10 days and the results were measured using the seed germination index (GI), relative seed germination (RSG) and relative root elongation (RRE).

Results and Discussion

Biochemical Oxygen Demand
BOD stands for the amount of oxygen required for biological breakdown of organic materials. BOD estimation in water and wastewater is a commonly used method for estimating total organic load and contamination extent. The effluent was initially characterised as having a high BOD content, which was reduced further by the studied organisms (Table 2 and Figure 3).

The reduction in BOD using Aspergillus flavus and Aspergillus aculeatus is as follows:
Aspergillus flavus: 621.36±0.05 to 478.1±0.05 (raw effluent), 416.13±0.057 to 278.5±0.1 (75% dilution), 286.56±0.11 to 181.1±0.11 (50% dilution) and 137.7±0.55 to 79.8±0.11 (25% dilution) (Table 2). The percentage reduction of BOD in raw effluent, 75%, 50% and 25% dilution were 21.84%, 33.07%, 36.80% and 42.04% (Figure 3).

After treatment, it was found that Aspergillus flavus was effective at reducing BOD at dilutions of 50% and 25%. Similar findings were reported by Lanka et al., (2018), who worked on reduction of organic load in oil mill effluent using fungal strains. The selected fungal strains efficiently reduced the concentration of BOD by 50.25% in the effluent.

Aspergillus aculeatus: 621.36±0.05 to 485.6±0.05 (raw effluent), 416.13±0.057 to 278.5±0.1 (75% dilution), 286.56±0.11 to 181.1±0.11 (50% dilution) and 137.7±0.55 to 79.8±0.11 (25% dilution) (Table 2). The percentage reduction of BOD in raw effluent, 75%, 50% and 25% dilution were 21.84%, 33.07%, 36.80% and 42.04% (Figure 3).

To lower the concentration of BOD in wastewater, two fungal strains were used in this investigation. The effluent was evaluated for phytotoxicity on Arachis hypogaea seeds after treatment to see whether it affected their germination. The germination index was calculated by calculating the percentage of seed germination in treated and untreated samples. The results showed that the treated effluent had a lower germination index compared to the control, indicating a reduction in phytotoxicity.

Phytotoxicity study

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**Table 1.** Macroscopic and microscopic characteristics of fungal strains

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Macroscopy</th>
<th>Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ544387 (Aspergillus flavus)</td>
<td>The upper surface of colonies was olive green with white edge, granular surface and white coloration on the reverse side</td>
<td>Nature of hyphae: Non-septate; Conidia shape: Rough, irregular</td>
</tr>
<tr>
<td>MZ569631 (Aspergillus aculeatus)</td>
<td>The colonies were widely spread, black, spongy surface densely packed and brown on reverse side</td>
<td>Nature of hyphae: Non-septate; Conidia shape: Ellipsoidal</td>
</tr>
</tbody>
</table>

**Table 2.** BOD (mg/l) at different dilutions before and after treatment with study organisms

<table>
<thead>
<tr>
<th>Effluent dilution</th>
<th>BOD Aspergillus flavus Before treatment</th>
<th>After treatment</th>
<th>BOD Aspergillus aculeatus Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>621.36±0.05</td>
<td>478.1±0.05</td>
<td>621.36±0.05</td>
<td>485.6±0.05</td>
</tr>
<tr>
<td>75%</td>
<td>416.13±0.057</td>
<td>265.6±0.20</td>
<td>416.13±0.057</td>
<td>278.5±0.1</td>
</tr>
<tr>
<td>50%</td>
<td>286.56±0.11</td>
<td>163.7±0.15</td>
<td>286.56±0.11</td>
<td>181.1±0.11</td>
</tr>
<tr>
<td>25%</td>
<td>137.7±0.55</td>
<td>68.5±0.30</td>
<td>137.7±0.55</td>
<td>79.8±0.11</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (Standard deviation)

**Table 3.** Effect of effluent treated with Aspergillus flavus and Aspergillus aculeatus on the germination and growth of Arachis hypogaea

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
<th>Germination (%)</th>
<th>GI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Af</td>
<td>Aa</td>
<td>Af</td>
<td>Aa</td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
<td>13</td>
<td>19.5</td>
<td>19.5</td>
</tr>
<tr>
<td>Raw</td>
<td>2.3</td>
<td>2.3</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Treated</td>
<td>7</td>
<td>5.8</td>
<td>19.2</td>
<td>18.9</td>
</tr>
</tbody>
</table>
germination, shoot length, and root length (GI). The study’s findings are reported and discussed below.

The proportion of seed germination in the control group was 100%. It was 70% in wastewater treated with Aspergillus flavus. It was 60% in Aspergillus aculeatus treated wastewater and 40% in raw effluent. Seeds grown with treated wastewater grew more roots and shoots than seeds grown in raw effluent. Raw effluent had a low GI percentage (22.1% for A. flavus and 12% for A. aculeatus), however effluent treated with A. flavus had a GI of 68.92% and effluent treated with A. aculeatus had a GI of 58.15% (Table 3). GI values less than 50% are considered extremely hazardous, values between 50% and 80% indicate mild toxicity, and GI values greater than 80% are considered non-toxic.

Conclusion
Contamination of water is a serious issue today. Dairy is one of the sectors that pollutes the environment by producing a lot of effluent. Exposure to inadequately treated or untreated wastewater can have toxic effects on aquatic life and human health. The purpose of this work was to see how efficient selected fungal strains were at decreasing BOD levels in dairy effluent. Aspergillus flavus was shown to be more effective than Aspergillus aculeatus at reducing BOD levels. BOD levels were reduced by 50.25% in Aspergillus flavus and 42.04% in Aspergillus aculeatus. The BOD levels dropped dramatically after pretreatment. The toxicity of effluent before and after treatment on the germination of Arachis hypogaea was investigated in a phytotoxicity research. Seed germination, root and shoot length reductions are all signs of effluent toxicity. Arachis hypogaea germination was much higher in 25% diluted effluent than in raw effluent. According to the phytotoxicity results, the diluted effluent had positive impact on plants and can be used for crop cultivation. As a result, Aspergillus flavus and Aspergillus aculeatus can be recommended for dairy wastewater bioremediation.

Acknowledgement
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