Eco. Env. & Cons. 29 (May Suppl. Issue) : 2023; pp. (S289-S293)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i03s.052

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*

Chaithra C.[1], Hina Kousar[2*], Dhanushree M.S.[3] and Akshatha K.U.[4]

Department of P.G. Studies & Research in Environmental Science, Kuvempu University, Shivamogga 577 451, Karnataka, India

(Received 8 December, 2022; Accepted 13 February, 2023)

ABSTARCT

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-

([1,4]Research Scholar, [2]Professor, [3]Guest Lecturer)

cording 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 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 ^{0}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 KUESCCHK-1 and MZ569631 for *Aspergillus aculeatus* isolate KUESCCHK-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 examination 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).

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

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

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

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:

A B C

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

A B C

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

Aspergillus flavus: 621.36±0.05 to 478.1±0.05 (raw effluent), 416.13±0.057 to 265.6±0.20 (75% dilution), 286.56±0.11 to 163.7±0.15 (50% dilution) and 137.7±0.55 to 68.5±0.30 (25% dilution) (Table 2). BOD reduction were 23.05% (raw effluent), 36.17% (75% dilution), 42.87% (50% dilution) and 50.25% (25% dilution) (Figure 3).

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)

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.

Phytotoxicity study

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

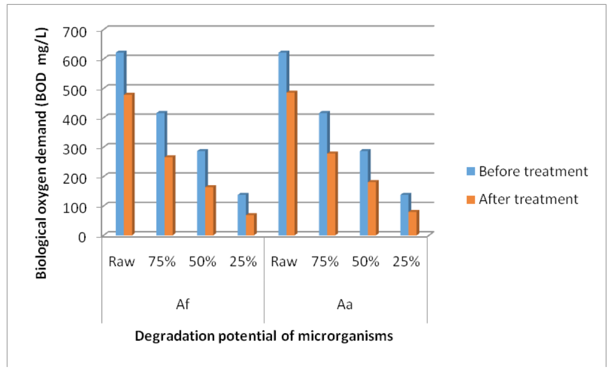

Fig. 3. BOD reduction by study organisms at different effluent dilutions

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

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

Effluent dilution	BOD *Aspergillus flavus*		BOD *Aspergillus aculeatus*	
	Before treatment	After treatment	Before treatment	After treatment
Raw	621.36±0.05	478.1±0.05	621.36±0.05	485.6±0.05
75%	416.13±0.057	265.6±0.20	416.13±0.057	278.5±0.1
50%	286.56±0.11	163.7±0.15	286.56±0.11	181.1±0.11
25%	137.7±0.55	68.5±0.30	137.7±0.55	79.8±0.11

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*

Sample	Shoot length (cm)		Root length (cm)		Germination (%)		GI (%)	
	Af	Aa	Af	Aa	Af	Aa	Af	Aa
Control	13	13	19.5	19.5	100	100	100	100
Raw	2.3	2.3	3.9	3.9	40	40	22.1	12
Treated	7	5.8	19.2	18.9	70	60	68.92	58.15

Note: Af- *Aspergillus flavus and Aa- Aspergillus aculeatus* 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 *A. flavus*. It was 60% in *A. 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

The Author acknowledges the Department of P.G. Studies and Research in Environmental Science, Kuvempu University, Shankaraghatta, Shimoga, Karnataka, India for providing the laboratory and other facilities for carrying out the research.

References

American Public Health Association (APHA), Standards Methods for the Examination of water and Wastewater, twenty–third ed. American Public Health Association, American Water Work Association, Water Work Federation, Washington DC, (2017)

APHA. 2017. *Standard Methods for the Examination of Water and Wastewater*. APHA, U.S.A.

Chaithra, C. and Kousar, H. 2022. Molecular Identification of Fungal Strains isolated from Textile Effluent and Contaminated Soil using 16s Rrna Sequencing. *Research Journal of Biotechnology.* 17(6): 1-6.

Iqbal, K., Ahmed, A., Ansari, Y., Rashid, A. and Khan, A. 2020. Environmental Impact of Dairy Effluent. *International Journal of Advanced Science and Technology.* 29 (6s): 4010-4017.

Joshiba, G. J., Kumar, P. S., Femina, C. C., Jayashree, E., Racchana, R. and Sivanesan, S. 2019. Critical review on biological treatment strategies of dairy wastewater. *Desalination and Water Treatment.* 160 : 94-109.

Kousar, H., Chaithra, C. and Akshatha K, U. 2020. Role of certain bacterial and fungal strains in bioremediation of textile industrial effluent- A Review. *Journal of Advanced Scientific Research.* 11(4 Supple 8): 77-79.

Kousar, H., Chaithra, C. and Akshatha, K. U. 2021. Use of Aspergillus niger for the reduction of Biological Oxygen Demand in textile industry effluent. *Journal of Advanced Scientific Research.* 12 (2 Suppl 11): 351-354.

Lanka, S. and Pydipalli, M. 2018. Reduction of organic load from palm oil mill effluent (POME) using selected fungal strains isolated from POME dump sites. *African Journal of Biotechnology*. 17(36) : 1138-1145.

Mehrotra, R., Trivedi, A. and Mazumdar, S. K. 2016. Study on characterisation of Indian dairy wastewater. *International Journal of Engineering Applied Sciences and Technology.* 1 (11): 77-88.

Navitha, K. R. and Kousar, H. 2018. A comparative study on the potential of Aspergillus niger and Aspergillus flavus for the treatment of coffee processing effluent. *International Journal of Environment, Ecology, Family and Urban Studies (IJEEFUS).* 8 (4): 17-22.

Porwal, H. J., Mane, A. V. and Velhal, S. G. 2015. Biodegradation of dairy effluent by using microbial isolates obtained from activated sludge. *Water Resources and Industry.* 9 : 1-15.

Shete, B. S. and Shinkar, N. P. 2013. Comparative study of various treatments for dairy industry wastewater. *IOSR J Eng.* 3 (8): 42-47.

Shivsharan, V. S. and Wani, M. 2017. Anaerobic-Aerobic Activated Sludge Treatment to Dairy Effluent. *Research Journal of Pharmaceutical Biological and Chemical Sciences.* 8 (3): 2303-2307.