Pollution 2022, 8(2): 611-619
DOI: 10.22059/POLL.2021.332245.1215

RESEARCH PAPER

Myco-remediation of Dairy Wastewater by Naturally Attenuated *Aspergillus* sp. Responsible for Sulfate Reduction

Neha Sharma[1,*], Alok Kumar Meher[2], Harshita Bhagwani[3], Darshan Chahar[3], Neetu Yadav[3], Vipra Kohli[4], Bhavyam Shourie[4], Kalirajan Arunachalam[5] and Munaska Siankuku[6]

1, 4. Research and Development Centre, Maharishi Markandeshwar Medical College and Hospital, Maharishi Markandeshwar University, Solan- 173229, Himachal Pradesh, India
2. Instrumentation Laboratory Central Pollution Control Board Parivesh Bhawan, East Arjun Nagar Shahdara, Delhi- 110032, India
3. Department of Zoology, Poddar International College, Jaipur-302020, Rajasthan, India
5. Department of Science Engineering and Technology, School of Science, Engineering and Technology, Mulungushi University, Kabwe.80415, Zambia
6. University of Zambia, School of Natural Sciences, Department of Biological Sciences, Lusaka-32379, Zambia

Received: 13.09.2021, Revised: 30.12.2021, Accepted: 31.12.2021

ABSTRACT

Dairy industries generate enormous volumes of waste water which are significantly rich in organic compounds; contributing to high BOD, COD and sulfates. As a mandate to 'treat' effluents generated by different unit operations in a dairy industry, current treatment methods rely on physico-chemical, mechanical and conventional biological interventions. This approach remains unviable because of cost intensiveness and excessive energy usage. Additionally, the significant lowering of pollution indicators remains a daunting task with inlet and outlet parameters. With these identifiable gaps, our study was aimed to screen bio-efficacious, naturally attenuated fungal isolates to lower exceeding levels of sulfate in effluents released by dairy industry. Effluent samples were collected from Effluent Treatment Plant (ETP) of Jaipur Dairy, Rajasthan Dairy Co-operation Limited (RCDF), Jaipur. For mycological investigations, qualitative screening was carried out in Potato Dextrose Agar (PDA) supplemented with Calcium Sulfate (CaSo4) (0.1g/L). The most promising fungal isolates belonging to *Aspergillus* sp. was characterized based on its cultural and microscopic characteristics. Microcosm study was conducted by supplementing *Aspergillus* sp. in Untreated Dairy Effluent (UDE) for a period of 7 days at Room Temperature (RT) under static conditions. Following the incubation phase, mycelial mesh (plug) was indicative of exponential fungal growth. Effluent seeded with *Aspergillus* sp. and abiotic controls were spinned at 5000 rpm for 15 minutes to eliminate biomass. Sulfate estimation was carried out in Cell Free Extract (CFE) of both experimental and control group. A significant reduction of 67.3% was observed ($p<0.05$) with respect to positive control and 8.4% when contrasted with abiotic control.

Keywords: Bio augmentation, Effluent Treatment Plant, Microcosm, Rajasthan Dairy Co-operation Limited (RCDF), Sulfate removal

INTRODUCTION

Milk is regarded as one of the prime food worldwide and India ranked first for the milk production (Samal & Pattanaik, 2014). In the third world, the agro processing industries have

* Corresponding author Email: nehamicrobiologist@gmail.com

grown tremendously both in terms of numbers and production capacities (Carvalho et al., 2013). It has been calculated that 2.0 to 2.5 liters of wastewater being generated for processing of 1.0 liter of milk, which is laden with biochemical moieties like carbohydrate, proteins, fats, oil/grease, along with other nutrient parameters (Sharma & Dwivedi, 2017). Therefore, effluent generated from the dairy industries loaded with numerous environmental pollutants contribute to elevated concentrations of turbidity, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrates, phosphates and sulfates. The elevated level of BOD deteriorates the water bodies by lowering the availability of the dissolved oxygen for the aquatic organisms and slowing down the rate of decomposition of the organic matter and contribute to eutrophication (Shete et al., 2013).

Sulfate is one of the most abundant anions occupying every possible ecological nice (Brahmacharimayum et al., 2019). It exists in the form of insoluble salts like Barite ($BaSO_4$), Epsomite ($MgSO_4 \cdot 7H_2O$) and gypsum ($CaSO_4 \cdot 2H_2O$), oxidized form of sulfide ores in Acid Mine Drainage (AMD) (Neculita et al., 2007). Effluents released by industries like food processing, agro-processing, mining, animal husbandry, detergents and synthetic dyes are influxed with excessive sulfates (Lens & Hulshoff Pol, 2000). Amongst the various treatment options available for removal of sulfate from effluent systems, most efficacious is the bioreduction of sulfate (Liamleam & Annachhatre, 2007) attributed to minimal sludge formation, operational feasibility and viability with cost effectiveness.

Dairy wastewater has been considered as a potential reservoir of both sulfate reducing bacteria (SRB) and lactic acid bacteria (LAB) which in addition to possessing probiotic properties have also been explored for their probable role in pollution abatement (Sharma et al., 2018). However, it's imperative to treat the dairy wastewater prior to discharge to any environmental matrices namely land, surface water bodies or oceans to circumvent toxicological implications. The common strategies for the treatment of dairy wastewater involve the primary, secondary and tertiary units in the Effluent Treatment Plant (ETP). Effluents released by dairy industries have been tested for their outlet and inlet parameters to assess the performance of an ETP (Sharma et al., 2013). In the primary unit the excess oil, grease and fats are being removed by the application of skimmers or oil-water separators, screens to remove the floatable solids, flow equalization tanks and cleaners to separate the suspended solids. The secondary treatment scheme deals with aerobic and anaerobic biological treatment methods to reduce the soluble organic load and partial removal of the nutrient parameters such as nitrogen and phosphorous. The aerobic treatment process involves the processes namely activated sludge, sequencing batch generator, trickling filters, revolving biological contactor or the aerated lagoons. Some of the anaerobic treatment schemes may be listed as Up-flow Anaerobic Sludge Blanket (UASB), Anaerobic Sequencing Batch Reactors (ASBR), Continuous-Flow Reactor or the Hybrid Anaerobic Digesters (Mainardis et. al, 2020). In general, the anaerobic treatment scheme is a flexible and cost effective option as compared to the aerobic one for the of treatment wastewater loaded with elevated concentration of organic components or effluents with high BOD level, such as dairy wastewater, due to some key advantages namely low energy requirement, lower sludge generation and possibility of biogas generation (Karadag et al., 2015). Moreover, the undesirable concentration of sulfate may be effectively removed by anaerobic process by the sulfur reducing bacteria (SRB). However, the dairy wastewater induces a strong deficit in the dissolved oxygen concentration, which leads to production of the H_2S content by the activities of the SRB (Slavov, 2017). The elevated concentrations of the H_2S is toxic for the human health and also causes corrosion issues in the treatment plant (Jiang et al., 2016). The anaerobic dairy wastewater treatment method involves the application of sulfur reducing and methane forming micro-organisms. The bacteriological sulfate reduction mechanism involves the utilization of sulfate as a terminal electron acceptor in the

electron transport chain. In this context, sulfate reduction by of remain a major issue due to higher kinetics of the SRB as compared to the methanogens, because of the toxicity of the generated sulfide by SRB to both the microorganisms (Mannucci et al., 2014). Sulfide is also known as a potential inhibiting agent for the heterotrophic de-nitrification process, which is an important step in the wastewater treatment plant for the reduction of nitrates (Liang et al., 2020).

Dairy wastewater is a promising substrate for the application of fungi for its treatment, because of requirement of minimal supplements for microbial growth, which is a distinct advantage over the bacterial wastewater treatment process, which requires the Electron Donors as the supplement (Liamleam & Annachhatre, 2007; Sankaran et al., 2010). Filamentous fungi are advantageous attributed to growth kinetics, easy separation from the bulk media after treatment process and elimination of possibilities of behavior as opportunistic pathogens owing to obligatory acidophilic properties. Mechanism of myco-remediation involves the oxidation of reduced organic or inorganic compounds to the reduction of sulfate or other oxidized sulfur compounds, producing sulfide as metabolic product (Bardone et al., 2012).

Current focus of the projected microbiological studies has aimed to screen bio-efficacious naturally attenuated fungal isolates promiscuous enough to lower exceeding levels of sulfate in the untreated effluent generated from the dairy industry; thereby minimizing the adverse eco- toxicological implications in the receiving waterbodies. We are reporting first fungal assisted bio-removal of sulfate from dairy effluent. This pilot study would pave a path for unlocking metabolic machinery behind bio-prospective study. Additionally, role of bacterial-fungal consortium could be explored for pollution abatement in industrial effluents by virtue of their bio-stimulation and bio-augmentation attributes.

MATERIALS AND METHODS

Effluent Treatment Plant (ETP) indigenous to Jaipur Dairy, Rajasthan Co-operative Dairy Federation Limited (RCDF), Jaipur was selected for the planned study. Untreated Dairy Effluent (UDE) samples were collected (in triplicates) in pre-sterilized cans from inlet of ETP and stored under refrigerated conditions at 4ºC until further analysis (APHA, 2005) (Figure 1). Inlet is a common raw effluent drainage point where wastewater generated by different unit processes in a dairy unit enters ETP (Porwal et al., 2015).

Fig 1: Sampling Site

Site specific parameters like temperature, pH, odor and color were observed in accordance with standard procedures (Figure 2). For sulfate analysis, samples were collected, transported and processed (APHA, 2005) (Figure 3).

Fig 2: Site specific parameters

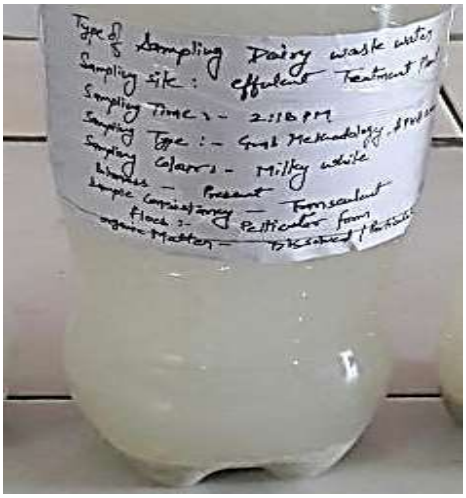

Fig 3: Sample for sulfate analysis

Freshly prepared sterile Potato Dextrose Agar (PDA) (Hi Media) spiked with Calcium Sulfate ($CaSO_4$) (0.1%w/v) with the composition was used for mycological screening. By serially diluting UDE samples (10^{-6}), inoculation was carried out by spread plate method (Richards, 2002). The plates were incubated at 20-25°C for 5-7 days for a visible mycelial growth.

Actively growing mycelial plugs (approximately 0.7 cm) were aseptically transferred to 100 ml sterilized fermentation medium, Potato Dextrose broth (PDB) (Hi Media) with pH adjusted to 6.0 ± 0.2. The plates were incubated for 8 days at 20-25 ° C until a thick scum was developed (Asses et al., 2018).

In line with our previous bio-prospective studies conducted on textile and dairy effluents, the study was based on microcosm approach by seeding sterilized effluent with screened strains (Sharma et al., 2014) (Figure4). Briefly, 1000 ml refrigerated UDE was used for microcosm experiments. 500ml UDE was pre-sterilized and dispensed into two Erlenmeyer flasks A (Experimental) and B (Abiotic Control) (250 ml each). Additionally, unsterilized 250 ml fraction was run as a positive control (C).

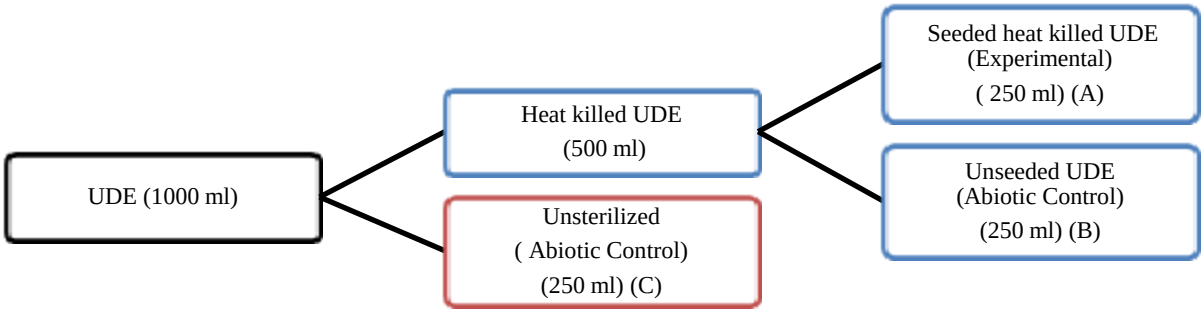

Fig 4: Microcosm experiment for fungi assisted biodegradation

To the experimental set, a loopful (0.1 w/v) of actively growing mycelial mesh was inoculated in heat killed UDE under aseptic conditions and incubated as described previously. Initial sulfate estimation was carried out in experimental group and contrasted with abiotic

and positive control. Following 48 hours incubation, aliquots (50 ml each A, B and C) were aseptically withdrawn and spinned at 5000 rpm for 15 minutes to remove fungal biomass (Schneider & Topalova, 2013). Sulfate estimation was carried out in Cell Free Extract (CFE) of all samples according to protocol devised by (Thangiah, 2019). For a week's duration, reduction in sulfate was monitored until a significant lowering was observed ($p<0.05$).

RESULTS AND DISCUSSION

In continuation with microbiological interventions to mitigate pollution indicators like phosphate and nitrate from effluents released by Jaipur Dairy, our current study was aimed to screen bio-efficacious fungus to lower down levels of sulfate (Sharma et al., 2018; Sharma et al., 2013). The functionality of ETPs is based on *end of pipe treatment* strategy which has been substituted for a decentralized approach (Shah, 2016). Biotechnological advancements for resource recovery from waste water systems have gained momentum in recent past (Puyol et al., 2017).

Color of UDE was milky white, attributable to unit manufacturing of milk and its products lost post production (spilled milk, spoiled milk, skimmed milk and curd pieces); starter cultures and by-products of processing operations (Slavov, 2017). Typically, dairy waste water is white in color (Carvalho et al., 2013). Its removal from inlet across outlet is correlated to vigorous phyco-logical activity (Verma and Madamwar, 2003).

Temperature of UDE was 22° C$\pm 2^{\circ}$ C. Annual temperature range of dairy effluents have been reported to be warmer than municipal waste water, a characteristic that enhances biodegradation (Water Environment Federation, 2007).

pH of sample was found to be slightly acidic 5.6±0.4. Acidic pH has been correlated with cheese manufacturing plants with maximum utilization of sweet whey (Venetsaneas et al., 2009). Optimum pH required for biological treatment of dairy effluents is 6-9.

Qualitatively screening of the most promiscuous fungal isolate capable of reducing sulfate in UDE was characterized as *Aspergillus* sp. based on its cultural and staining characteristics (Figure 5). The role of *Aspergillus niger, Mucor hiemalis* and *Galactomyces geotrichum*in biodegradation of raw dairy effluent has been well established (Djelal & Amrane, 2013). Aerobically, yeast has been found to lower pollution indicators like BOD, Oil & Grease, turbidity and COD (Porwal et al., 2015). Bacteria mediated reduction of nitrate in dairy effluents has been reported previously owing their natural attenuation property (Sharma & Dwivedi, 2017).

Fig 5: Pure culture of *Aspergillus* sp.

SmF was carried out in Potato Dextrose Broth (PDB) under fastidious conditions by amending $CaSO_4$ as sole source of sulfate for *Aspergillus* sp. to undertake gratuitous

metabolism. *Aspergillus niger* GH1 has been explored for its biofficacy to degrade tannins under both SmF and Solid State Fermentation (SMF) (Jose Carlos et al., 2020).

Fig 6: Submerged Fermentation

UDE (1000 ml) was divided into three groups (A, B and C). Set A was spiked with a loopful (0.1w/v) of actively growing *Aspergillus* sp. Set B and C were left uninoculated to check for abiotic losses and role of resident microflora in lowering pollution indicators over a period of time. Following incubation period, at regular intervals of 24 hours, sulfate estimation was carried out in CFE and compared with abiotic and positive control. Percent reduction (%) was expressed with respect to uninoculated samples. A significant reduction in the range of 23-67 ±0.7% with respect to positive control and 3.5-8.4 ±0.3% with respect to abiotic losses was observed (Figure 7). Anaerobic reductive pathway of sulfate has been implemented at an industrial scale for sulfate contaminated waste waters, validated by its operational cost effectiveness utilizing organic and inorganic carbon sources (Liamleam & Annachhatre 2007). A study exploring role of *Desulfotomaculum ruminis* was conducted which reported a 50% reduction of sulfide in dairy effluent (Wawrzak, 2014). Myco-remediation by *Alternaria* sp., *Fusarium* sp. and *Aspergillus* sp. for reducing organic and inorganic load in dairy effluents has been studied in detail (Al Wasify et al., 2017).

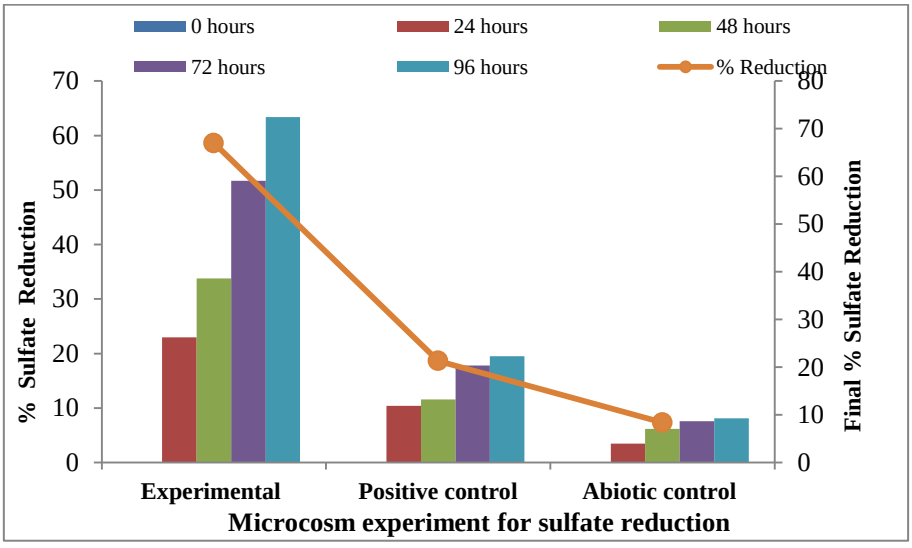

Fig 7: *Aspergilus* sp. mediated sulfate removal from UDE with respect to positive and abiotic control ($p<0.05$)

CONCLUSION

Bio-prospecting study aimed to reduce the levels of sulfate in untreated dairy effluents was conducted to screen for autochthonous fungi. Until recently, much attention has been given to bacteria mediated biodegradation studies. Inspired by our previous study on textile effluents, lab scale microcosm (*In situ* bioremediation) was devised to affirm bio-efficacy of fungal counterparts (in monoculture) under sterile conditions (Heat killed effluent). To nullify the abiotic losses, both positive and negative controls were contrasted with experimental group. *Bio-enriched and bio-augmented Aspergillus*sp., by virtue of its ubiquitous and natural attenuation properties, reduced sulfate in the range of 23-67% ±0.7 from heat killed effluent. However, a non-significant reduction with respect to abiotic and positive controls was reportedly ineffective in lowering down the sulfate levels in UDE. Through this preliminary study, we suggest role of innate fungi as potential *pollutant reducers* for decelerating pollution load reflective as an increased level of sulfates, nitrates and phosphates in dairy effluents. Conventional and centralized *End of Pipe* treatment strategy undoubtedly remains the preferred option for treating industrial effluents as it mainly relies on physic-chemical treatment. Though, biological models including bacterial-fungal consortium for pollution abatement in industries are being investigated for their efficacy to bio-degrade and *bio-mineralize recalcitrant toxicants* from waste water systems. An underlying mechanism of *sulfate scavenging* needs to be explored to address toxicological implications. That said, secondary metabolites released by gratuitous metabolism needs to be investigated for probability of secondary amines; considerably manifesting eco-toxicological threat. Insights into metagenomics and metabolomics needs to be dictated for unlocking field centric bioremediation pathways to rule out the toxicological adversities. Additionally, microbial ecology and community dynamics of waste water systems remains sparsely understood.

ACKNOWLEDGEMENT

Authors would like to acknowledge Maharishi Markandeshwar Medical College and Hospital (MMMC&H) Maharishi Markandeshwar University, Solan (Himachal Pradesh) and Poddar International College, Jaipur, for providing requisite infrastructure and administrative approvals to carry out the research project. Sincere thanks to Department of Science and Technology, Government of Rajasthan, for allocating funds to carry out the project.

FUNDING

Research work undertaken was a part of project entitled “Bioprospecting of Dairy Waste Water for Nitrate Reduction”, funded by Department of Science and Technology (DST), Government of Rajasthan. [F-(7) Vi. Pro/S.P./2017/286].

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

LIFE SCIENCE STATEMENT

No Life Science threat was practiced in this research

REFERENCES

Al-Wasify, R.L., Ali, M.N. and Hamed, S.R. (2017). Biodegradation of dairy wastewater using bacterial and fungal local isolates. Water Sci. Technol., 76(11); 3094–3100. https://doi.org/10.2166/wst.2017.481

APHA/AWWA/WEH (2005). Standard Methods for the Examination of Water and Wastewater, 21st Ed., American Public Health Association/ American Water Works Association/Water Environment Federation. (Washington, DC, USA)

Asses, N., Ayed, L., Hkiri, H. and Hamdi, M. (2018). Congo Red Decolorization and Detoxification by *Aspergillus niger*: Removal Mechanisms and Dye Degradation Pathway. Biomed. Res. Int., https://doi.org /10.1155/2018/3049686

Bardone, E. (2012). Associazione Italiana di Ingegneria Chimica, (3rd International Conference on Industrial Biotechnology, Palermo, Italy. 27: 175-180

Bejarano, Ortiz D.I., Thalassso, F., Cuervo Lopez, F. de M. and Texier, A.C. (2013). Inhibitory effect of sulphide on the nitrifying respiratory process. J. Chem. Technol. Biotechnol., 88; 1344-1349. https://doi.org /10.1002/jetb.398

Brahmachrimayum, B., Mohanty, M.P. and Ghosh, P.K. (2019). Theoretical and practical aspects of biological sulfate reduction: A Review. Glob. Nest. J., 21(2); 222-244. https://doi.org /10.30955/gnj.002577

Carvalho, F., Prazeres, A.R. and Rivas, J. (2013). Cheese Whey Waste Water: Characterization and Treatment. Sci. Total Environ., 15; 445-446, 385-96. https://doi: 10.1016/j.scitotenv.2012.12.038

Djelal, H. and Amrane, A. (2013). Biodegradation by bioaugmentation of dairy wastewater by fungal consortium on a bioreactor lab-scale and on a pilot-scale. J. Env. Sci., 25(9); 1906-12. https://doi:10.1016/S1001-0742(12)60239-3

Jiang, J., Chan, A., Ali, S., Saha, A., Haushalter, K.J., Macrina Lam, W., Glasheen, M., Parker, J., Brenner, M., Mahon, S.B., Patel, H.H., Ambasudhan, R., Lipton, S.A., Pilz, R.B., Boss, G.R. (2016). Hydrogen Sulfide—Mechanisms of Toxicity and Development of an Antidote. Sci. Rep., https://doi.org/10.1038/srep20831

José Carlos L M, Leonardo S, Jesús MC, Paola MR, Alejandro ZC, Juan AV, Ristóbal Noé A. (2020). Solid-State Fermentation with *Aspergillus niger* GH1 to Enhance Polyphenolic Content and Antioxidative Activity of Castilla Rose (*Purshia plicata*). Plants (Basel). 9(11);1518. https://doi: 10.3390/plants9111518

Kardag, G., Koroglu, O.E., Ozkaya, B. and Cakmacki, M. (2015). A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. Proc. Biochem., 50 (2); 262-271. https://doi: 10.1016/j.procbio.2014.11.005

Lens, P. and Hullshoff Pol, P.W. (2000) Environmental Technologies to Treat Sulphur Pollution: Principles and Engineering, (IWA Publishing, London)

Liamleam, A.W. and Annachhatre, A.P. (2007). Electrical Donors for Biological Sulphate Reduction. Biotechnol. Adv., 25(5); 452-6. https://doi: 10.1016/j.biotechadv.2007.05.002

Liang, Z., Sun, J., Zhan, C., Wu, S., Zhang, L. and Jiang, F. (2020). Effects of sulfide on mixotrophic denitrification by *Thauera*-dominated denitrifying sludge**.** Environ. Sci.: Water Res. Technol**.,** 6**;** 1186-1195. https://doi.org/10.1039/C9EW01014A

Mainardis M, Buttazzoni M, Goi D. (2020) Up-Flow Anaerobic Sludge Blanket (UASB) Technology for Energy Recovery: A Review on State-of-the-Art and Recent Technological Advances. Bioengineering (Basel). 7(2); 43. https://doi: 10.3390/bioengineering7020043

Mannucci, A., Munz, G., Gualtiero, G. and Lubello, C. (2014). Factors Affecting Biological Sulphate Reduction in Tannery Wastewater Treatment. Environ. Eng. Manag. J., 13(4); 1005-1012

Neculita, C.M., Zagury, G.J. and Busiere, B. (2007). Passive Treatment of Acid Mine Drainage in Bioreactors using Sulfate-Reducing Bacteria: Critical Review and Research Needs. J. Environ. Qual. 36(1);1-16. https://doi.org/10.2134/jeq2006.0066

Porwal, H.J., Mane, A.V. and Velhal, S.G. (2015). Biodegradation of dairy effluent by using

microbial isolates obtained from activated sludge, Water Resour. Indus., 9,1-15. https://doi.org/10.1016/j.wri.2014.11.002

Puyol, D. and Batstone, D.J. (2017). Resource Recovery from Wastewater by Biological Technologies. Front. Microbiol., 8; 998. https://doi.org/10.3389/fmicb.2017.00998

Richards, O.W. (2002) Effect of calcium Carbonate on the growth and fermentation of yeast. J. Am. Chem. Soc., 47(6). https://doi.org/10.1021/ja01683a023

Salvov, A.K. (2017). General Characteristics and Treatment Possibilities of Dairy Wastewater – A Review. Food Technol. Biotechnol., 55(1); 14–28. https://doi.org/ 10.17113/ftb.55.01.17.4520

Samal, L. and Pattnaik, A.K. (2004) Dairy Production in India - Existing Scenario and Future Prospects. Int. J. Livest. Res., 4(2); 105-113. https://doi.org/10.5455/ijlr.20131002065611

Sankaran, S., Khanal, S.K., Jasti, N., Jin. B., Pometto III, A.L. and Van Leewen, J.H. (2010). Use of Filamentous Fungi for Wastewater Treatment and Production of High Value Fungal Byproducts: A Review. Crit Rev Environ Sci Tec., 40(5); 400-449. https://doi.org/10.1080/10643380802278943

Schneider, I., and Topalava, Y. (2013). Microbial Structure and Functions of Biofilm During Wastewater Treatment in the Dairy Industry, Biotechnol. Biotechnol. Equip., 27(3); 3782-3786. https://doi.org/ 10.5504/BBEQ.2013.0015

Shah, M.P. (2016). Industrial Wastewater Treatment: A Challenging Task in the Industrial Waste Management. Adv Recycling Waste Manag., https://doi.org/ 10.4172/2475-7675.1000115

Sharma, N. and Dwivedi, A (2017) Bioremediation of Dairy Waste Water for Nitrate Reduction. World J. Pharm. Sci., 3(1); 375-384

Sharma, N. Saxena, S. Fatima, M., Iram, B., Datta, A. and Gupta, S. (2014). Microcosm Analysis of Untreated Textile Effluents by Autochthonous Bacteria. Int. J. Curr. Res. Pharm. Sci., 1(5); 15-23

Sharma, N., Chatterjee, S. and Bhatnagar, P. (2013.) An Evaluation of Physico-chemical Properties to Assess Quality of Treated Effluents from Jaipur Dairy. Int. J. Chem. Pharm. Res., 4 (4-3); 54-58

Sharma, N., Yadav, N., Bhagwani, H., Chahar, D. and Singh, B. (2018). Screening of lactic Acid Bacteria from Effluents of Jaipur City. Int. J. Waste. Resour., https://doi.org/10.4172/2252-5211.1000332

Shete, B.S. and Shinkar, N.P. (2013). Dairy Industry Wastewater Sources, Characteristics & its Effects on Environment. Int. J. Curr. Eng. and Technol. 3; 1611-1615

Thangiah, A.S. (2019). Spectrophotometric Determination of Sulphate and Nitrate in Drinking Water at Asia-Pacific International University Campus, Muak Lek, Thailand. Rasayan J. Chem., 12 (3); 1503 – 1508. http://dx.doi.org/10.31788/RJC.2019.1235201

Venetsaneas, N., Antonopoulou, G., Stamatelatou, K., Kornaros, M., Lyberats, G. (2009). Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. Bioresour. Technol., 100; 3713-3717

Verma, P. and Madamwar, D. (2003). Comparative Study on Transformation of Azo Dyes by White Rot Fungi. Ind. J. Biotechnol., 1; 393-396

Wawrzak, D. (2014). Microbiological Reduction of Sulphates to Sulphides used in Dairy Waste Water Treatment. Inzynieria Mineralna 14(2);109-114