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Research Article

Application of effective microorganisms technology on dairy wastewater treatment for irrigation purposes

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Abstract

Due to the massive amounts of freshwater consumed in dairy industries, as a result, thousands of liters of wastewater were produced as one liter of milk produces 10 liters of wastewater which represents a major threat to the surrounding environment and aquatic life. The application of a promising technology called "effective microorganism (EM)" was the key solution due to its low operating cost, low technology, and eco-friendly condition. Three different effective microorganisms were used, such as Bacillus bacteria (EM1), Staphylococcus bacteria (EM2), and EM stoste + Molasses (EM3). EM1 and EM2 were isolated from the dairy wastewater by using streaking for isolation on an agar plate process, while EM3 was prepared by mixing 12 % EM stoste, 6% molasses, and 82% distilled water. A laboratory pilot consists of aeration and final settling tanks, both tanks followed by an activated carbon filter. Four trials were performed, the first trial was without any EM, the second trial was adding EM1 with a dose of 50 mL to the aeration tank, the third trial was EM2 with a dose of 50 ml to the aeration tank, finally adding EM3 with a dose of 30 mL to the aeration tank. Results showed that using Bacillus bacteria (EM1) was the most effective trial as it was effective in reducing TSS (total suspended solids), BOD (biological oxygen demand), COD (chemical oxygen demand), TN (total nitrogen), and TP (total phosphorous) concentrations by removal efficiency of 93%, 96.2%, 95.9%, 94%, and 64%, respectively which were below the limitations of the Egyptian code for reuse for irrigation purposes.

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Introduction

Nowadays, Egypt is one of the countries that paid remarkable attention to industries due to the rapid economic development and the massive growth rate of the population. A big sector of these industries depends basically on the water to operate its processes. The water requirements of the industrial sector in Egypt is about 5.4 billion m^3 per year (Abd Ellah, 2020), as a result, large amounts of wastewater produced due to the increase of the growth's rate of these industries which represent a critical issue, especially in a country like Egypt which exposed to a probable and future water sacristy that will affect each sector depend on freshwater resources. Food industries occupy a big sector of the industries which produced a large amount of effluent that affect in a negative way on the surrounding environment and the aquatic life (Mekonnen and Gerbens-Leenes, 2020).

Dairy industry is one of the most popular food industries that specialize in converting raw milk into products also produces thousands of liters of wastewater effluent as one liter of milk produces 6 to 10 liters of wastewater effluent (Verma et al., 2012). This effluent is rich with organic containments, fats, proteins, suspended solids (Al-Wasify et al., 2017), methane, and hydrogen sulfide derivatives which are the main causes of the bad and high intense odor which causes a hazardous effect on the surface water (Desai et al., 2012) in addition to lactose and inorganic salts. Another problem related to the dairy effluent is nutrients such as phosphorous and nitrogen which lead to eutrophication if it derived directly to the surface water as it decreases the dissolved oxygen levels leading to the death of aquatic life in the receiving water. Also, dairy industry produces a large amount of sludge during the aerobic treatment which is about 0.5 kg per 1 kg of the removed COD (Porwal et al., 2015).

As a result of the high strength- polluted wastewater produced from dairy industry, disposal of dairy effluent became a critical issue. In order to achieve an effective water conversion, several technologies are used for dairy wastewater treatment such as activated sludge process (ASP) (Elmagd and Mahmoud, 2014), aerated lagoons, membrane biofilm reactor (MBR) (Naghizadeh et al., 2011), and moving bed biofilm reactor (MBBR) (Luo et al., 2014). But, a promising biological treatment process appeared in 1970 called effective microorganisms (EM). This technology depends on employing different cultures of microbes which have a reviving effect on aquatic life and the environment by converting the polluted environment into safe, clean, and eco-friendly conditions.

Using the effective microorganisms combines the advantages of using the aerobic and non-aerobic treatment methods as it is described as a hybrid culture of aerobic and anaerobic microorganisms (Syzmanski and Patterson, 2003). Also, effective microorganisms technology offers several advantages over the conventional methods as its usage is very simple with low operating costs and low technology which made it more economical compared to the other treatment methods (Al-Wasify et al., 2017).

Effective microorganisms technology produces safe, clean, non-toxic, and eco-friendly end products in addition to less amount of sludge which provides it a wide public acceptance (Porwal et al., 2015). Effective microorganisms include several species of microbes that can be employed for wastewater and wastes produced from different industries, such as lactic acid bacteria, yeasts, fermenting fungi, and photosynthetic bacteria (Syzmanski and Patterson, 2003). In this study, a scale pilot was established to perform a simulation of biological treatment of dairy wastewater by employing the effective microorganisms isolated from the dairy effluent in order to reduce the concentrations of organic matter and nutrients to allowable limits to reuse it for irrigation purposes.

Materials and Methods

Collection of dairy wastewater sample

Fresh dairy wastewater was collected from a dairy factory located in Giza, Egypt. Five plastic containers were used to collect raw wastewater samples; each container was 10 liters in volume. Before filling the plastic containers with samples, plastic containers were washed with alcohol and distilled water to prevent any change to the wastewater characteristics and left to dry at room temperature. After the container's purification, containers were filled with dairy wastewater samples via a plastic cone and then transported to the national research center in Cairo where the laboratory experiments were taking place. Samples stored at very low temperature (4ºC) in order to prevent any change in the physicochemical characteristics of the raw samples.

Isolation of microorganisms

The microbial isolation process was performed by using "Streaking for isolation on an agar plate" which depend on the continuous dilution of microorganisms in order to make the microbial cells at an occasional density that allowed its cells to separate physically in an obvious way to recognize each microbial colonies. The microbial isolation was performed as follows:

1. Dairy wastewater samples were continuously diluted from (1/10 to 1/1000000).
2. Erlenmeyer flask with a volume of 250 mL was disinfected in order to be ready for use.
3. The erlenmeyer flask was filled with a proper nutrient broth to enhance the bacteria's growth.
4. 10^{-6} diluted dairy wastewater was inoculated into the erlenmeyer flask.
5. The erlenmeyer flask was left to inoculated at (20º C) at a rotary shaker operating at 100 rpm for about 48 hours.
6. A looped of enriched microbial samples were poured into nutrient agar petri dishes.
7. The nutrient agar petri dishes remained in an isolated place at a temperature of 33º C for one day. Figure 1 describes all steps of the microbial isolation process.

Specific culture media

After the isolation process, several microbial colonies were observed due to their different formations and color. A milk broth was prepared by adding 5 grams of peptone and 3 grams of yeast to 10 mL of milk then it was poured into Erlenmeyer flask with a volume of 250 mL. The microbial colonies were inoculated into a rotary shaker for 35º C for 48 hours and then transferred into nutrient agar Petri dishes and inoculated for 24 hours.

Identification of microbial isolates

Several microbial colonies were identified using Biolog's microbial identification system (Biolog® Gen III, USA).

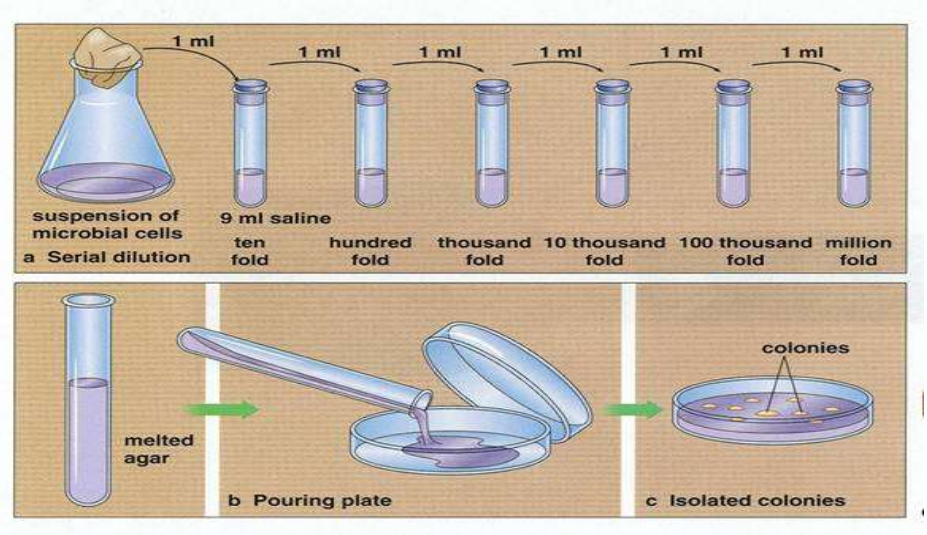

Figure 1. Steps of isolation of microorganisms.

Steps of inoculum preparation

1. 0.1 mL of each recognized microbial isolates were inoculated into 100 mL of nutrient broth
2. The flaker was placed into a rotary shaker which operated at 150 rpm at 35° C for one day.
3. After 24 hours, the active microbial cultures were washed with sterile deionized water.
4. The rotary shaker was operated at 10000 rpm for about 10 minutes to get wet pellets.

Preparation of pre-prepared effective microorganism (EM3)

In this study, effective microorganisms stoste and the molasses were obtained from (Emoted for chemical industries), Cairo, Egypt. EM culture solution was prepared by mixing 12 % EM stoste, 6% molasses, and 82% distilled water, as shown in Figure 2. The mixing process was performed at a temperature of 28° C in order to activate the microorganisms.

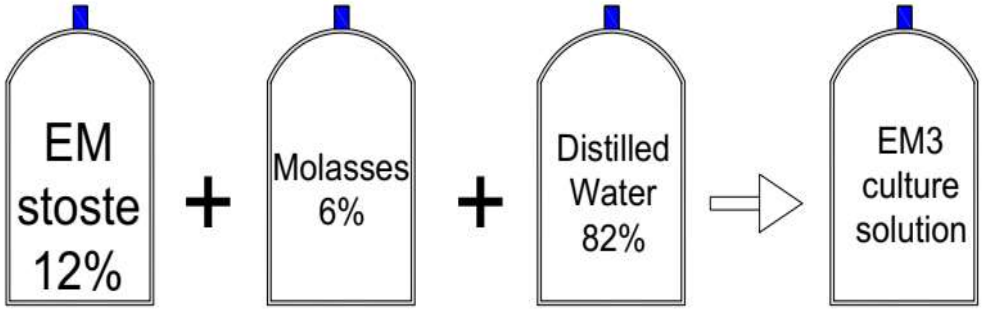

Figure 2. Preparation of EM3 culture solution.

Description of the laboratory pilot

The pilot consists of two glass rectangular tanks followed by a filter, as shown in Figure 3. The first tank was an aeration tank where the microorganisms biodegrade the organic matter into inorganic substances. The aeration tank was equipped with an air blower (SPT500) to provide the sufficient dissolved oxygen required for microorganisms. The aeration tank followed with a final settling tank where the inorganic substance produced from the aeration tank allowed settling under gravity and followed by a filter of activated carbon which settled on layers of 10 cm. Table 1 illustrates the description of pilot tanks in addition to the operational parameters of the experimental operation.

Description of the experimental operation

The aeration tank was fed with 48 liters of dairy raw wastewater. The aeration tank was equipped with an air blower to provide sufficient oxygen in order to provide the dissolved oxygen required by the effective microorganisms for the biodegradation of the organic matter. Then, the effective microorganisms were fed into the aeration tank, as shown in Table 2.

Table 1. Full description of the laboratory pilot.

Tank	Parameter	Value
Final settling tank	water volume	450 liters
	Length × width × depth	60 × 30× 30 cm
	Hydraulic retention time	2 hours
Aeration tank	Length × width × depth	60 × 30× 30 cm
	water volume	450 liters
	Hydraulic retention time	12 hours
Air blower	Type	SPT500
	Rate of flow	350 liters/hour
Membrane	Material	Activated carbon
	Layer thickness	20 cm

Table 2. Doses of effective microorganisms.

Type of EM	Bacillus bacteria	Staphylococcus bacteria	EM stoste + Molasses
Code of EM	EM1	EM2	EM3
Dose (mL)	50	50	30

The aeration process extended for 12 hours, after that, the dairy wastewater was transferred into the final settling tank for 2 hours or the membrane basin to remove the non-organic substances produced from the biodegradation of organic matter and allowed to settle under gravity. In this experimental study, four attempts were performed as follows:

1. Aeration cycle for hydraulic retention time (HRT) of 12 hours without adding any effective microorganisms followed by final settling tank for HRT= 2 hours then passed to activated carbon membrane.
2. Aeration cycle for hydraulic retention time (HRT) of 12 hours with adding Bacillus bacteria (EM1) followed by final settling tank for HRT= 2 hours then, passed to activated carbon membrane.
3. Aeration cycle for hydraulic retention time (HRT) of 12 hours with adding Staphylococcus bacteria (EM2) followed by final settling tank for HRT= 2 hours then, passed to activated carbon membrane.
4. Aeration cycle for hydraulic retention time (HRT) of 12 hours with adding EM stoste + Molasses (EM3) followed by final settling tank for HRT= 2 hours then, passed to activated carbon membrane.

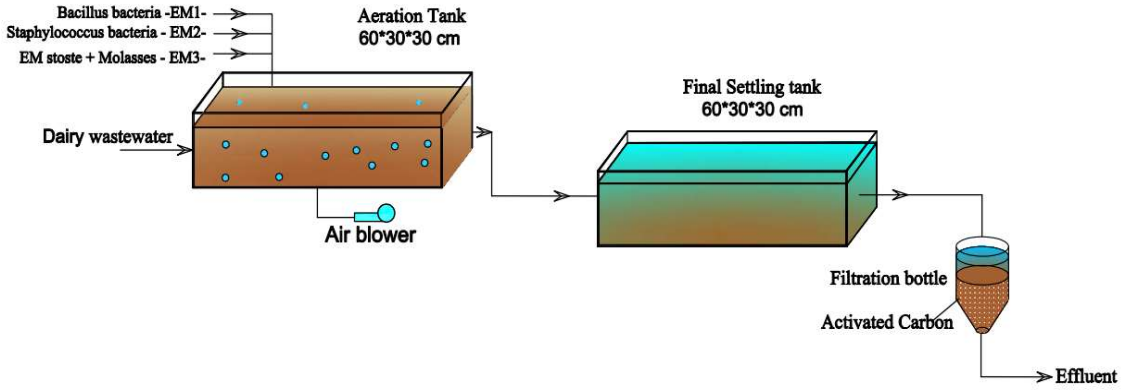

Figure 3. Scheme of the Experimental pilot.

Measured physical and chemical parameters

- The collected industrial wastewater samples were analyzed for characterization. Samples were also collected after each processing stage.
- The temperature (T°C) was measured daily before the wastewater samples were collected.
- The measured parameters were: Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), pH, Total Phosphorous (TP), and Total Nitrogen (TN). These parameters were measured according to standard methods.

Results and Discussion

Characteristics of raw dairy wastewater

The physicochemical characteristics of dairy wastewater are discussed in Table 3. From Table 3, the average pH of dairy wastewater was 6.6 which tends to an acidic state. Other studies reported the range of the pH of the dairy wastewater what was from 4.1 to 8.8 (Shete and Shinkar, 2013). This acidic condition was due to the lactose formulation in milk which is the base of all dairy products, lactose is able to break down into lactic acid causing this low pH value (Al-Wasify et al., 2017). The raw dairy wastewater was characterized by high concentrations of total suspended solids that reached up to 700.3 mg/L as shown in Table 3. This high TSS concentration was the same value that reported by other studies. These studies illustrate the TSS concentration was at the range of 630 mg/L (Porwal et al., 2015). Disposal of the dairy wastewater with this high concentration of TSS may prevent light from penetrating the surface water causing several problems to the aquatic life. The dairy wastewater was loaded with high concentrations of organic matter as the COD and BOD concentrations were 2203.5 and 1284.8, respectively. Other researches illustrate that the range of organic matter concentrations of raw dairy wastewater was 1200 to 1800 mg/L for BOD, and 1900 to 2700 mg/L for COD (Shete and Shinkar, 2013). These high COD and BOD concentrations were because the dairy wastewater is rich in fats and proteins causing high organic matter concentrations.

Table 3. The physicochemical characteristics of raw dairy wastewater in mg/L.

Parameters	Raw 1	Raw 2	Raw 3	Raw 4	Average	Standard deviation
pH	6.7	6.4	6.4	6.8	6.6	0.2
Total suspended solids, TSS	720	678	714	689	700.3	20.0
Biological oxygen demand, BOD	1310	1248	1287	1294	1284.8	26.3
Chemical oxygen demand, COD	2120	2240	2304	2150	2203.5	84.2
Total nitrogen, TN	230	217	225	207	219.8	10.0
Total phosphorous, TP	41	48	40	39	42.0	4.1

Performance of using several types of effective microorganisms for dairy wastewater treatment

Table 4 shows the final concentrations of all cycles of dairy wastewater treatment. In order to use treated wastewater instead of fresh water for agriculture purposes such as restricted irrigation. All the results were compared with the Egyptian standards for reusing the treated wastewater in irrigation.

Table 4. The physicochemical characteristics of dairy wastewater after each stage.

Trials	Parameters	pH	TSS	BOD	COD	TN	TP
	Units		mg/L	mg/L	mg/L	mg/L	mg/L
Trial 1	Raw	6.8	689	1294	2150	207	39
	Aeration tank (12 hr.)	7.1	447.85	414.08	623.5	101.43	17.94
	F.S (2 hr.)	7.4	124.02	232.92	365.5	74.52	12.48
	Filter	7.4	75.79	168.22	344	62.1	10.14
Trial 2	Raw	6.4	714	1287	2304	225	40
	aeration tank (12 hr.) + EM1	6.6	464.1	296.01	437.76	29.25	17.2
	F.S (2 hr.)	6.6	85.68	115.83	161.28	13.5	15.6
	Filter	6.6	49.98	48.906	94.464	13.5	6.4
Trial 3	Raw	6.4	678	1248	2240	217	48
	aeration tank (12 hr.) + EM2	6.5	457.65	199.68	313.6	52.08	27.36
	F.S (2 hr.)	6.5	88.14	99.84	129.92	39.06	22.56
	Filter	6.4	54.24	74.88	100.8	26.04	20.64
Trial 4	Raw	6.7	720	1310	2120	230	41
	aeration tank (12 hr.) + EM3	6.9	532.8	882.94	1454.32	163.3	23.78
	F.S (2 hr.)	6.9	424.8	602.6	1102.4	82.8	16.4
	Filter	6.8	338.4	510.9	890.4	73.6	14.35
Law 48 (1982)		6 to 9	60	40	100	……..	10

Remarks: BOD = Biological Oxygen Demand, COD = Chemical Oxygen Demand, TSS = Total Suspended Solids, TN = Total Nitrogen TP = Total Phosphorous.

Effect of using effective microorganisms on pH of the solution

From Table 4, using EM1, EM2, and EM3 in the aeration tank for HRT=12 hours has an effect on the pH as it increases the pH at trial 1 from 6.8 to 7.1, from 6.4 to 6.6 at trial 2, from 6.4 to 6.5 at trial 3, finally from 6.7 to 6.9 at trial 4. So, all pH increase was tended to neutralization. Some researchers studied the effect of using EM on the pH and delivered the same result such as (Noorjahan and Jamuna, 2012). This increase in pH may be due to the accumulation process of organic matter performed by each type of effective microorganisms (EM1, EM2, and EM3). Also, using the activated carbon filter has no effect on the pH of the solution that agreed with the other studies (Al-Wasify et al., 2017).

Effect of using EM1, EM2, and EM3 on the total suspended solids (TSS) concentrations

Table 4, illustrates the ability of EM1 and EM2 for the removal of TSS from dairy wastewater. At trial 1, EM1 and EM2 were able to decrease the TSS concentrations after HRT= 12 hours at the aeration tank by removal efficiency up to 35 % and 32%, respectively. That was similar to the results reported by other researchers which documented the ability of EM1 and EM2 to remove TSS by removal efficiency up to 33.5 % (Al-Wasify et al., 2017). The TSS removal efficiency obtained from this experimental study was higher than the results achieved by other studies was due to the high dose of effective microorganisms that reached 30 mg/L. The final TSS concentration shown in Table 4 was achieved after the settling and filtration stage which ensures the high ability of activated carbon to remove TSS as the TSS removal efficiency reached 89% and 93% in case of using EM1 and EM2, respectively, that was due to its high absorption capacity of the activated carbon (Namane et al., 2005). Using EM3 achieved the lowest TSS removal efficiency as it was able to decrease the TSS concentration by 26 % after HRT= 12 hour at the aeration tank that may be due to the loss of efficiency in degrading the organic substances that reflect in the TSS removal as reported in (Zhou et al., 2013).

Effect of using EM1, EM2, and EM3 on the organic matter concentrations

From Table 4, EM1 was able to reduce the COD and BOD concentrations after the aeration stage by removal efficiency up to 81% and 77%. Also, using EM2 was able to decrease the COD and BOD concentration after HRT of 12 hours at the aeration tank by removal efficiency of 86% and 84%. Finally, using EM3 at trial 4 was able to reduce COD and BOD concentrations by a removal efficiency of 31.4% and 32.6%, respectively. From the previous results, EM1 and EM2 achieved high performance in removing the organic matter concentrations up to 80% while other studies reported that the most reduction occurred in the case of using EM1 and EM2 was 74.2% (Al-Wasify et al., 2017) that may be due to the high dose of effective microorganisms (50 mL) used in this experimental study leading to the increase of the biodegradation rate of organic matter by microorganisms that explained the increase in COD and BOD removal. Results obtained from trial 4 were slightly lower than the results achieved by other studies which reached up to 33.92% (Zhou et al., 2013). The reason behind the reduction in organic matter removal was due to the low hydraulic retention time (12 hours) as EM3 reached 33.93% removal efficiency after a fermentation time of 12 days.

Effect of using EM1, EM2, and EM3 on nutrients (nitrogen and phosphorous) removal

From Table 4, the application of effective microorganisms has an effective role in removing TN and TP by removal efficiency of 94% and 84%, respectively in case of using EM1 and 88% and 57% while using EM2, finally decreased to 68%, and 65% in case of using EM3. Other studies reported that the high performance of effective microorganisms technology in reducing nutrients concentrations by 88% for TN and 50% for TP (Li et al., 2020). So, the TP removal efficiency obtained from this experimental study was at the range of the other studies as the phosphorous compounds found in dairy wastewater was almost inorganic which does not need any biological treatment so the final settling tank and the activated carbon filter has the main role in removing phosphorous compounds (Slavov, 2017). TN removal efficiency achieved by this experimental study was less than results reported by the other studies, as other studies providing a high retention time reached 8 days in order to get sufficient time for the slow-growing bacteria to grow up and to perform the nitrification process.

Conclusion

Due to the lack of fresh water resources which consider the backbone of all life aspects such as industry and agriculture, big attention was paid to the treatment of dairy wastewater as an additional source of water, in addition to provide safe disposal of dairy wastewater to the surface water due to the high concentrations of organic matter, fats, proteins. Application of effective microorganisms by using Bacillus bacteria (EM1), Staphylococcus bacteria (EM2), and EM stoste + Molasses (EM3) was very effective in reducing the COD, BOD, TN, TP, and TSS. Depending on Bacillus bacteria (EM1) was more effective than EM2 and EM3 as it reduced the organic matter concentration up to 90%. Also, using EM1, EM2, and EM3 has a slight difference in removing the TN and TP concentration by removal efficiency up to 50%.

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References

Abd Ellah, R.G. 2020. Water resources in Egypt and their challenges, Lake Nasser case study. *Egyptian Journal of Aquatic Research* 46(1): 1-12, doi: 10.1016/j.ejar.2020.03.001.

Al-Wasify, R.S., Ali, M.N. and Hamed, S.R. 2017. Biodegradation of dairy wastewater using bacterial and fungal local isolates. *Water Science and Technology* 76(11): 3094-3100, doi: 10.2166/wst.2017.481.

Desai, S.S., Sangam, V.S., Joshi, L., Smita, K., Namazi, A. and Swetha, G. 2012. Development and formulation of effective microbial (EM) technology for dairy industrial effluent treatment. AICERA 2012 - *Annual International Conference on Emerging Research Areas: Innovative Practices and Future Trends*, December, 1-6, doi: 10.1109/AICERA.2012.6306702.

Elmagd, A.M.A. and Mahmoud, M.S. 2014. Tannery wastewater treatment using activated sludge process system (lab scale modelin). *International Journal of Engineering and Technical Research* 2(5): 21-28.

Li, X., Guo, Q., Wang, Y., Xu, J., Wei, Q., Chen, L. and Liao, L. 2020. Enhancing nitrogen and phosphorus removal by applying effective microorganisms to constructed wetlands. *Water (Switzerland)* 12(9): 1-14, doi: 10.3390/w12092443.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Kang, J., Xia, S., Zhang, Z. and Price, W.E. 2014. Removal and fate of micropollutants in a sponge-based moving bed bioreactor. *Bioresource Technology* 159: 311-319, doi: 10.1016/j.biortech.2014.02.107.

Mekonnen, M.M. and Gerbens-Leenes, W. 2020. The water footprint of global food production. *Water (Switzerland)* 12(10): 2696, doi: 10.3390/w12102696.

Naghizadeh, A., Mahvi, A.H., Mesdaghinia, A.R. and Alimohammadi, M. 2011. Application of MBR technology in municipal wastewater treatment. *Arabian Journal for Science and Engineering* 36(1): 3-10, doi: 10.1007/s13369-010-0007-7.

Namane, A., Mekarzia, A., Benrachedi, K., Belhaneche-Bensemra, N. and Hellal, A. 2005. Determination of the adsorption capacity of activated carbon made from coffee grounds by chemical activation with $ZnCl_2$ and H_3PO_4. *Journal of Hazardous Materials* 119(1-3): 189-194, doi: 10.1016/j.jhazmat.2004.12.006.

Noorjahan, C.M. and Jamuna, S. 2012. Physico-chemical characterisation of brewery effluent and its degradation using native fungus -*Aspergillus Niger*, Aquatic Plant - Water Hyacinth-*Eichhornia sp.* and Green Mussel - Pernaviridis. *Journal of Environment and Earth Science* 2(4): 31-40.

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, doi: doi.org/10.1016/j.wri.2014.11.002.

Shete, B.S. and Shinkar, N.P. 2013. Dairy industry wastewater sources, characteristics and its effects on environment. *International Journal of Current Engineering and Technology* 3(5): 1611-1615.

Slavov, A.K. 2017. General characteristics and treatment possibilities of daity wastewater - a review. *Food Technology and Biotechnology* 55(1): 14-28, doi: 10.17113/ftb.55.01.17.4520.

Szymanski, N. and Patterson, R.A. 2003. Effective Microorganisms (EM) and Wastewater Systems in Future Directions for On-site Systems: Best Management Practice. *Proceedings of On-site '03 Conference by Patterson*, R.A. and Jones, M.J. (Eds). Held at University of New England, Armidale 30th September to 2nd October 2003. Published by Lanfax Laboratories Armidale. ISBN 0-9579438-1-4 pp 347-354.

Verma, A., Singh, A., Mathur, N. and Atri, R. 2012. Physico-chemical analysis of effluent from dairy industry. *International Journal of Chemical Sciences* 10(4): 2061-2066.

Zhou, Y.X., Zhen, S.C. and Zhai, Y.M. 2013. The application of EM-treated dairy effluent on the flue-cured tobacco irrigation. *Journal of Food, Agriculture and Environment* 11(1): 960-964.