Biological Forum – An International Journal **15(5a): 151-156(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Efficient Biodegradation of Food Processing Wastewater using Microbial Consortium: A Case Study

***Gandhi S.N.*[1*] *and Raval A.A.*[2]**
[1]*Ph.D. Scholar, Department of Microbiology, Arts, Science and Commerce College, Kholwad.*
Affiliated to Veer Narmad South Gujarat University, Surat-3940180, (Gujarat), India.
[2]*Assistant Professor, Department of Microbiology, Arts, Science and Commerce College, Kholwad,*
Affiliated to Veer Narmad South Gujarat University, Surat-3940180, (Gujarat), India.

(Corresponding author: Gandhi S.N.)*
(Received: 11 March 2023; Revised: 27 April 2023; Accepted: 06 May 2023; Published: 15 May 2023)
(Published by Research Trend)

ABSTRACT: Food products are consumed for their nutritional value and flavourful taste. The colour of food not only stimulates appetite but also enhances its aesthetic appeal to consumers. Wastewater generated by the food sector is significantly loaded with organic substances, nitrogen, phosphorus, dyes, and potentially heavy metals. These waste byproducts, especially the dyes, constitute an estimated 15% of all dyes emitted globally, causing serious harm to aquatic ecosystems. To address this issue, the exploration of an environmentally friendly and self-sustaining treatment method is crucial. This study investigates the use of a microbial consortium consisting of three Enterobacter species that were previously isolated from various textile industries and have demonstrated to be highly effective at removing heavy metals and textile dyes both, individually and in consortia. The objective of this study was to evaluate the potential of this pre-established consortium in treating food processing wastewater. Our findings suggest that this microbial consortium effectively removes up to 60% of colour and 44% of organic load from food processing wastewater. Consequently, the utilization of microbial consortia, with proven effectiveness in treating textile wastewater, offers substantial promise for application in the food processing industry as well, providing a viable, environmentally sustainable approach to wastewater treatment.

Keywords: Food colours, biodegradation, coagulant-flocculent, microbial consortium, Enterobacter.

INTRODUCTION

Food processing wastewater management is a significant global issue (US EPA, 2017). Food processing industries produce wastewater with high levels of organic compounds, nutrients, suspended solids, and colorants. Excess nitrogen and phosphorus present in food industry waste streams is a common environmental issue. If untreated, food manufacturing wastewater promotes the growth of microorganisms, induces eutrophication of freshwaters, and affects aquatic and human life. (Ghimpusan *et al.*, 2017). The BOD and COD of food processing industries are 10–100 times higher compared to domestic wastewater. (Vymazal *et al.*, 2014; Benyakhlef *et al.*, 2007; Kengne *et al.*, 2014; Meul *et al.*, 2009). Apart from organic pollutants, every year, approximately 8 million tons of food colorants are produced by industries. Most food dyes are azo dyes which widely used in food, cosmetics, pharmaceutical products, and the textile industry. Dye-containing wastewater is discharged from numerous industries, such as textiles, leather, paint, food, and pharmaceuticals, deteriorating the aquatic environment and posing a threat to living organisms. Azo dyes include one or more R1-N=N-R2 bonds that are degraded by enzymatic processes into aromatic amines. Several azo dyes are often used in beverages, puddings, icings, jellies, dairy products, spices, and many baked goods, whereas yellow azo dye is utilized in dressings, yogurt, baked goods, snack foods, and so on (Olas *et al.*, 2021). Dye containing wastewater is discharged from numerous industries, such as textiles, leather, paint, food, and pharmaceuticals, deteriorating the aquatic environment and posing a threat to living organisms. These dyes are aromatic compounds that are usually stable and highly water-soluble. In the USA and European countries, some azo dyes have been banned as food additives due to their toxic, mutagenic, and carcinogenic side effects (Chung, 1983). Food colours can be either natural or synthetic. Synthetic colours are inexpensive and stable compared to natural colours, which are costly and less stable. Some examples of commonly used food colours are mentioned in Table 1. However, synthetic colours have adverse effects on human health, including live and kidney damage (Mahmoud, 2006). The most common natural dyes used are carotenoids, chlorophyll, anthocyanin, and turmeric. Various physical and chemical techniques are used for the removal of dyes, including filtration, adsorption by activated charcoal, coagulation by chemical coagulants, and advanced oxidation techniques. However, these techniques have some disadvantages, such as not being not cost-effective, time-consuming, and generating residuals. Biological techniques involve the use of microbial biomass for the degradation and decolourization of dyes through enzymatic degradation (Knackmuss, 1996; Moosvi *et al.*, 2007). The intermediate compounds formed are aromatic amines, which are sometimes toxic, mutagenic, and carcinogenic by nature (IARC, 2009, Sani *et al.*, 1999). Unfortunately, most organic dyes are non-degradable, heat stable, and resistant to decomposition with respect to biochemical oxidation due to their large or complex sizes and inert nature. These characteristics have rendered organic dyes difficult to decolourization using conventional methods of wastewater treatment.

Moreover, the varied composition and high pollutant load of wastewater from food processing make treatment difficult (Pervez *et al.*, 2021). The utilization of microbial consortia for the biodegradation of wastewater from food processing has been investigated by numerous studies (Lashani *et al.*, 2023). According to the findings (Kuo & Dow 2017), degradation efficiency has improved, and in some cases, useful byproducts like biogas have been produced. Due to their functional diversity and synergistic interactions, it has been demonstrated that using a consortium of microorganisms rather than a single strain increases the efficiency of degradation (Cao *et al.*, 2022). Depending upon the wastewater composition, different consortia have been developed to degrade specific pollutants and their performance can be optimized. (Zhang & Zhang 2022).

In the present study, three potent bacterial strains were isolated from different textile effluents and assessed for their potential in treating effluent from the food industry. Various physicochemical parameters were determined before and after treatment, including pH, turbidity (NTU), colour, total organic carbon (TOC), total dissolved solids (TDS), total suspended solids (TSS), total hardness, and total alkalinity.

No.	FD & C designation	Name	Colour
1	**Blue no. 1**	**Brilliant blue FCF**	**Blue**
2	**Blue no.2**	**Indigotine**	**Indigo**
3	**Green no.3**	**Fast green FCF**	**Turquoise**
4	**Red no.3**	**Erythrosine**	**Pink**
5	**Red no.40**	**Allure red AC**	**Red**
6	**Yellow no.5**	**Tartrazine**	**Yellow**
7	**Yellow no.6**	**Sunset yellow FCF**	**Orange**

Table 1. Food colours approved by the U.S. Food and Drug Administration. FD&C stands for laws passed by the U.S. Congress in 1938, called the Federal Food, Drug, and Cosmetic Act (U.S. Food & Drug Administration, 2018).

MATERIAL AND METHODS

1. Collection of samples: A wastewater sample was collected from a local food processing industry in Bharuch district, Gujarat, India. The sample was labelled and preserved under refrigeration according to standard methods. The next day, all physicochemical parameters were performed as mentioned in Standard Methods for the Examination of Water and Wastewater, 24th Edition (American Public Health Association *et al.*, 2023).

2. Turbidity (NTU), pH, and colour: The turbidity of the sample before and after treatment was determined using a turbidimeter (model: TL2360, make: HACH). pH was measured using a pH meter. Colour was determined by measuring absorption at the maximum wavelength using a UV-Vis spectrophotometer (model: Lambda 365, make: Perkin Elmer).

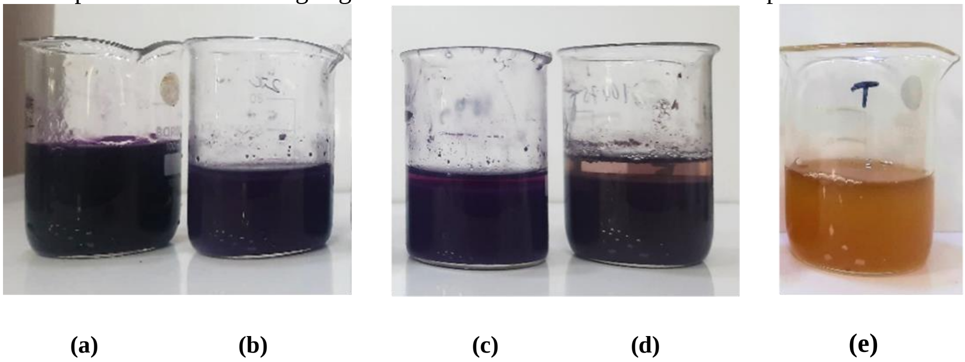
3. Electrical conductivity (EC), total solids, total suspended solids, and total dissolved solids (TS, TSS, and TDS), Total Organic Carbon (TOC): Electrical conductivity (EC) was determined using an ion analyzer (model: Orion Versa Star, make: ThermoScientific), where the electrode was directly dipped into the sample to display the result on a digital scale after calibrating the instrument. TS, TSS and TDS were determined by the standard gravimetric method. TOC (mg/L) of a diluted sample was measured using a TOC analyzer (model: TOC-L, make: Shimadzu) and COD (mg/L) was determined using a COD digester using the dichromate open reflux method.

4. Total hardness and alkalinity: Hardness (mg/L) and alkalinity (mg/L) were determined using standard titrimetric methods.

5. Chloride and total phosphorus: Chloride (%) was determined by iodometric titration, and phosphorus was determined using the vanadomolybdate method followed by spectrophotometric analysis.

6. Coagulant-flocculent treatment: Polyaluminum chloride (PAC) and decolourant Vytal 630 (products from Grasim Industries Ltd. India) were used as coagulant and decolourant. 1 g of each PAC-18 liquid and decolourant Vytal 630 were dissolved in 100 mL of distilled water to prepare 10,000 ppm stock solution. Different doses ranging from 50 ppm to 1000 ppm were added to the sample, followed by the addition of a flocculent. Coagulation studies for dose optimizations were performed using a jar test apparatus with set parameters of rapid mixing with addition of coagulant or decolourant and flocculent at 200 rpm for 1 min, slow mixing at 30 rpm for 15 mins to allow flocculation and sedimentation for 30 mins.

7. Microbial treatment. A microbial consortium in optimised ratio was prepared from individual strains isolated from various textile effluents based on their dye degradation potential. An overnight-grown culture was added to the sample at a rate of 10% inoculum.



(a) Control, (b) PAC treated, (c) Vytal 630 low dose (d) Vytal 630 high dose, (e) Bacterial consortia treated
Fig. 1. Various treatments of coagulant, decolourant and bacterial consortia.

RESULT AND DISCUSSION

1. Physicochemical characterisation of sample. Turbidity reflects the amount of particulate matter present, which might include pollutants or contaminants. Turbidity was observed on the higher side, exceeding 90 NTU, indicating high levels of solids and organics. pH determination is critical as it influences the biological and chemical processes, which can significantly affect wastewater treatment efficiency (Peavy *et al.*, 1985). Generally, food processing industries have an acidic pH due to manufacturing processes and microbial fermentation (Vanereker *et al.*, 2013; Alao *et al.*, 2010). However, the values obtained meet the wastewater discharge standards of pH between 6.5 and 8.5 (CPCB MoEF, PCLS4/2000-2001). Simultaneously, a colour change was observed from the darkest brown to dark purple. Electrical conductivity provides information about the overall salinity or ionic strength of the wastewater. Hardness and alkalinity are crucial parameters as they can impact the effectiveness of various wastewater treatment processes. High alkalinity was observed due to the presence of substantial amounts of dissolved organics, inorganics, suspended solids, and bicarbonates in the water. High conductivity indicated elevated levels of total dissolved solids (TDS) (Metcalf & Eddy 2003). The measurement of total solids, suspended solids, dissolved solids, and total organic carbon (TOC) helps in understanding the total pollutant load in the wastewater (Metcalfe & Eddy 2003). High levels of these parameters can indicate the presence of organic matter, salts, or other pollutants.

2. Effect of coagulants and decolourant on removal of colour. Coagulation and flocculation are widely practiced methods for removing colour from textile and tannery wastewaters. Coagulation involves the addition of a coagulant, such as Polyaluminum Chloride (PAC 18), which neutralizes the charge of fine particles in the wastewater, allowing them to come together by sweep floculltation. Polyaluminum chloride (PAC 18) was used in varying doses from 50 to 1000 ppm, but no coagulation or settling was observed, resulting in no colour removal as observed in Fig. 1(b). This suggests that PAC 18 may not be an effective coagulant for the specific types of colour-causing pollutants present in this particular wastewater sample. Decolourant Vytal 630 was used to remove colour. Significant colour reduction was observed Fig. 1(c), but at very high doses Fig.1(d), which may generate a large amount of sludge and not be cost-effective. The disposal of this sludge can pose its own environmental and logistical challenges (Mehedi, 2019).

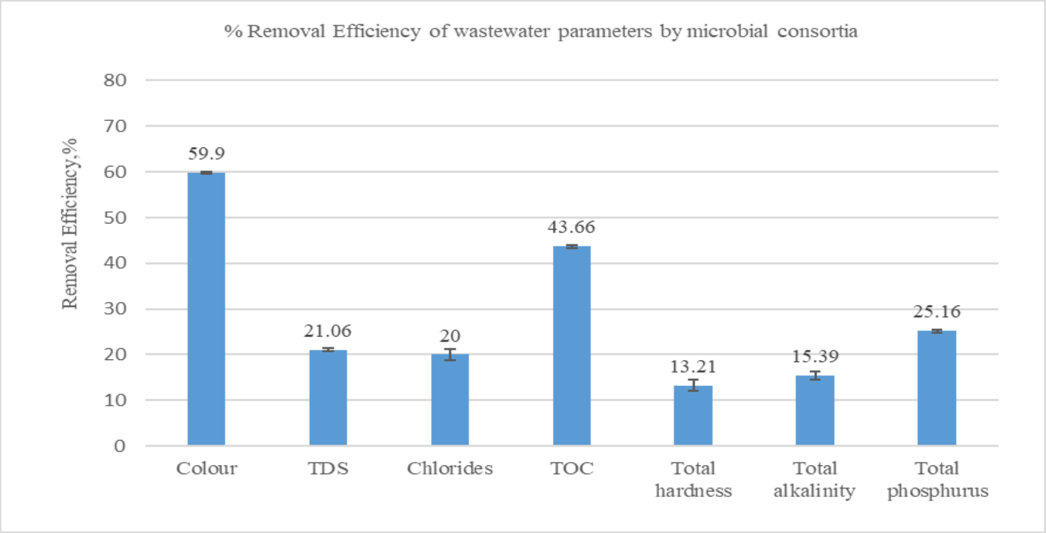
3. Effect of bacterial consortium on removal of colour. A bacterial consortium comprising three potent isolates was added to a fixed volume of the sample. A 10% acclimatized culture was added and kept under static conditions at ambient temperature for 7 days. After 7 days, the percentage of decolourization was evaluated using spectrophotometric analysis Fig. 1(e). Upon shaking the sample, no reoccurrence of colour was observed, indicating complete enzymatic degradation by the bacteria. The isolates used for the experiment were previously isolated from textile effluents, purified, maintained, and preserved as glycerol stocks under refrigerated conditions. All isolates belong to the *Enterobacteriaceae* family. All isolates were identified by the 16S rDNA sequencing method, as summarized in Table 2.

Table 2. Molecular identification of isolates.

Isolate	Close relative	NCBI GenBank Accession number
ABG -38	*Enterobacter asburiae*	OQ428176
ABG -39	*Enterobacter hormaechei*	OQ435375
ABG-PB	*Enterobacter ludwigii*	OQ540964

Table 3: Physico-chemical analysis of wastewater parameters before and after treatment.

Sr. No.	Parameter	Pre-treatment analysis	Post treatment analysis
1	**Turbidity (NTU)**	92.6	94.6
2	**Conductivity(mS/cm)**	133	109
3	**pH**	7.02	8.49
4	**Colour**	Dark brown to dark purple	Light yellow
5	**Colour (Abs @ λmax:410 nm)**	26.45	10.58
6	**Odour**	Fermented unpleasant	Less odour
7	**TDS (mg/L)**	85,053	67,140
8	**TSS (mg/L)**	340	586.66
9	**Chloride (%)**	4.60	3.68
10	**TOC (mg/L)**	8423	4745
11	**Total hardness(mg/L)**	1400	1215
12	**Total alkalinity(mg/L)**	10,200	8630
13	**Total phosphorus(mg/L)**	153	114.5



*Mean of three replicates ±Std. Dev.

Fig. 2. Graph showing % removal of various parameters after treatment.

4. Impact of biologically treated sample on removal of other parameters. Along with colour removal, other wastewater parameters were also checked using standard methods. Considerable removal was observed in TOC, dissolved solids, phosphorus, and chlorides, as shown in the graph, Fig. 2. The percentage removal efficiency (%) for each parameter was calculated using the formula:

$$\text{Removal (\%)} = (C_i - C_f) / C_i \times 100$$

Where Ci and Cf are the initial and final concentration for each parameter, respectively.

Removal of colour and TOC. The colour of the effluent was found to be highly concentrated by visual observation due to the presence of a mixture of food dyes like Carmoisine Red, Tartrazine Yellow, Brilliant Blue, and Fast Green. Dye degradation by the bacterial consortium takes place through enzymatic breakdown or cleavage of the azo bond (-N=N-) into intermediate compounds like aromatic amines. These amines further get mineralized into various metabolic pathways of the organism (Ajaz *et al*., 2020). A 60% dye degradation was observed with no reoccurrence of colour upon aeration. The bacterial consortium was able to remove 43% of the TOC, which is a considerable achievement that further enhances the effectiveness of the treatment process.

Removal of total solids. Both organic and inorganic dissolved solids raise TDS levels in wastewater to a high extent. TDS was removed by 21% after bacterial treatment, which can be attributed to the adsorption of ions on the surface of bacterial cells followed by bacterial assimilation. TSS was found to be increased after treatment as compared to the control, which can be due to the presence of bacterial biomass and nutrients in the sample (Tchobanoglous *et al*., 2014).

Removal of total hardness and alkalinity. Hardness and alkalinity were removed in the range of 13-15% by microbial metabolic activity during the degradation process.

Removal of chlorides and total phosphorus. Chloride level is a general indicator of the salinity of the wastewater, while high phosphorus levels can lead to eutrophication in receiving waters, causing excessive growth of algae and other aquatic plants (Yang *et al*., 2005). High chloride contributes to high TDS in wastewater (Hong *et al*., 2023). Chlorides were removed by 20 % and total phosphorus by 25 % respectively. Chlorides are removed by bio sorption mechanisms by certain bacteria. Bacteria remove elemental phosphorus by assimilation, converting them to orthophosphates (Sengupta *et al*., 2015).

CONCLUSION

The present experimental studies on the treatment of food industry effluent using a potent microbial consortium shown significant reduction of various parameters. The efficiency of biodegradation can be credited to the diverse mechanisms inherent to each bacterial strain within the consortium. Of these, colour and Total Organic Carbon (TOC) reductions were most prominent, although other parameters also shown substantial reduction. Each strain present in the consortium has its unique capabilities to remove each parameter efficiently, and collectively they exhibit synergistic approach and enhanced efficiency. It was observed that decolourization began 24 hours into the experiment, reaching its peak after an incubation period of 7 days, with no further changes observed thereafter. The findings suggest that the microbial consortium, primarily comprising Enterobacter species, originally proven efficient in degrading textile dyes, is equally efficient at removing food dyes. The versatility and efficiency of the microbial consortium can be used as an effective treatment for diverse industrial effluents.

FUTURE SCOPE

Determination of the consortium's potential for degradation of pollutants, other than dyes.

Acknowledgement. I extend my sincere gratitude to Dr. Arti Raval (major advisor) for giving me proper guidance throughout the course of the study. I also sincerely thank Late Dr. Sanjeev Gupta and R & D department, Grasim Industries Ltd., Bharuch for providing lab infrastructure to undergo this project efficiently.

Conflict of interest. The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES

Alao Olajumoke, Arojojoye Oluwatosin, Ogunlaja Olumuyiwa and Famuyiwa Abimbola (2010). Impact Assessment of Brewery Effluent on Water Quality in Majawe, Ibadan, Southwestern Nigeria. *Researcher*, *2*, 21-28.

Ajaz, M., Shakeel, S. & Rehman, A. (2020). Microbial use for azo dye degradation—a strategy for dye bioremediation, *International Microbiology*, *23*, 149-159.

American Public Health Association, American Water Works Association, Water Environment Federation. Lipps, W.C., Braun-Howland, E.B. & Baxter, T.E. (eds) (2023). *Standard Methods for the Examination of Water and Wastewater*. *24th edn*. Washington DC: APHA Press.

Benyakhlef, M., Naji, S. & Belghyti, D. (2007). Caractérisation des rejets liquides d'une conserverie de poissons, *Bull. Soc. Pharm. Bordeaux*, *146*, 225-234.

Cao, Z., Yan, W., Ding, M. & Yuan, Y. (2022). Construction of microbial consortia for microbial degradation of complex compounds, *Frontiers in Bioengineering and Biotechnology*, *10*, 1051233.

Chung, K.T. (1983). The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes, *Mutation Research/Reviews in Mutation Research*, *463*(1),11-21.

Environmental Standards for Ambient Air, Automobiles, Fuels, Industries and Noise (2000-2001). Pollution Control Law Series PCLS4/2000-2001. New Delhi: CENTRAL POLLUTION CONTROL BOARD (Ministry of Environment & Forests, Government of India).

Ghimpusan, M., Nechifor, G., Nechifor, A. C., Dima, S. O. & Passeri, P. (2017). Case studies on the physical-chemical parameters variation during three different purification approaches destined to treat wastewaters from food industry, *Journal of Environmental Management*, *203*, Part 2, 811-816.

Hong, Y., Zhu, Z., Liao, W., Yan, Z., Feng, C. and Xu, D. (2023). Freshwater Water-Quality Criteria for Chloride and Guidance for the Revision of the Water-Quality Standard in China, *International Journal of Environmental Research and Public Health*.

IARC (2009). A review of human carcinogens: chemical agents and related occupations. Lyon, France: *International Agency for Research on Cancer.*

Olas, B., Białecki, J., Urbańska, K., Bryś, M. (2021). The Effects of Natural and Synthetic Blue Dyes on Human Health: A Review of Current Knowledge and Therapeutic Perspectives. *Adv. Nutr.*, *12*, 2301–2311.

Knackmuss, H. J. (1996). Basic knowledge and perspectives of bioelimination of xenobiotic compounds, *Journal of Biotechnology*, *51*, 287-295.

Kengne, E. S., Kengne, I. M., Nzouebet, W. A. L., Akoa, A., Viet, H. N. & Strande, L. (2014). Performance of vertical flow constructed wetlands for faecal sludge drying bed leachate: effect of hydraulic loading, *Ecological Engineering*, *71*, 384-393.

Kuo, J. & Dow, J. (2017). Biogas production from anaerobic digestion of food waste and relevant air quality implications, *Journal of the Air & Waste Management Association* (1995), *67*(9), 1000–1011.

Lashani, E., Amoozegar, M. A., Turner, R. J. & Moghimi, H. (2023). 'Use of Microbial Consortia in Bioremediation of Metalloid Polluted Environments', Microorganisms, *11*(4), 891.

Mehedi, M. (2019). Decolorization of Industrial Dyeing Wastewater by Coagulation with Alum, Poly Aluminum Chloride (PAC), Polyelectrolyte, and Oxidation using Chlorine, *Journal of Industrial Pollution Control*, *34*.

Meul, M., Nevens, F. & Reheul, D. (2009). Validating sustainability indicators: Focus on ecological aspects of Flemish dairy farms, *Ecological Indicators*, *9*(2), 284-295.

Moosvi, S., Kher, X. & Madamwar, D. (2007). Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2, *Dyes and Pigments*, *74*, 723-729.

Metcalf & Eddy, Inc. (2003). Wastewater Engineering: Treatment and Reuse. 4th edn. New York: McGraw-Hill Education.

Mahmoud, Neveen (2006). Toxic Effects of the Synthetic Food Dye Brilliant Blue on Liver, Kidney and Testes Functions in Rats. *J. Egypt Soc Toxicol.*, *34*.

Pervez, M. N., Mishu, M. R., Stylios, G. K., Hasan, S. W., Zhao, Y., Cai, Y., Zarra, T., Belgiorno, V. & Naddeo,

V. (2021). Sustainable Treatment of Food Industry Wastewater Using Membrane Technology: A Short Review, *Water*, *13*(23), 3450.

Peavy, H. S., Rowe, D. R. & Tchobanoglous, G. (1985). Environmental Engineering. New York: McGraw-Hill Education.

Sani, R. K. & Banerjee, U. C. (1999). Decolorization of triphenylmethane dyes and textile and dyestuff effluent by *Kurthia* sp, *Enzyme and Microbial Technology*, *24*, 433-437.

Sengupta, S., Nawaz, T. & Beaudry, J. (2015). Nitrogen and Phosphorus Recovery from Wastewater, *Current Pollution Reports*, *1*(3), 155-166.

Tchobanoglous, G., Burton, F. L. & Stensel, H. D. (2003), *Wastewater Engineering: Treatment and Reuse*. 4th edn. New York: McGraw-Hill Education.

U.S. EPA (Environmental Protection Agency). (2017). Toxics Release Inventory – 2017 National Analysis Dataset. https://www.epa.gov/trinationalanalysis

U.S. Food & amp; Drug Administration. (2018). Color Additives Approved for Use in Food. Retrieved from https://www.fda.gov/food/food-additives-petitions/color-additives-approved-use-food.

Vanerkar, A. P., Satyanarayan, S., & Satyanarayan, S. (2013). Treatment of food processing industry wastewater by a coagulation/flocculation process. *International Journal of chemical and physical sciences*, *2*(3), 63-72.

Vymazal, J. (2014). Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological engineering*, *73*, 724-751.

Yang, X. E., Wu, X., Hao, H. L., & He, Z. L. (2008). Mechanisms and assessment of water eutrophication. *Journal of Zhejiang University. Science. B*, *9*(3), 197–209.

Zhang, T., & Zhang, H. (2022). Microbial Consortia Are Needed to Degrade Soil Pollutants. *Microorganisms*, *10*(2), 261.

How to cite this article: Gandhi S.N. and Raval A.A. (2023). Efficient Biodegradation of Food Processing Wastewater using Microbial Consortium: A Case Study. *Biological Forum – An International Journal, 15*(5a): 151-156.