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Biotreatment of sugar industry effluent using the tank cleaner, *Pterygoplichthys pardalis*

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Abstract

Wastewater can be considered as a precious resource that can be used to fulfill our growing demands of water. In the present study, physico-chemical parameters of the sugar industry effluent were analyzed. The sailfin catfish, *Pterygoplichthys pardalis* were exposed to 10, 20, 30 and 40 % concentrations of sugar industry effluent for 1, 2, 3, 4 and 5 days of treatment. The physico-chemical parameters such as pH, total solids, total dissolved solids, total suspended solids, total alkalinity, chlorides, total hardness, nitrates, sulphates, total phosphorus, ammonia and BOD were gradually reduced after treatment with *P. pardalis* for all the exposed concentrations of sugar industry effluent. Students' 't" test was used to test the level of significance for the variation between control and experimental sets for all the parameters. Ammonia, pH and chlorides exhibited more significant difference than the other parameters. Results revealed that *P. pardalis* is highly efficient in the treatment of sugar industry effluent. Biotreatment with *P. pardalis* may provide an economical and environmentally sustainable treatment method in future.

Keywords: Tank cleaner; *Pterygoplichthys pardalis*; Waste water treatment; Sugar industry effluent; Physicochemical parameters

1. Introduction

The management and disposal of agro-industrial residues have recently received attention because of the indiscriminate discharge of many effluents in to the environment. Since each type of industrial effluent has a specific character and impact on the biota, it is essential to assess the contribution of each pollutant. Water is essential for all known life forms but still water pollution and the destruction of ecosystems continue to increase. Water contamination is now a major problem in the global context as a consequence of industrialization, population growth, urbanization and warfare combined with increased wealth and more extravagant lifestyles [1]. This results in serious public health issues. For biodegradable wastes, biological treatment may be the best option which could be through either aerobic or anaerobic methods. Anaerobic treatment converts the wastewater organic pollutants into small amount of sludge and large amount of biogas as source of energy [2]. As a result of unprecedented human growth, fresh water will become increasingly scarce and is expected to be the primary constraint for increased food production. The use of treated wastewater for crop irrigation has been suggested as one of the possible ways out of a looming water crisis [3].

Indian sugar mills generate $0.16 - 0.76 \text{ m}^3$ of waste water for every tonne of cane crushed by them. The pollution standards stipulate that the BOD of waste water should be less than 30 mg/L for disposal into inland surface waters and less than 100 mg/L for disposal on land whereas, the combined sugar mill waste water had a BOD of 1,000 to1,500 mg/L [4]. Sugar industry effluent is usually an acidic compost (pH: 3.5–5), dark brown slurry, with a high organic content and an unpleasant odour to humans. Despite the wealth that this industry generates, there are problems from the planting to the harvest of sugarcane. Sugar mill effluents are having a very bad odour and lot of

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organic pollutants. The industries have not yet developed any concern about the treatment of the effluent till today. The elemental pollutants present in the sugar mill effluents are phosphates, nitrogen in the form of nitrates, various volatile solids, high TDS and suspended solids and various organic pollutants with High COD level. These may cause toxicity in soil if spoil and also due to a high COD, cause harmful effect on environment [5-8].

Fish is an important aquatic animal and is particularly sensitive to wide variety of toxicants. It is used as bioindicator of metal pollution in particular and industrial pollution in general [9]. *Pterygoplichthys* spp. is also highly tolerant to poor water quality and is commonly found in polluted waters [10-12]. The present study investigates the possibilities of employing the tank cleaner, *P. pardalis* as an agent in the biotreatment of sugarcane industry effluent by analysing changes in certain physico-chemical parameters.

2. Material and methods

Effluent samples were collected from local sugarcane industry at Alanganallur near Madurai, Tamil Nadu, India and stored in a refrigerator for further analysis. Physico-chemical parameters such as total solids, total dissolved solids, total suspended solids, pH, BOD, chlorides, total phosphates, nitrates, total hardness, total alkalinity, ammonia and sulphates were analyzed based on Standard methods for the examination of water and wastewater [13]. The fish were brought from Aquagardens fish farm, Madurai, Tamil Nadu, India. Their mean weight was 14.21 ± 1.08 g. Then they were acclimatized to laboratory conditions for a period of one week. Later they were exposed to different concentrations of sugar industry effluent to determine the tolerance level and finally the working concentrations were chosen as 10, 20, 30 and 40 %. Students' 't' test was applied to analyze the level of significance for the difference between control and experimental sets for all the parameters tested.

2.1. Total dissolved solids

A dry, clean evaporating dish was taken, and weighed. 100 ml of sample was filtered through filter paper and taken in an evaporating dish. The sample was evaporated on a hot water bath. When whole water was evaporated, the weight of evaporating dish was noted after cooling it in a desiccator and the following formula was used for calculation.

$$TDS (g/L) = (A - B / V) \times 1000$$

Where, TDS= total dissolved solids; A= final weight of evaporating dish; B= initial weight of evaporating dish; V= volume of sample taken.

2.2. Total solids

To compute total solids, same procedure was followed as above, except that here the sample was not filtered.

2.3. Total suspended solids

Total suspended solids were calculated as below:

Total suspended solids = Total solids - Total dissolved solids

2.4. Total alkalinity

50 ml of sample was taken in an Erlenmeyer flask and 2-3 drops of phenolphthalein indicator were added. The solution was titrated against sulphuric acid until the solution becomes colourless. 2-3 drops of methyl orange indicator were added in the same flask and titrated against sulphuric acid until yellow colour of solution turns into orange. The reading was noted as t and the following formula was used for calculation.

Where, S= volume of sample; and t= total volume of titrant used for the two titrations.

2.5. Total hardness

50 ml of sample was taken in a conical flask and 1ml of ammonia buffer solution and 4-5 drops of Eriochrome black-T indicator were added and titrated against EDTA solution until the wine red colour of solution turns blue. The total hardness was calculated by the following formula.

Total hardness (mg/L) = T x 1000 / V

Where, T = volume of titrant; and V = volume of sample.

2.6. Chlorides

10 ml of sample was taken in a flask and 5-6 drops of potassium chromate indicator were added. The colour of the sample became yellow and it was titrated against silver nitrate solution until a persistent brick red colour appeared. The chlorides were then calculated by the given formula.

Chlorides (mg/L) =V x N x 35.457 x 1000 / S

Where, V=volume of titrant; N= normality of titrant (0.02N); and S= volume of sample.

2.7. Sulphates

The sample was filtered through filter paper and 50 ml of filtrate was taken in a conical flask. To this 10 ml of NaCl-HCl solution and 10 ml of glycerol–ethanol solution were added. The sample was stirred in a magnetic stirrer for about an hour. Then the absorbance was measured at 420 nm using spectrophotometer. The sulphate content of the sample was deduced in mg/l by comparing the absorbance of sample with standard curve.

2.8. Ammonia

20 ml of sample was taken in a 25 ml conical flask. To this 2ml of phenol-nitroprusside solution and 2 ml of alkaline hypochlorite solution were added. It was made up to 25 ml by adding ammonia-free distilled water and kept in a dark place at 25 °C for about one hour. The absorbance was recorded in a spectrophotometer at 635 nm by using distilled water as blank. The concentration of ammonium ions in sample was deduced in mg NH4 -N/L by comparing the absorbance of sample with the standard curve.

2.9. Nitrates

25 ml of sample was taken in a glass beaker and evaporated to dryness on a hot water bath. 0.5 ml of phenol disulphonic acid was added to the residue and dissolved with the help of a glass spatula. 5 ml of distilled water and 1.5 ml of potassium hydroxide solution were added. It was subjected to thorough mixing. The supernatant with yellow colour was taken and its absorbance was read on a spectrophotometer at 410 nm by using distilled water as blank. The standard nitrate solutions were processed in a similar manner and the absorbance was noted for each. The level of nitrates in the sample was deduced by comparing the absorbance of sample with the standard curve and the result was expressed in mg NO_3/L .

2.10. Total phosphorus

25 ml of sample was taken in a conical flask and evaporated to dryness. It was cooled and the residue was dissolved in one ml of perchloric acid. The flask was heated gently so that the contents become colourless. It was cooled and 10ml of distilled water and two drops of phenolphthalein indicator were added to the contents. It was titrated against sodium hydroxide solution until the appearance of slight pink colour and made up to the volume of 25 ml by adding distilled water. One ml of ammonium molybdate solution and three drops of stannous chloride solution were added. A blue colour appeared and the absorbance was recorded in a spectrophotometer at 690 nm. The total phosphorus content of the sample was determined by comparing its absorbance with standard curve and the total phosphorus was expressed in mg/L.

2.11. Biochemical oxygen demand

Dilution water was prepared by aerating the BOD-free distilled water in a glass container for about half an hour. To one litre of this water, one ml each of phosphate buffer solution, magnesium sulphate solution, calcium chloride solution, and ferric chloride solution were added. The pH of the sample was adjusted to neutral (7.0) using 1N sulphuric acid or 1 N sodium hydroxide solution. To ensure that not all the oxygen of sample is exhausted during incubation, the sample was diluted with the dilution factor of 100. Two sets of BOD bottles were filled with this sample and one ml of allyl thiourea solution was added to each bottle. The dissolved oxygen was estimated in one set immediately following Winkler's method of oxygen estimation. The other set of BOD bottles were incubated at 20 °C for five days in a BOD incubator. The bottles were taken after five days and their dissolved oxygen content was determined.

 $BOD_5 20 \degree C (mg/L) = (D_0 - D_5) x dilution factor$

Where, D_0 = initial dissolved oxygen in the sample; and D_5 = dissolved oxygen left out in the sample after five days of incubation.

3. Results

Table 1 divulges the physico-chemical characteristics of the raw sugar industry effluent collected from Alanganallur near Madurai, Tamil Nadu, India. Samples were used for the analysis of twelve parameters such as pH, total solids, total dissolved solids, total suspended solids, total alkalinity, chlorides, total hardness, nitrates, sulphates, total phosphorus, ammonia and BOD.

Sr. No.	Parameters (unit)	Values
1.	рН	6
2	Total solids (g/l)	27.5
3	Total dissolved solids (g/l)	3.7
4	Total suspended solids (g/l)	23.8
5	Total alkalinity (mg/l)	1100
6	Chlorides (mg/l)	248.19
7	Total hardness (mg/l)	1148
8	Nitrates (mg/l)	0.59
9	Sulphates (mg/l)	20.4
10	Total phosphorus (mg/l)	290
11	Ammonia (mg/l)	0.11
12	BOD ₅ 20 °C (mg/l)	1060

Table 1 Physico-chemical characteristics of sugar industry effluent

Figure 1 describes the changes in the levels of pH in sugar industry effluent after treatment with *P. pardalis*. The initial pH was maintained at 6 throughout the treatment period. But after first and second day of treatment, pH was found to be 7. Figure 2 shows changes in the levels of total solids in sugar industry effluent after treatment with *P. pardalis*. In the experimental tank, the level of total solids has been gradually reduced from 12.4 to 0.9 g/l during treatment period. Based on the results the lowest level of total solids of 0.9 g/l was recorded at 10 % concentration of sugar industry effluent after five days of treatment. In control, the level of total solids has also been gradually reduced from 13 to 1.8 g/l.



Figure 1 Changes in the levels of pH in Sugar industry effluent after treatment with P. pardalis



Figure 2 Changes in the levels of total solids (g/l) in sugar industry effluent after treatment with P. pardalis

In the experimental tank, the level of total dissolved solids in sugar industry effluent has been decreased gradually from 1.2 to 0.1 g/l after *P. pardalis* treatment (Fig.3). The minimum level of total dissolved solids of 0.1 g/l at 10 % concentration of sugar industry effluent after five days of treatment was observed. In control, total dissolved solids level in sugar industry effluent has been also gradually decreased from 1.4 to 0.2 g/l. Sugar industry effluent treatment with *P. pardalis* greatly reduced total suspended solids level from 11.6 to 0.8 g/l in the experimental tanks (Fig.4). Lowest level of total suspended solids of 0.8 g/l at 10 % concentration of sugar industry effluent after five days of treatment was observed. In control, total suspended solids level in sugar industry effluent after five days of treatment was observed. In control, total suspended solids level in sugar industry effluent after five days of treatment was observed. In control, total suspended solids level in sugar industry effluent after five days of treatment was observed. In control, total suspended solids level in sugar industry effluent has been also gradually decreased from 11.6 to 1.1 g /l. The minimum total suspended solids of 1.1 g/l at 40 % concentration of sugar industry effluent, after the five days was noticed in the control tank.



Figure 3 Changes in the levels of total dissolved solids (g/l) in sugar industry effluent after treatment with P. pardalis



Figure 4 Changes in the levels of total suspended solids (g/l) in sugar industry effluent after treatment with P. pardalis

Figure 5 exhibits the changes in the levels of total alkalinity in sugar industry effluent after treatment with *P. pardalis*. The level of total alkalinity has been gradually reduced from 830 to 462 mg/l during treatment period. Based on the results, lowest level of total alkalinity of 462 mg/l at 10 % concentration of sugar industry effluent after five days of treatment was recorded. In control, the level of total alkalinity has been also gradually reduced from 910 to 494 mg/l during treatment period. Lowest level of total alkalinity of 494 mg/l at 10% concentration of sugar industry effluent after five days was recorded in the control. Sugar industry effluent treatment with *P. pardalis* greatly reduced the level of chlorides from 205.65 to 92.18 mg/l (Fig.6). Lowest level of chlorides of 92.18 mg/l at 40 % concentration of sugar industry effluent after five days of treatment was observed in experiment. In control, the level of chlorides has been also gradually reduced from 234.01 to 163.11 mg/l. The lowest level of chlorides of 163.11 mg/l at 30 % and 40 % concentration of sugar industry effluent after five days was noticed in the control.



Figure 5 Changes in the levels of total alkalinity (mg/l) in sugar industry effluent after treatment with P. pardalis



Figure 6 Changes in the levels of chlorides (mg/l) in sugar industry effluent after treatment with P. pardalis

Figure 7 shows changes in the levels of total hardness in sugar industry effluent after treatment with *P. pardalis*. Total hardness seemed to be fluctuating but minimal level was observed on the second day at 20 and 30% concentration of sugar industry effluent. Sugar industry effluent treatment with *P. pardalis* greatly reduced the level of total harness from 698 to 400 mg/l. The lowest level of total hardness of 400 mg/l at 10 % concentration of effluent after five days of treatment was observed in the experimental tank. In control, the level of total hardness has been also fluctuating on the second day at 20 and 30 % concentration of sugar industry effluent. In control, the level of total hardness has been also fluctuating on the second day at 20 and 30 % concentration of sugar industry effluent. In control, the level of total hardness has been also reduced from 930 to 410 mg/l. The lowest level of total hardness of 410 mg/l at 10 % concentration of sugar industry effluent after five days was noted in the control. The level of nitrates was highly reducing which is shown in Fig.8. In the experimental tank, the level of nitrates has been reduced from 0.33 to 0.06 mg/l during treatment period using *P. pardalis*. The lowest level of nitrates has been also decreased from 0.41 to 0.11 mg/l. The lowest level of nitrates of 0.11 mg/l at 10 % concentration of sugar industry effluent after five days was noted in the control.



Figure 7 Changes in the levels of total hardness (mg/l) in sugar industry effluent after treatment with P. pardalis



Figure 8 Changes in the levels of nitrates (mg/l) in sugar industry effluent after treatment with P. pardalis

Figure 9 exhibits the changes in the levels of sulphates in sugar industry effluent after treatment with *P. pardalis*. The level of sulphates has been reduced from 10.2 to 0.3 mg/l during treatment period. Lowest level of sulphates of 0.3 mg/l at 40 % concentration of sugar industry effluent was observed after five days of treatment period in the experimental tank. In control, the level of sulphates has been also reduced from 13.6 to 2 mg/l. The lowest level of sulphates of 2 mg/l at 10 % concentration of sugar industry effluent after five days was noticed in the control. The level of total phosphorus is highly reduced by using *P. pardalis* which is shown in Fig.10. Maximum level was observed in 40 % concentration at first day. In this study, the lowest level of total phosphorus of 20 mg/l was found at 10, 20 and 30 % concentration on first day. In this study the lowest level of total phosphorus as 30 mg/l was found at 20 % concentration of sugar industry effluent after five days of treatment.



Figure 9 Changes in the levels of sulphates (mg/l) in sugar industry effluent after treatment with P. pardalis



Figure 10 Changes in the levels of total phosphorus (mg/l) in sugar industry effluent after treatment with P. pardalis

Figure 11 exhibits the changes in the levels of ammonia in sugar industry effluent after treatment with *P. pardalis*. In the experimental tank, the level of ammonia highly increased from 0.24 to 0.36 mg/l during treatment period. Highst level of ammonia of 0.36 mg/l was found at 40 % concentration of sugar industry effluent after five days of treatment. The level of ammonia was reduced from 0.16 to 0.01 mg/l in the control and the lowest level of ammonia was 0.01 mg/l. Changes in the levels of BOD in sugar industry effluent with *P. pardalis* are shown in Fig.12. The level of BOD has been reduced gradually from the first day to fifth day of treatment period. The level of BOD has been reduced from 180 to 10 mg/l during treatment period in the experimental tank. In control, the level of BOD was also reduced from 280 to 90 mg/l.



Figure 11 Changes in the levels of ammonia (mg/l) in sugar industry effluent after treatment with P. pardalis



Figure 12 Changes in the levels of BOD₅ 20 °C (mg/l) in sugar industry effluent after treatment with P. pardalis

Table 2 indicates the Students "t" test applied to analyse the level of significance for the difference between control and experimental sets for all the parameters tested. Ammonia, pH and chlorides exhibited more significance than the other parameters.

		Level of significance (P < 0.5) Effluent concentration (%)				
Sr. No	Parameters					
		10	20	30	40	
1.	pH	S	S	S	S	
2.	Total solids	NS	NS	NS	S	
3.	Total dissolved solids	NS	NS	NS	NS	
4.	Total suspended solids	NS	NS	NS	S	
5.	Total alkalinity	NS	NS	NS	NS	
6.	Chlorides	NS	S	S	S	
7.	Total hardness	S	S	NS	NS	
8.	Nitrates	S	NS	NS	NS	
9.	Sulphates	S	NS	NS	NS	
10.	Total phosphorus	NS	NS	NS	NS	
11.	Ammonia	S	S	S	S	
12	BOD₅ 20 °C	NS	S	NS	NS	

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4. Discussion

The physico-chemical properties such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen contents and microbial population were higher in samples collected from sugar industry wastes dumped than in the no dump sites [14]. Sugar mill effluents and soil samples exhibited high metal content than the permissible limits. Plant samples also indicated the maximum accumulation of Fe followed by Mn and Zn in root, shoot, leaves and seeds of wheat and mustard. The sugar mill effluent was acidic and yellowish in colour. It showed high levels of total suspended and dissolved solids with high BOD and COD. Higher amount of chlorides, calcium,

magnesium, sodium, potassium and iron were also reported in the effluent. These effluents severely affected the plants and soil properties when used for irrigation [15].

Waste water is the combination of liquid or water carried wastes originating in the sanitary conveniences of dwellings, commercial or industrial facilities and institutions, in addition to any ground water, surface water and storm water that may be present [16]. Untreated waste water generally contains high levels of organic compounds, countless pathogens, as well as nutrients and toxic compounds. It thus entails environmental and health hazards and, must immediately be disposed away from its generation sources and treated appropriately before final disposal [17]. The ultimate goal of wastewater management is the protection of environment in a manner agreeable with public health and socio-economic concern. Wastewater treatment is gaining importance due to depleting water resources, and increasing disposal problems [18].

The study of physico-chemical characters could help in understanding the structure and function of particular water body [19]. The results obtained from four different fisheries from British Columbia showed pH in the range of 5.7–7.4 with an average pH of 6.48. During the production of fish meal, fish condensate is produced where pH ranged from 9 to 10. Ammonia excretion and protein degradation are influenced by pH. In this study the pH range 6 - 7 was maintained in sugar industry effluent. The highest level of total solids was recorded in sugar industry effluent during initial day of treatment with *Pterygoplichthys pardalis* and the level of total solids was reduced later. The level of total solids in effluents represents the colloidal form and dissolved species. The probable reason for the fluctuation of values in total solids and dissolved solids was due to content collision of these colloidal particles [20-23].

Total dissolved solids in sugar industry effluent have been gradually decreased from 1.2 to 0.1 g/l during the treatment with *P. pardalis*. In control, TDS in sugar industry effluent has also been decreased, because microbes have also been involved in biotreatment. The high level of TDS may impart taste or odour. Dissolved solids in wastewater destined for agriculture purposes might be as high as 2000 mg/l, as but not more than this limit. TDS, potassium, sulphates, variable phosphorus content and biodegradable organic pollutants are generated in high concentrations [24-28].

Suspended solids may reduce light penetration and thus affect aquatic life. In British Columbia, the effluent total solids concentrations were generally high (2000–3000 mg/L). Total suspended solids account for approximately 10–30 % of total solids. In general, fish processing wastewater contains high levels of suspended solids which are mainly proteins and lipids [29]. Total suspended solids do not mean that they are floating matters and remain on top of water layer. They deplete oxygen level in water. The highest value of total suspended solids was recorded in sugar industry effluent during initial day and after treatment with *P. pardalis*. The level of total suspended solids was reduced in both control and experiment. But in the experiment the reduction was higher than that of control [30].

Excess quantity of nitrogen and phosphorus may cause proliferation of algae and affect aquatic life in water bodies. For biological treatment, a ratio of N: P of 5:1 is recommended for proper growth of the biomass. The high nitrogen levels are likely due to the high protein content (15–20 % of wet weight) of fish and marine invertebrates [31]. In the present study, total phosphorus and nitrates were reduced more in the experiment than that of control.

As reported by a few fish processing plants, the overall ammonia concentration ranged from 0.7 to 69.7 mg/L. Environment Canada, Atlantic Region reported an effluent ammonia concentration of 42 mg/L for salmon processing and 20 mg/L for ground fish processing. Phosphorus also partly originates from the fish, but can also be introduced with processing and cleaning agents [32]. The levels of ammonia in sugar industry effluent after treatment with *P. pardalis* increased more in the experiment than that of control.

BOD of effluents must be less than 30 mg/L for disposal into inland surface waters and less than 100 mg/L for disposal on land. But the combined sugar mill effluent had a BOD of 1,000 to 1,500 mg/L [33]. Nath and Sharma [34] observed that the sugar mill effluent is toxic to plants when used for irrigation without treatment. Increased organic matter results in excess oxidation of organic matter to carbon dioxide and the water creates an atmosphere of oxygen depletion and results in high BOD level. The highest value of BOD was recorded in sugar industry effluent during initial day after treatment with *P. pardalis* and the level of BOD was reduced later for both control and experiment. But the degree of BOD reduction was more in the experimental tank. The level of total alkalinity has been gradually reduced from 830 to 462 mg/l during treatment period. Based on the results lowest level of total alkalinity of 462 mg/l at 10 % concentration of sugar industry effluent after five days of treatment was recorded in experimental tank. According to the permissible level suggested by Bureau of Indian standards (BIS) almost all the water quality parameters in the sugar industry effluents have been found to be very high and well above the permissible limits [35].

Sugar industry effluent treatment with *P. pardalis* greatly reduced the level of chlorides from 205.65 to 92.18 mg/l. The level of total hardness in sugar industry effluent was reduced after treatment with *P. pardalis*. Total hardness seemed to be fluctuating but minimal level was observed on the second day of 20 and 30% concentration of sugar industry effluent due to hardness of the wood chips, they require longer contact times. Total hardness (342 mg/l) according to the permissible level suggested by Bureau of Indian standards (BIS) in the sugar effluents have been found to be very high and well above the permissible limits. The changes in the levels of sulphates in sugar industry effluent after treatment with *P. pardalis* were observed. Sulphates (540 mg/L), according to the permissible level suggested by Bureau of Indian standards (BIS) in the sugar effluent advect be permissible level suggested by Bureau of Indian standards (BIS) in the permissible level suggested by Bureau of Indian standards (BIS) in the sugar effluents have been found to be very high and well above the permissible limits have been found to be very high and well above the permissible limits have been found to be very high and well above the permissible limits have been found to be very high and well above the permissible limits have been found to be very high and well above the permissible limits have been found to be very high and well above the permissible limits [36].

5. Conclusion

The analysis of physico-chemical properties in the treated sugar industry effluent showed that *P. pardalis* has the capacity to remediate sugar industry effluent. Therefore it can be employed as an agent of biotreatment of sugar industry effluent. We also urge for further studies to determine exact mechanism of biotreatment, isolation and identification of microbes from *P. pardalis* to improve the efficiency of waste water treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors namely Karthika D, Surendran Appasamy and Joseph Thatheyus Antony state that the article has not been published in another publication and is not being submitted simultaneously to another journal.

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