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Simulated Sugar Factory Wastewater Lagooning with Pseudomonas Putida

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Abstract: The generation of organic wastewater such as from sugar factories is a source of water pollution particularly in developing countries like Pakistan, where sugar industry is the second largest after tanneries and a major source of environmental nuisance. Simulated sugar factor wastewater (SFW) was investigated for its degradation using aerobic bacteria such as Pseudomonas putida (PP) in terms of different parameters like pH, DO, temperature and COD. The mass inoculation of allochthonous or external PP was performed in a lagoon type rectangular tank. The synthetic version of real sugar mills wastewater (RSMW) was made via mixing the appropriate contents of components found in RSMW such as organic loading (COD), nitrogen and phosphorous. The SWF biodegradation revealed that the pH decreased by 1.9 per day, whereas DO reduction of 6 mg/l/d occurred as a result of its uptake by the bacteria. COD profile in the tank transpired that it was decreasing for the first 20 hr followed by a slight increase in it when DO was a limiting factor in the medium.

Keywords: Lagooning, sugar factory, wastewater, Pseudomonas Putida, Cell cultivation

1. INTRODUCTION

Sugar industry, a potential source of organic pollution caused by its wastewater discharges, is the second largest industry in Pakistan where 400 million gallons of industrial wastewater is daily discharged untreated into the sea and around 1% or lesser of generated wastewater is treated before its disposal (Rehman, 2011). Approximately, 15 m^3 of water is consumed per metric ton of sugarcane (or 15 l water per kg cane) processed (Zver and Glavic, 2005). Sugar factories generate wastewater of different characteristics from multiple sources mostly of organic origin that are quite homogenous in their characteristics (Rittmann, 2008). The soluble, biodegradable organic matter in the wastewater is composed of carbohydrates, which can be either readily degradable starches and sugars or cellulose. The microorganisms easily metabolize the starches and sugars, while the cellulose compounds are degraded at a much slower rate due to the issues such as complex structure and larger size. The overall objective of biological remediation of wastewater is to transform or oxidize dissolved and particulate biodegradable constituents into acceptable and preferably useful products by removing or reducing the concentration of organic and inorganic compounds present in wastewater. Microorganisms such as algae and bacteria are used to oxidize or convert the dissolved carbonaceous organic matter into simple products with additional biomass. When organic matter is removed or taken up from water by aerobic microorganisms, two basic phenomena occur: oxygen is consumed by the organisms for energy, and new cell mass is synthesized. The simulated sugar factory wastewater (SFW) is a characteristic replica of organic wastewater generated by sugar factories. SFW was selected as a case study because sugar factories produce more waste including solid (mud and ash) and liquid (wastewater) than the main product (sugar), by-products (pulp or bagasse and molasses) combined (Asadi, 2007). Pseudomonas putida (PP) is an aerobic bacteria from pseudomonad family, which is well known for its tendency to takeup sugar and other organics for its growth (Memon *et al*., 2014).

The objective of this research was to investigate the biodegradation potential of PP in an organic- rich medium like sugar factory wastewater. This was achieved via the determination of operational variables such as pH, DO, temperature and COD.

2. EXPERIMENTAL

2.1 Simulated sugar factory wastewater

Based on the analyzed contents of real sugar factory wastewater, SFW was prepared accordingly. Fine size sugar crystals with mean aperture size of about 200-300 µm were used in the preparation of SFW. **(Table 1)** shows the chemicals representing the respective contaminants in SFW together with the contaminant concentration present in the sugar factory wastewater, along with the relative amounts of chemicals added for the preparation of SFW (Guven *et al*., 2009).

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Table 1: Simulated sugar factory wastewater compositional values

Surrogate substance or chemical used	Concentration in Sugar factory wastewater, mg l^{-1}	Weight in g for 13 l SSFW
Sugar for COD (100%)	13,000-15,000	13 x 13 = 169
Ammonium bicarbonate for nitrogen (5%)	43-49	0.65 x 13 = 8.5
Potassium dihydrogen phosphate for phosphorous (1%)	7-10	0.13 x 13 = 1.7
Calcium hydroxide for Calcium (4%)	14-19	0.52 x 13 = 6.76

2.2 Pseudomonas Putida

Obligate aerobe *Pseudomonas Putida* (*PP*) is a harmless and best-characterized saprophytic that has retained its ability to survive and function in the environment. For biodegradation of SSFW organics, *PP* strain KT2440 (ATCC No. 47054) was selected based on its organic degradation ability as well as being a good biofilm former (Perni *et al*., 2006). The average size of *PP* ranges between 1.0 and 4.0 µm and they may be of straight, curved or rod shape. *PP* is a member of *Pseudomonad* family, which consists of 178 or more different types of strains (Bakker *et al*., 2004). *PP* is gram-negative, non-spore forming, typically motile with its polar flagella and non-pathogenic due to the absence of virulence factors in its system like certain exotoxin genes and type III secretion systems, which are found in other members of *Pseudomonad* family such as *P. Aeruginosa* containing these genes (Nelson *et al*., 2002). *PP* strain was purchased from LGC standards Middlesex, UK, which was received as a lyophilized cell line in a tube of around 5 ml. The lyophilized *PP* cell-line after its delivery was immediately put in a freezer for cryogenic preservation at around -80^0C until its use later. The culture vial was put into refrigerator at around 4^0C for overnight in order to start the revitalization process 24 hr prior to its culturing. Before the strain was transferred into culturing bottle, the refrigerated cell line was thawed at 30^0C for 4 hr using hotplate. Luria Bertani broth medium, containing Tryptone (10 g l^{-1}), Sodium chloride (5 g l^{-1}) and Yeast Extract (5 g l^{-1}), was used for *PP* culturing.

2.3 Lagooning tank

Lagooning of SFW was carried out in a rectangular lagooning tank (LT) with flat bottom. The LT is 120 cm in length to ensure maximum dilution and saturation of inoculum in the tank. The tank is partitioned from inside across the middle to induce wall turbulence for homogeneity of the tank contents. The LT is spaced into six sampling points including three front (1, 2, and 3) and as many back points. The recirculation flow rate was set at 40 ml min^{-1}.

3. RESULTS AND DISCUSSION

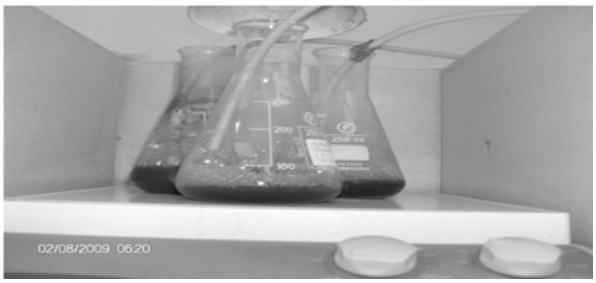
3.1 Pre-culturing of *Pseudomonas Putida*

Pseudomonas Putida (*P. Putida*) strain was cultured for 48 hr at 37^0C in the bacteria culturing unit, which was setup in the lab. Visual observation of the cell growth in culture flasks was done periodically after every 8 hr and suggested that there was no growth visible after the lapse of 24 hr. This was also checked by determining the optical density (OD) test of the cultures using UV-Vis Spectrophotometer. **Table 2** presents the data related with the OD determination of the culturing flasks. The data in the Table show that until 48 hr of sub-culturing, the cultures had not grown much so as to give optimum OD value of above 0.7 (70% growth), which was obtained after 56 hr of subculturing regime.

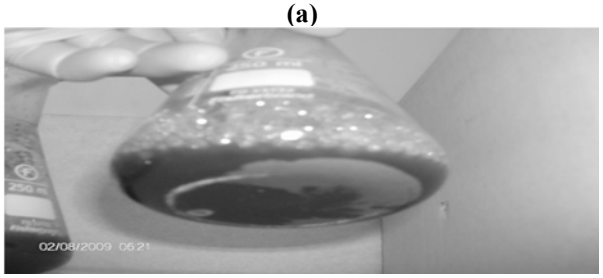
Table 2: Optical density values during *PP* culturing at different times

Culturing time after, hr	Optical density at 660 nm		
	Flask 1	Flask 2	Flask 3 (blank)
0	0.05	0.04	0.01
24	0.16	0.12	0.01
30	0.31	0.26	0.02
48	0.56	0.49	0.02
56	0.76	0.71	0.03

Fig 1 shows two images taken during *PP* sub-culturing with frame (a) showing the formation of a colony inside the culturing flask. A close look at the colony suggested that it was a large homogenous cluster of the cells with no other colony existing in the flask, hence indicating purity of the culture growth, since a single colony in the bacterial cultures permit to visualize purity of the culture and the presence of more than one colony is indicative of a contaminated culture (Madigan *et al*., 2009).



(a)



(b)

Fig 1: *P. Putida* sub-culturing showing in: (a) bacteria sub culturingunderway on a hot plate at controlled temperature of 37^0C, and (b) growth of *P. Putida* colony.

3.2 Simulated sugar factory wastewater lagooning with *P Putida*

13 l of prepared SFW was poured into LT and analyzed for initial values of pH, DO, temperature and COD. 10 ml of centrifuged cultured *PP* cells (0.76 ml/l SW) were inoculated progressively into LT via sterilized pipette with 5 ml each at points 1 and 2.

3.2.1 pH analysis

Fig 2 shows the pH data at the surface at the middle points 2 and 5 and side points 1 and 6 in LT during 24 hr of lagooning with *PP*. The pH values at the bottom being identical to the surface data are not presented separately. The graph shows 24 hr pH profile after the inoculation of the bacterial culture into SFW indicating the impact of bacterial culture on SFW pH in a 24-hr cycle. These results suggest that there was no reduction in the pH for the first 4 hr of lagooning. However, for the next 16 hr the pH was reduced by 10%, with maximum reduction of 53% taking place in the final 4 hr of lagooning. Overall, pH decreased by 26% in 24 hr after *PP* inoculation with a decreasing rate of 1.9 d^{-1}. Moreover, the decreasing pattern at all the points, as depicted by the curves, is almost symmetrical implying towards the unanimous growth of the cells followed by the spread of *PP* all over the tank under the influence of medium recirculation.

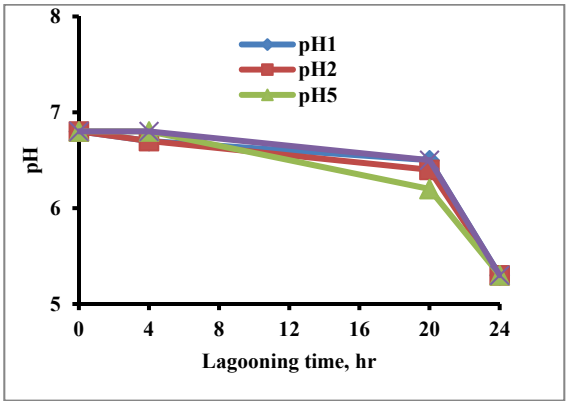


Fig 2: pH at the surface of LT at points 1, 2, 5 and 6 during 24 hr of SFW lagooning with 10 ml *PP* (mean, n=3).

3.2.2 DO and Temperature

The DO results at points 1, 2, 5 and 6 in LT are presented in **Figure 3** along with the temperature data. Like the pH results, the impact of inoculated *PP* culture was apparent on the DO results in only 24 hr of lagooning. The data in the graph suggested that DO decreased by 35% in the first 20 hr likely due to the onset of bacterial activity consuming SFW organics from the medium. In addition, it also indicated that the rapid degradation of SFW organics by the allochthonous *PP* culture started after 20 hr, which may be deemed as the beginning of log phase growth time for the *PP* cells. The overall kinetics of this organic biodegradation suggested that SFW DO decreased by 88% at 6 mg l^{-1} in 24 hr (0.25 mg l^{-1} hr^{-1}). The average temperature in LT was observed to be higher by 1^0C after 24 hr than it was at the start.

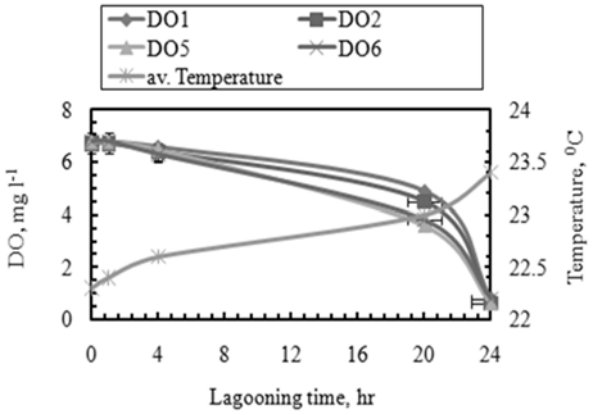


Fig 3: DO and average temperature profile at the surface at points 1, 2, 5 and 6 during 24 hr of SFW lagooning with 10 ml PP (mean, n=3).

3.2.3 COD analysis

Fig 4 shows COD data obtained during SSFW lagooning with *PP* for 24 hr, with reference to observed DO values. The graph highlights the influence of bacterial culture on the degradation of SFW organics in terms of variation in the COD values, which is also correlated with the consumption of SFW DO during the process. The study of the curves in the graph suggested that until the DO became limited factor after 20 hr of SFW lagooning with *PP* cells COD was observed as being stabilized for the first 20 hr with 14% COD reduction occurring during that time. However, during the last 4 hr with SFW medium with depleted DO, COD was increased by 9% implying towards non-consumption of the substrate by *PP* likely due to hypoxic conditions prevailing in the medium. In the event of non-availability of DO in the medium, it was likely that the bacterial cells released soluble fraction of synthesis products in the medium, thus increasing the COD level resulting in a halt in COD reduction.

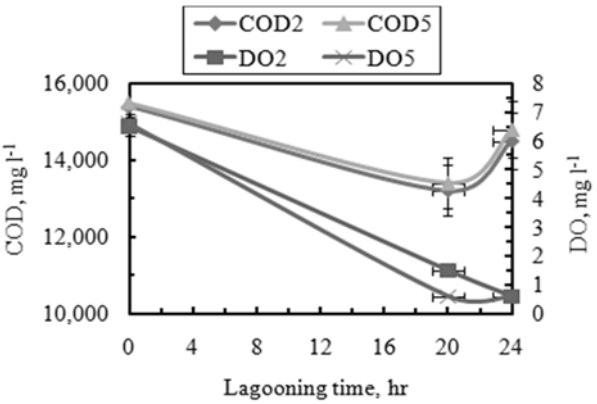


Fig 4: COD at points 2 and 5 in reference to DO values after 24 hr of SFW lagooning with 10 ml *PP* (mean, n=3).

4. CONCLUSION

This research was done to analyze the influence of *P. Putida* inoculation in simulated sugar factory wastewater (SFW). Allochthonous *P. Putida* activity during SFW lagooning yielded in DO reduction of 6 mg l^{-1} d^{-1} along with COD pattern that was observed to be increasing after 20 hr of lagooning, when DO was a limiting factor in the medium. This also meant that for efficient biodegradation of the organics, a continuous source of oxygen supply must be ensured to the bioreactor for process sustainability. COD decomposition by *PP* also suggested that this bacteria is an efficient degrader of the organics present in sugar industry wastewater and thus could be a best option for efficient removal of the pollutants from SFW.

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