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An eco-friendly approach towards treatment of tannery lime liquor using halo tolerant alkaliphile of extreme origin

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ABSTRACT

The management of tannery lime liquor using halo tolerant bacterium of extreme origin (accession number MTCC 5899) is reported in the present study. The isolate is capable to bring simultaneous reduction in pH as well as Total Dissolved Solids (TDS) of highly alkaline lime liquor (~13.6 pH) within a reasonable time period. The strain is found to be growing at a wide range of pH (9.0–13.6) and salt concentration of 0–10%. Factors like amount of inoculum, dilution of liquor, type and concentration of carbon and nitrogen source used and initial TDS levels were observed to have a profound effect on the neutralization and reduction in TDS levels of lime liquor. A reduction in BOD5 and COD by ~62% and 65% respectively was observed when the liquor was added with suitable carbon source. The present study was targeted to exploit the ability of alkaliphilic bacteria to reduce pH in order to provide an economic process for the nullification of highly alkaline lime liquor along with reduction in TDS, eventually making the liquor environmentally safe for discharge. This study paves way to greener route towards the treatment of tannery lime liquor, for the first time using halo alkaliphilic *Micrococcus* sp.

1. Introduction

The discharge of wastewaters, containing hazardous compounds into natural bodies adds substantially to the accumulation of recalcitrant pollutants (Kumar et al., 2005). The leather industry has been classified as one of the highly polluting industries and there is concern that leather making activity can have adverse impact on the environment (Kanagaraj et al., 2006). Hence, despite of the industry making traceable and visible impacts on socio-economics through employment generation and export earnings, the industry has gained negative image in the society owing to the resulting pollution (Thanikaivelan et al., 2004).

The leather making process can be split into four main categories: i) storage and beam house operations ii) tan yard operations iii) post-tanning operations and iv) finishing operations (Durai and Rajasimman, 2011). The use of sodium chloride during raw material preservation is a major contributor of TDS in tannery wastewater, however, other processing units viz., soaking, liming-deliming, bating and degreasing also contribute to the TDS load as each unit discharges effluent containing inorganic components like sulphates, sulphides, carbonates, bicarbonates and calcium (Kumar et al., 2005). The TDS of soak liquor varies from 33,000 to 40,500 ppm while that of lime liquor varies from 38,500 to 45,500 ppm (Pachpande and Ingle, 2004). The use of high quantities of lime and sodium sulphide creates an extremely alkaline environment resulting in the pulping of hair and its subsequent removal. The disadvantages include contribution of 70–80% of total Chemical Oxygen Demand (COD) of effluent, sulphide being highly toxic with obnoxious odor when left untreated causes major problems in the sewer and poses health hazards due to severe alkaline conditions (Sanghi and Singh, 2012). Among the problems in complying with standards, high TDS levels in the tannery effluent is yet to be resolved. While the treated tannery effluent has TDS value of about 7000–10,000 ppm, the Indian standard for the same is prescribed to be 2100 ppm (Sivakumar et al., 2005). Although there are well established Common Effluent Treatment Plant (CETP's) for the management of effluents from various tanning operations, the lime liquor needs special attention since the available chemical treatments are not very effective and add

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substantially to the pollution load. Systematic studies on the world wide treatment technologies and advances for pollution prevention from tannery chemicals and wastewater have been undertaken (Lofrano et al., 2013; Kowalik -Klimczak and Gierycz, 2014). Several methods such as membrane separation (Alves and de Pinho, 2000; Viero et al., 2002), reverse-osmosis (Cassano et al., 2001), electrodialysis (Raghava Rao et al., 1989) etc., have been employed for the treatment of tannery effluent. However, these methods pose problems like rapid scaling and befouling of the membrane resulting in reduced flux rate and performance, frequent membrane cleaning and replacement may therefore be necessary, which would also enhance the maintenance and operating cost of the technology (Ranganathan and Kabadgi, 2011; Shreesadh et al., 2013; Yusif et al., 2016). Therefore, the most sustainable solution of TDS reduction is to adopt process innovations (Sivakumar et al., 2005).

Total Dissolved Solids provide information on the amount of both organic and inorganic compounds which may in many cases remain persistent and result in a cumulative toxic effect (Genschow et al., 1996). The variations in the amounts of Dissolved Solids can be detrimental since the density of TDS determines the flow of water in and out of an aquatic organism's cell. High concentration of TDS may reduce the water clarity leading to a decrease in photosynthesis and when added with toxic compounds/heavy metals, leads to increase in temperature. This can often be harmful to many aquatic forms (Kumar et al., 2005).

In a recent study, liming and pickling streams were mixed at different ratios with a view to develop a simple and economically viable method for tannery effluent treatment. This resulted in effluent with pH very close to neutral and caused a remarkable reduction in COD (88%), BOD (94%) and TS (97%) when the lime: pickle ratio was kept at 1: 4 (Mottalib et al., 2014).

One of the major obstacles in treating the lime liquor through biological means is its high alkaline pH (12.7–13.4) (Pachpande and Ingle, 2004) as terrestrial microbes cannot grow/survive in such harsh condition. One ideal option to treat such effluents would be the use of alkaliphiles which would not only survive but grow luxuriously in such environment. Certain group of microorganisms demonstrates growth in highly alkaline conditions and in turn reduces the pH of the medium through its biochemical pathways. Reports suggest that more than 3–4 units of reduction in pH were observed by group of halophiles and alkaliphiles. Hence, making use of those species to reduce the pH of the lime liquor by natural means is found to be attractive and once the pH is reduced, the effluent may be treated in CETP along with other sectional streams.

The biological treatment of alkaline effluent generated from paper and pulp (Kumar et al., 2005a), textile (Kumar et al., 2005b; Kumar and Kumar, 2006), beverage (Kumar and Kumar, 2006a; Kulshreshtha et al., 2010) and chlor-alkali industries (Jain et al., 2011), have established the prospective use of alkaliphiles for bioremediation which further needs to be proved for lime liquor of leather industry. The ability of extremophilic bacteria growing at high pH could be exploited for treatment of this type of wastewater.

In the present study, an attempt is made to address two major concerns of the lime liquor viz., exceedingly alkaline pH and elevated levels of TDS. A process was established wherein; the ability of extreme alkaliphilic bacteria to cause changes in pH even in a well buffered, carbohydrate containing medium and requirement of Na^+ for their growth was used to neutralize the liquor. To the best knowledge of the investigators there are no reports on simultaneous reduction of pH and TDS using bacteria with special emphasis on lime liquor. Management of lime liquor through biological means will certainly reduce the pollutant load to CETP in addition to making the process more environmental friendly.

2. Materials and methods

2.1. Sample collection and isolation of alkaliphiles

Lime liquor and sludge were collected from pilot plant of CSIR-CLRI, Chennai, India. Bacteria were isolated immediately after collection of sample on modified Horikoshi medium (Horikoshi, 1999) containing g/L of glucose 10, peptone 5, yeast extract 5, K_2HPO_4 1, $MgSO_4.7H_2O$ 0.2 and agar 20, pH was adjusted to 10.0 using a saturated solution of NaOH. Isolation of bacteria was done by conventional methods of serial dilution (Yapici et al., 2008). For sludge samples nearly 1.0 g of sludge was suspended in 100 mL sterile saline solution, mixed thoroughly and subsequently subjected to serial dilution. 200 μL from each dilution was plated in triplicates on medium described above and incubated at 35 ± 2 °C for 24 h. The isolated bacteria were purified by repeated sub culturing on the same medium and preserved on slants at 4 °C.

2.2. Studies on pH and salt tolerance and neutralization of modified Horikoshi medium

The distinctly different bacteria obtained as a result of screening experiment were tested for their ability to grow in liquid medium with different pH viz., 6.0, 8.0, 10.0, 11.5, and 13.5. A different set of experiment was also conducted to check the salt tolerance ability by supplementing the liquid medium (pH 10) with sodium chloride in the range of 2.0–10%. A control was maintained which did not contain any salt added. After inoculation, the tubes were monitored visually for the growth of bacteria for 24 h.

The cultures screened above were subjected to neutralization of modified Horikoshi broth (now on referred as growth medium) and dispensed in 100 mL of this medium into different flasks and autoclaved at 121 °C for 15 min. After autoclaving, pH was adjusted to 10.0, 12.0 and 13.6 with separately autoclaved saturated solution of NaOH. Inoculum was prepared by adding a loopful of the selected cultures into the growth medium, incubating at 35 ± 2 °C at 150 rpm and allowed to grow for 16–18 h (till it reached an OD of ~2.0, approx.1.8 × 10^4–1.8 × 10^6 cells/mL). This was then added at a ratio of 100:10 (Medium: Inoculum) to the growth mediums with different pH. Reduction in pH was monitored regularly.

2.3. Neutralization of tannery lime liquor

The potential isolate obtained, was exploited for neutralization of lime liquor (pH ~13.6) collected from pilot plant of CSIR-CLRI, Chennai. This liquor was enriched with 1.0% glucose and 0.5% peptone (separately autoclaved) and added to the sterilized lime liquor in aseptic conditions. Inoculum was prepared as mentioned in previous section and added into flasks containing 100 mL of enriched liquors. A control flask with enriched lime liquor but devoid of inoculum was also maintained. These were incubated at 35 ± 2 °C at 150 rpm and reduction in pH and TDS was monitored at regular time intervals. The experiment was repeated three times independently each with three replicates. The pH was monitored with pH meter (Eutech, Thermo Scientific) and TDS determined according to standard method (APHA, 1998).

2.4. Identification of the promising bacterium

Bacterium was identified on the basis of morphological, physiological and biochemical procedures. The identification was further confirmed by 16S rDNA sequence analysis. Cell morphology was examined by light microscopy (Nikon E600). Genomic DNA was isolated from purified culture pellet. Using rDNA sequence specific consensus primer, ~1.5 kb fragment was amplified using high