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Sequential oxic-anoxic bio reactor for the treatment of tannery saline wastewater using halophilic and filamentous bacteria

Maharaja P, Mahesh M, Chitra C, Kalaivani D, Srividya R, Swarnalatha S, Sekaran G*

Environmental Science and Engineering Division, Council of Scientific & Industrial Research (CSIR), Central Leather Research Institute (CLRI), Adyar, Chennai 600 020, India

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ABSTRACT

The present investigation was focused on the removal of dissolved organics and suspended solids from tannery saline wastewater $[TSWW]_{Raw}$, known as soak liquor, by sequential oxic-anoxic bio reactor (SOAR) using halophilic bacteria 3and filamentous bacteria (FB). The high concentration of Sodium Chloride (NaCl) in tannery wastewater retards biodegradation of dissolved organics. The aim of this study was to cultivate halophilic bacteria and facultative bacteria from tannery soil supported sewage culture using soak liquor nutrient, for the degradation of organics in $[TSWW]_{Raw.}$ SOAR was used for the maximum removal of proteins, 47.4%; carbohydrates, 44.8% and lipids, 70.4% from $[TSWW]_{Raw}$ at optimum HRT of 12 h. The Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and suspended solids were removed by 36.8%, 31.2% and 72.6% respectively from $[TSWW]_{Raw}$ containing total dissolved solids (TDS) concentration of 6–7% (w/v) without the addition of any coagulants. The degradation of proteins (albumin and globulin) in $[TSWW]_{Raw}$ was evaluated and confirmed through circular dichroism (CD) studies. Further, the removal of organic compounds was characterized by UV–vis spectroscopy and fluorescence spectroscopy, TGA-DSC and FT-IR studies. The presence of filamentous bacteria was confirmed through SEM analysis.

1. Introduction

Agro-food industries, gelatine producing industries, leather manufacturing industries and petroleum industries discharge huge volume of wastewater with high concentration of Total Dissolved Solids (TDS). Amongst these industries, tanning industry generates huge volume of saline wastewater [1]. The presence of heavy metals, toxic chemicals, chloride, lime, suspended solids and other by-products formed during tanning processes retard the efficiency of biological treatment of tannery wastewater [2]. The tanning process can be classified into four main categories, storage and beam house operations, tanyard (chrome tanning or vegetable tanning) operations, post-tanning operations and finishing operations. The natural wet raw skin collected from slaughter house contains 60–65% moisture content (MC). In order to prevent microbial attack during the transportation sodium chloride is being used as a preservative. Soaking process is being carried out in tannery to remove added salts onto raw skins/hides, dirt and dung. The hyper saline wastewater generated in pickling and soaking of hides/skins contains NaCl by about 80 g/L. The discharge of untreated wastewater containing high Total Dissolved Solids (interms of Sodium chloride/Calcium Chloride) and high organic content into water bodies adversely affect the aquatic life, water potability and agriculture [3–7]. At present, the soak liquor is being collected and evaporated in solar evaporation pans/thermal evaporators to get dry residue. The evaporated residue lacks reusability characteristics due to the presence of organic pollutants. Hence, the residues are being stored under shelter as hazardous solid waste to prevent ground water contamination by leaching. However there is a scope for reusability of the evaporated residue, if these organic pollutants are removed

Abbreviations: COD, Chemical Oxygen Demand; BOD_5, Biochemical Oxygen Demand; TDS, Total Dissolved Solids; TS, Total Solids; TSS, Total Suspended Solids; APHA, American Public Health Association; TOC, Total Organic Carbon; TKN, Total Kjeldahl Nitrogen; TGA, Thermo Gravimetric Analysis; DSC, Differential Scanning Colorimetry; SEM, Scanning Electron Microscopy; FT-IR, Fourier Transform Infra Red Spectroscopy; UV–vis, Ultraviolet Visible Spectroscopy; CD, Circular Dichroism analysis; HRT, Hydraulic Retention Time; ORP, Oxidation- Reduction Potential; SOAR, Sequential oxic-anoxic bio reactor; MP's, Mucopolysaccharides; FB, Filamentous bacteria; $[TSWW]_{Raw}$, Raw tannery saline wastewater; $[TSWW]_{SOAR}$, Tannery saline wastewater after treatment in SOAR.

* Corresponding author at: Environmental Science and Engineering, Central Leather Research Institute, Adyar, Chennai, 600 020, Tamil Nadu, India.
E-mail address: sekaransabari@gmail.com (G. Sekaran).

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from the $[TSWW]_{Raw}$ and thus the burden on disposal of evaporated residue can be avoided [8,9].

Various treatments, such as physico-chemical processes, solar evaporation, mechanical evaporation, thermal evaporation and biological processes were attempted to treat saline effluents. However, the physico-chemical techniques are more energy-consuming, and their capital investment and operation costs are high. Therefore, the saline wastewater is currently treated by biological processes using varied microorganisms. But the activities of microorganisms are generally affected by high salt concentration, consequently COD removal efficiency was retarded and bulking of the activated sludge was evident. Conventional bacterial cultures have poor efficiency to treat saline wastewater containing salt concentration at above 3% owing to plasmolysis of bacterial cells that results in loss of activity. This is considered to be the main challenge for the traditional biological treatment. Hence, identification of the efficient saline-tolerant microorganisms for the wastewater treatment to overcome the above mentioned issues has been the objective of researchers for many years [2,10–15].

Soak liquor contains suspended solids in the range 2000–3500 mg/L contributed by hair, clay, dirt and dung. The treatment of any kind of wastewater involves removal of suspended solids as the primary step preceeding any type of secondary biological treatment of wastewater [2,7]. Conventional technology for the treatment of $[TSWW]_{Raw}$ employs various coagulants and flocculants for the removal of suspended solids. The application of chemical flocculants and coagulants inhibit the treatment efficiency of biological wastewater treatment with substantial increase in cost of treatment process [16]. The conventional coagulants $Al_2(SO_4)_3{\cdot}18H_2O$ or $FeCl_3$ employed in the treatment of industrial wastewater, release ionised species of aluminium and iron into coagulated wastewater which have high hazardous potential to human health and environment. Further, the presence of residual concentrations of Al^{3+} and Fe^{3+} affect the performance of membrane separation process, for example, the scale formation in ultra filtration/reverse osmosis membranes in post biological treatment processes [17].

There are reports on the development of integrated biological and chemical treatment techniques for the management of saline wastewater [18–24]. $[TSWW]_{Raw}$ was treated in an aerobic biological sequential batch reactor with halophilic bacteria and it was observed that the microbial consortium was unable to adapt efficiently to the highest TDS levels (50 g/L) [25,26]. The concentration of suspended solids was increased in the treated tannery saline wastewater due to cell lysis during biological treatment processes. The application of filamentous bacteria to facilitate coagulation/flocculation of suspended solids in wastewater was attempted. Various attempts have been made to cultivate filamentous bacterial mats including glass wool, coconut mesh, polyester fiber, silica particles and grass silage as a growth scaffold for the treatment of saline wastewater [27]. The proposed SOAR process employed a circular plastic media for the growth of biofilm and for the removal of suspended solids. There are reports on the application of oxic-anoxic integrated reactors in the biological treatment of wastewater to gain certain advantages such as easy operation, avoiding undesirable product formation, sustainable long term operations and considerable reduction in sludge generation [28–32]. However, reports on the application of biological reactors for the removal of suspended solids from saline wastewater without application of coagulants are very few or perhaps nil.

Hence, this study was focused on to employ sequential oxic and anoxic processes in SOAR reactor for the removal of suspended solids and dissolved organics from tannery saline soak liquor with minimum sludge generation.

2. Materials and methods

2.1. Materials

All the chemicals used in this study for the determination of process parameters were organised from Merck (India) and other biological grade chemicals were purchased from Himedia, India.

2.2. Design and fabrication of sequential oxic-anoxic bio reactor (SOAR)

A rectangular shaped SOAR reactor of dimension $25.5 \times 15 \times 15$ cm was fabricated using polyacrylic sheet as shown in Fig. 1. The SOAR comprised of three chambers and each chamber consists of anoxic and oxic zones. The total reactor volume of SOAR was 3.6L (chamber I, 1.4 L; chamber II, 1.2 L and chamber III, 1.0 L). The six outlets in oxic zone and five outlets in anoxic zone were provided in the reactor for the withdrawal of samples. The chambers were filled with commercial grade corrugated high density cylindrical polyethylene plastic media of dimensions diameter, 21 mm and height, 25 mm. The plastic media provided active surface area to support the growth of biofilm for the removal of suspended solids and partial mineralisation of dissolved organic compounds present in $[TSWW]_{Raw}$. Each compartment was provided with outlets for the collection of samples at oxic and anoxic zones for characterization. Provision was made to maintain oxic and anoxic conditions to support the growth of the inoculated microorganisms on to the plastic media for the maximum removal of organic compounds in wastewater.

2.3. Generation and characterisation of tannery saline waste water $[TSWW]_{Raw}$

The saline wastewater, soak liquor, used in the present investigation was collected from a commercial tannery in Chennai. The soak liquor was allowed to settle for 2 h and filtered using poly ester cloth (sieve size 300 μ) to remove floating solids and coarse solids. The plain settled soak liquor was characterized and the results are presented in Table 1. The suspended solids in different forms such as coarse, fine and colloidal state were characterized. The $[TSWW]_{Raw}$ was screened sequentially through Whattman filter paper (NF), GF/A filtration (WF) and centrifuge (CENT) at 10000 rpm for 20 min. All the parameters were analysed to study the contribution of suspended solids to organic loading of wastewater before and after the treatment using SOAR process.

2.4. Isolation and identification of microorganisms for protease, lipase and amylase production under saline environment

The biofilm formed over the plastic media was collected using phosphate buffer (pH, 7) and it was serially diluted with sterile distilled water and the organisms were isolated using Nutrient Agar medium by pour plate method and followed by incubation at 40 °C for 24–48 h for the growth of halophilic microorganisms. Microbial colonies that appeared on the agar plates were pure cultured and subjected to qualitative screening for the identification of protease, amylase and lipase producing microorganisms on Skim Milk Agar (SMA) medium, Starch Casein Agar (SCA) medium and Tri-Butyrin Agar Medium (TBA) respectively. Extracellular enzymes producing halophilic microorganisms produced a clear zone of hydrolysis in the respective agar plates (SMA, SCA and TBA at 3% (w/v) NaCl) after incubation at 40 °C. Based on the size of the clear zones on the agar plates, the proteolytic, amylolytic and lipolytic organisms were screened and maintained in nutrient agar slants and stored at 4 °C.