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Biological treatment of saline wastewater using a salt-tolerant microorganism

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ABSTRACT

Biological aerobic treatment of saline wastewater provides the material of this study. A salt-tolerant microorganism (*Staphylococcus xylosus*) was isolated from a vegetable pickled plant containing about 7.2% salt. Selection, identification and characterization of the microorganism were carried out. The isolated microorganism was used as inoculum for biodegradation. An activated sludge reactor operated in a fed-batch mode was used for the treatment of synthetic saline wastewater using three different microbial cultures namely: activated sludge (100%), a mixture of *Staphylococcus* supplement by activated sludge (1:1) and pure *S. xylosus* (100%) at different salt concentrations ranging from 0.5 to 3% NaCl. The results obtained showed that at low NaCl concentration (1%), the removal efficiency of chemical oxygen demand (COD) using different microbial cultures were almost the same (80–90%). However, increasing the NaCl concentration to 2% and using *Staphylococcus*-supplemented mixture by activated sludge and *S. xylosus* alone improved the treatment performance as indicated by COD removal rates which reached 91% and 93.4%, respectively, while the system performance started to deteriorate when activated bacterial culture was used alone (74%). Furthermore, the increase in NaCl concentration up to 3% and with the inclusion of *Staphylococcus*-supplemented mixture by activated sludge increased the COD removal to 93%, while the use of *S. xylosus* alone further improved the COD removal rate up to 94%. Also, the use of *S. xylosus* alone proved to be capable for biological treatment of a real case study of a vegetable pickled wastewater containing 7.2% salinity; the removal efficiency of COD reached 88% at this very high concentration of NaCl.

1. Introduction

Saline impaired water originates from many sources such as sea water, ground water, concentrate from decantation plants, effluent from sewerage treatment plants, brine from natural salt lake or saline effluent storage basins, brine from salt harvesting activities, effluent from farming and irrigation schemes, effluent from pulp and paper, food. It also originates from fertilizers, chemicals, paint, ink, pharmaceuticals, produced water from oil and gas production, effluent from mining and mineral processing industries, pickling processes, meat packing and dyestuff, pesticides, herbicides, polyhydric compounds, organic peroxides, and pharmaceuticals [3,13].

Biological treatment of saline wastewater has not been easy and by far the most popular treatment method [13]. Salt removal operations by physico-chemical processes such as reverse osmosis [5], ion exchange [4] or electrodialysis [4] before biological treatment are rather expensive. The performance of the biological treatment process for saline wastewater usually has low chemical oxygen demand removal due to adverse effects of salt on microbial flora [7]. High salt concentrations (> 1%) cause disintegration of cells because of the loss of cellular water (plasmolysis) or recession of the cytoplasm which is induced by an osmotic difference across the cell wall and cause of outward flow of intracellular water resulting in the loss of microbial activity and cell dehydration. As a result, low removal performance of chemical and biological oxygen demands and increases of the effluent suspended solids especially at high salt concentrations (> 2%) occur [4,6,14].

However, the utilization of a salt-tolerant organism in biological treatment units seems to be a more reasonable approach for saline wastewater treatment [6]. Salt-tolerant halophilic organisms may be used singly or in activated sludge culture for effective biological treatment of the saline wastewater [6]. Biological treatment of hypersaline wastewater by pure halophilic bacteria has been studied in biofilms and in sequencing batch reactors [18,19]. Inclusion of halophilic bacteria in an activated sludge culture was shown to improve COD removal efficiency at high salt content in a rotating biological contactor [9]. High salt contents also adversely affect nitrification and denitrification of saline wastewater [10]. Various types of microbial cultures were tested for the treatment of saline wastewater in an aerated percolator unit. Salt-tolerant, *Halobacter*-supplemented mixture by an activated sludge culture was found to be the best of the cultures tested in terms of COD removal efficiency. Nearly 80% was obtained at a 5% salt concentration [8]. Therefore, the aim of this study is to elaborate and investigate the effect of salt concentrations up to 7.2% on the efficiency of biological activated sludge treatment using different microbial cultures.

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2. Materials and methods

2.1. Isolation and identification of a salt-tolerant microorganism

Wastewater produced from a vegetable pickled plant provides the media for isolation and selection of the most salt-tolerant microorganism for biological treatment of high saline wastewater. A small amount of industrial wastewater effluent produced from a pickled manufacturing plant (0.1 ml) was spread onto a sterilized surface plate of basal mineral salts BMS agar medium(3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 1 g yeast extract and 20 g agar) containing 100 ml pickled wastewater as a sole of carbon source [2], these plates were incubated at 37% for 48 h.

Five different morphological colonies with a good growth on a surface mineral medium agar plate were isolated and transferred to sterilized mineral broth containing 10% pickled wastewater. These tubes were incubated at 37% for 24 h. Each cultures was examined microscopically, one of these cultures gave high efficiency in the biodegradation of saline wastewater. The culture was purified and identified according to biochemical reactions such as catalase test, Voges–Proskaur test, urease, coagulase, tolerance of 15% NaCl, growth in presence of 40% bile broth and sugar fermentations (glucose, maltose, sucrose, raffinose, xylose, mannose, arabinose, fructose and trehalose). All reactions were carried out according to Bergey's manual [2,12,15,16].

2.2. Treatability study

2.2.1. Wastewater and reactor operation

A batch mode activated sludge treatment was applied for saline wastewater; four Plexiglas laboratory columns were used for this purpose (Fig. 1). The column diameter was 7 cm with 90 cm in Length and with a total volume of two liters. Each column was fed with 1700 ml synthetic sewage as mentioned in item (2iii) and inoculated with a pre-aerated sludge (300 ml) which was delivered from a near-by wastewater treatment plant its salt content was 360 ± 50 mg/l. The sludge was adapted in the laboratory for the high salt content, the adaptation period ranged from 20 to 60 days. The experiments were carried out at 23 ± 2 °C. An aerator was used as a source of air supply with a constant-flow rate for each run. Air supply to columns was adjusted to maintain a minimum concentration of 2 mg O_2/l and it was turned off twice a day and the sludge was allowed to settle for 60 min. One liter from the supernatant was drained and the columns were refilled again with wastewater. Two experimental runs were done simultaneously; the first one was for adaptation and the second for the actual experiment with the adapted microbial cultures. The same procedure was applied for a real wastewater from a vegetable pickled plant. The efficiency of the treatment processes was evaluated by measuring the residual COD and turbidity after reaching the steady state.

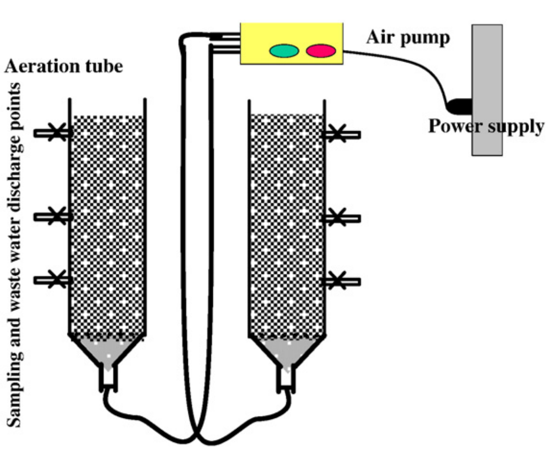

Fig. 1. Aerobic biological treatment reactor.

2.2.2. Preparation of salt concentration

Different salt concentrations (0.5, 1.0, 1.5, 2.0, and 3.0%) were prepared using NaCl analytical grade as a source of salt to be added to the inlet wastewater.

2.2.3. Wastewater composition

Synthetic wastewater similar to the concentration of natural sewage was prepared. It is composed of diluted molasses (97 g/l), NH_4Cl (6 g/l), $NH_4H_2PO_4$ (2.32 g/l) and meat extract (22.74 g/l).

2.3. Case study

Saline wastewater from a vegetable pickled production plant was treated biologically using the isolated culture from the same saline wastewater which was identified by biochemical reactions (*Staphylococcus xylosus*).

2.4. Analyses

After reaching the steady-state conditions, the physico-chemical analyses investigated covered the following parameters: pH value, COD, BOD, total solids at 105 °C, fixed residues at 550 °C, suspended solids, ammonia–nitrogen, organic-nitrogen, nitrite, nitrate, total phosphate, salinity, electrical conductivity and turbidity. The bacteriological examinations for the raw wastewater as well as the treated effluent were carried out. These included; total bacterial counts, total coliform group and fecal coliform group. The physico-chemical and bacteriological analyses of wastewater, unless specified, were conducted according to the American Standard Methods devised by [1].

3. Results and discussion

3.1. Isolation and identification of the most tolerant microorganism

A salt-tolerant microorganism was isolated from a vegetable pickled wastewater with 7.2% salinity and the pH was adjusted to 7.0 and at optimum detention time of 12 h (Table 1 and Fig. 2. This microorganism was inoculated to the biological treatment unit. The identification of bacteria was based on cellular, cultural and biochemical characteristics. The isolated strain (1) was examined microscopically as a gram-positive cocci arranged in clusters, *Staphylococci* is a positive catalase, urease and, Voges–Proskaur test but negative coagulase test and produce acid from sugar fermentation such as (glucose, maltose, sucrose, raffinose, xylose, mannose, arabinose, fructose and trehalose). Also the strain was able to grow in the presence of 40% bile salt, tolerance to 15% NaCl, and reduce nitrate. According to the results of biochemical reactions and the microscopic examinations, strain (1) was identified as *S. xylosus* to be used for the biological treatment through this study (Table 2).

Table 1
Isolation and selection of the most tolerant strain.

Run (1)	Stains	Residual COD[a] mg O_2/l	% R	Total Plate Count CFU/100 ml
Strain 1	G +	1470	82	3.5×10^5
Strain 2	G +	3186	61	3.8×10^5
Strain 3	G −	6371	22	4.1×10^5
Strain 4	G −	4656	43	4.4×10^5
Strain 5	G −	3921	52	5.6×10^5

[a] Initial COD: 8168 mg O_2/l, pH 7 and detention time 12 h.