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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.

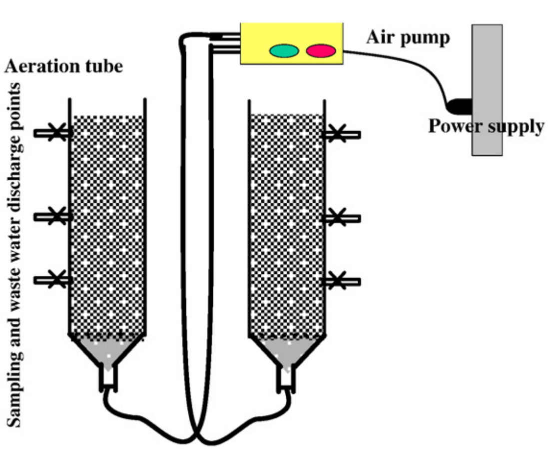

Fig. 1. Aerobic biological treatment reactor.

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.

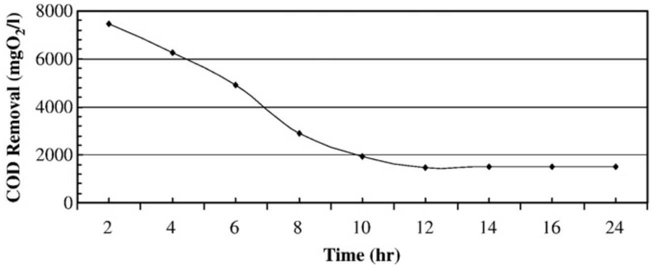

Fig. 2. Determination of the optimum detention time for biological degradation using *Staphylococcus xylosus*.

3.2. Biological treatment of saline wastewater

3.2.1. Pre-steady-state behavior of the treatment system at different NaCl concentrations

The system reached the steady-state conditions when three consecutive effluent's residual values of both COD and turbidity measurements were approximately the same. The steady-state conditions were reached after acclimatization period that ranged from 20 to 60 days for different cultures. The different cultures used were activated sludge culture, mixed culture (activated sludge and isolated *S. xylosus* with a ratio 1:1) and pure *S. xylosus* and at different NaCl concentrations namely, 5,10,15,20 and 30 g/l, respectively. Using 5 g/l NaCl, the acclimatization period was found to range from 20 to 30 days. Gradual increase of NaCl concentration to 10 g/l increases the acclimatization period to 25–35 days, then further increase of NaCl concentration from 10 to 15 g/l, the time required was about 40–48 days. By increasing the NaCl concentration to 20 g/l, the time required was 50–60 days. Finally, increasing NaCl concentration to 30 g/l, the time required for complete acclimatization took 50–60 days for the different cultures under investigation. The results obtained indicated that the time of acclimatization increased by increasing the salt content up to 30 g/l and the three different cultures behave in the same manner for adaptation. Generally, these results are in agreement with [17] which showed that adaptation and selection from mixed-activated sludge population took a long time at least 40 days, but was sufficient to achieve indicated removal.

Table 2
Identification and biochemical tests of *Staphylococcus xylosus*.

	Results
Properties and biochemical tests	
Gram staining	+
Cell morphology	Coccus in clusters
Catalase reaction	+
Coagulase (rabbit plasma)	−
Voges–Proskaur reaction	+
Tolerance to 15% NaCl	G
Growth in 40% bile broth	G
Nitrate reduction	+
Starch	−
Urease	+
Cellibiose	−
Mannitol	+
Ribose	+
Oxidase	−
B-galactosidase	+
Galactose	+
Utilization for different sugars	
Glucose	+
Maltose	+
Fructose	+
Sucrose	+
Xylose	+
Mannose	+
Trehalose	+
Salicin	+
Raffinose	−
Arabinose	+

G: growth.
Microscopic examinations were carried out when the organism appear in small extra cellular gram-positive cocci arranged in clusters.

3.2.2. Determination of the optimum time for biodegradation

After reaching the steady-state conditions, determination of the optimum detention time, which resulted in the best removal values of COD and turbidity with different salt concentrations and different cultures are shown in Fig. 3. It is obvious that 8 h was considered the optimum detention time which degrades the organic matters at salt concentrations ranging from 0.5 to 2%, however increasing the salinity to 3% increases the optimum retention time to 9 h.

3.3. Effect of salt concentration on system performance

Aerobic biological degradation for saline wastewater was carried out using three adapted cultures in separate reactors namely: activated sludge culture, salt-tolerant bacteria and a mixture of activated sludge and salt-tolerant bacteria with a ratio (1:1) at different NaCl concentrations namely: 5, 10, 15, 20 and 30 g/l NaCl. The treatment performance is shown in Fig. 4.

Starting with 5 g/l NaCl showed that activated sludge culture has the highest COD and turbidity removal values (92.8% and 73%). On the other hand, the pure culture (*S. xylosus*) resulted in the lowest efficiency since the optimal salt concentration for *S. xylosus* is nearly 15 g/l. This may be explained that 0.5% NaCl is probably an inhibitory for the pure culture and not high enough to be stimulatory.

The COD removal value using only activated sludge culture decreased with increasing the NaCl concentration to 10 g/l NaCl. It reached 79.6% and NTU removal was 62.5%. The best performance was obtained with a mixed sludge culture of activated sludge and *S. xylosus* resulting in nearly 90% COD removal rate and 65% for NTU removal. However, the removal efficiency of COD and NTU using the isolated culture alone (*S. xylosus*) decreased to 85% and 63.2%, respectively. These results confirmed that the isolated microorganism was not active enough at this salt concentration.

Further increase of NaCl concentration to 15 g/l, the COD removal of mixed and pure cultures were 90% and 89%, respectively and their corresponding NTU removal values were 70.5% and 71%. This can be explained that some synergistic interaction among bacteria in activated sludge culture and *S. xylosus* resulting in high and best COD and NTU removal efficiencies. From these results, it can be seen that saline wastewater containing 15 g/l NaCl is probably inhibitory for activated sludge culture, which result in low COD removal (≈75%) and NTU removal (65%). It was also reported that 1% salinity content was the threshold concentration for aerobic treatment system [11] using activated sludge only and in general, shifts of 0.5–2% salt will cause significant disruptions in system performance.

The results obtained in Fig. 4 indicted that a 2% NaCl concentration is probably inhibitory for activated sludge culture; COD removal rates obtained with the *Staphylococcus*-supplemented mixture by activated sludge culture was less than *Staphylococcus*-free activated sludge and greater than activated sludge culture. Inoculation of *S. xylosus* culture to the activated sludge was found to be beneficial by providing high COD removal rates which reached 91% and NTU removal of 75% compared with the activated sludge cultures which have COD removal rates of 75.2% and NTU removal of only 55%. It was also found that further increase in NaCl content from 1.5% to 2% for *S. xylosus* culture caused an increase in the removal rate of COD (93.4%) with a residual value about 33 mg O_2/l, while the turbidity removal reached 75%. This indicated stimulatory effect of high salt content of *S. xylosus*. These results are similar to that obtained by [4] which show that *Halobacter* addition produced significantly greater COD removal rates and

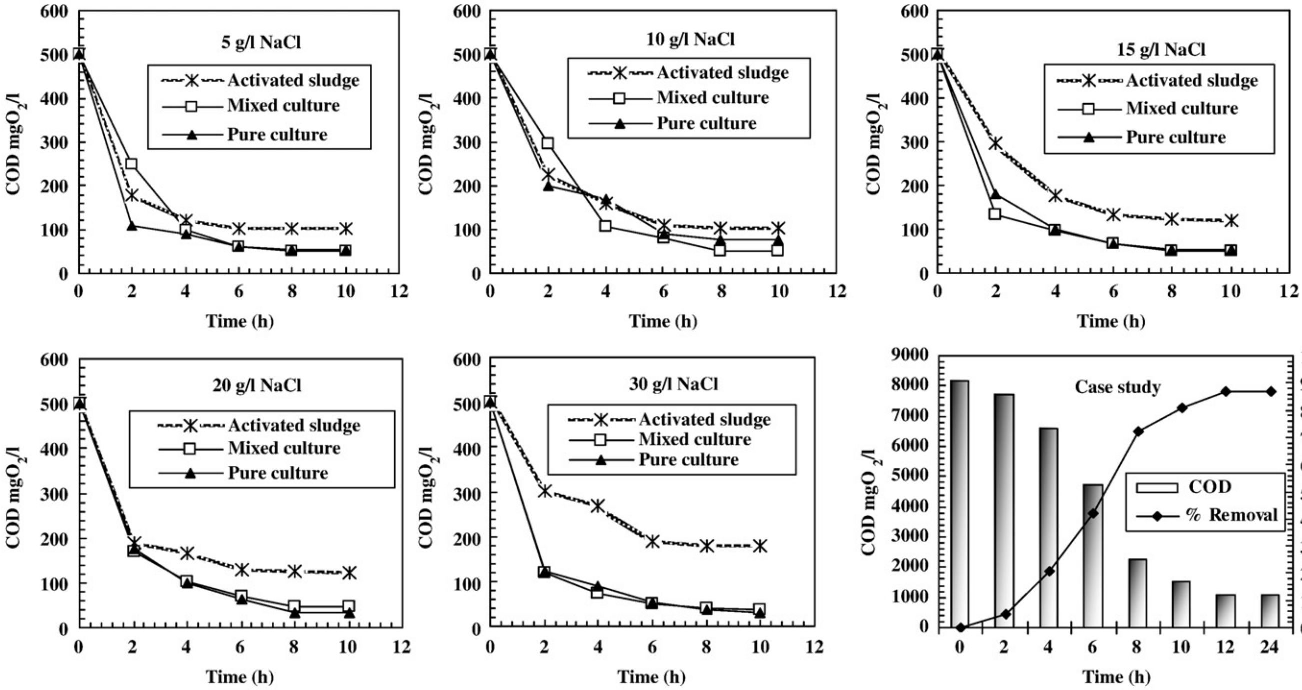

Fig. 3. Biodegradation of saline wastewater as a function of time using different cultures and different salt concentrations.

efficiencies at salt concentration greater than 2% salt, while at low salt concentrations, performance of both *Halobacter* and activated sludge cultures were comparable.

Finally, increasing NaCl concentration to 30 g/l at an optimum retention time of 9 h indicted that for *S. xylosus*, the COD residual value reached 30 mg O_2/l with a COD removal rate of 94%. At high NaCl concentration (3%), high activity of *S. xylosus* causes the increase of COD removal rate because *S. xylosus* (salt-tolerant microorganism) have special adaptation for survival at high salinities. However, using activated sludge culture alone decreased the removal value of COD from 94% to 64% with a residual value of 178 mg O_2/l. This may be attributed to the plasmolysis of the activated sludge organisms at high salt content. To overcome this problem a salt-tolerant *S. xylosus* was added to the activated sludge culture in order to alleviate salt inactivation effects. The residual COD value decreased from 178 mg O_2/l to 38 mg O_2/l with a COD removal rate of 92.4%. This indicated that the activated sludge organisms mixed with the *S. xylosus* populate and multiplies to become comparable in the total population with the pure *S. xylosus*.

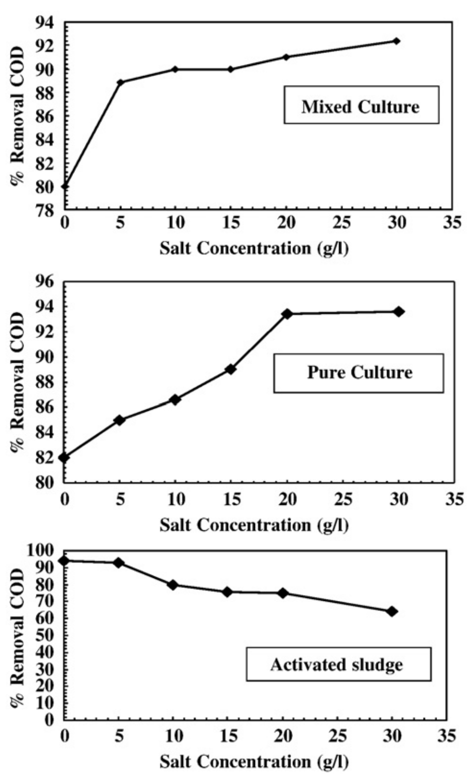

Fig. 4. Removal efficiency of COD as a function of NaCl concentrations using different cultures.

3.4. Case study

Based on the results obtained with synthetic sewage, the isolated *S. xylosus* was only used for treatment of a real wastewater for aerobic biodegradation of a vegetable pickled saline wastewater as a case study. The wastewater contains very high concentration of organic matters as represented by total COD values (8160) mg O_2/l, soluble COD (4834) mg O_2/l, total BOD (1700) mg O_2/l and TSS (392) mg/l. The pH value was acidic (3.63) and the salinity value was 72‰. The use of *S. xylosus* proved to be very efficient in treating such an extreme saline wastewater as shown in Table 3. The results indicated that *S. xylosus* has special adaptations for survival at high salinity and it can degrade the organic matters even at 7.2% salt concentration. The COD removal rates reached 88% with a residual value of 975 mg O_2/l which comply with the National Regulatory Standards for wastewater discharge into public sewage network.

4. Conclusions

Fed-batch aerobic biological treatment process using synthetic saline wastewater was carried out using different cultures namely: activated sludge culture, mixed culture (activated sludge and isolated *S. xylosus* with a ratio 1:1) and pure *S. xylosus* in an aerated reactor at different NaCl concentrations namely: 5, 10, 15, 20 and 30 g NaCl/l.

Table 3
Efficiency of Treated Vegetable Pickled Saline Wastewater using *Staphylococcus xylosus*.

Parameters	Unit	Raw wastewater	*S. xylosus-treated water*	% removal	[a]Ministerial decree 44/2000
pH	–	3.63	8.03	–	6.0–9.5
Chemical oxygen demand	mg O_2/l	8160	975	88	1100
Biological oxygen demand	mg O_2/l	1700	320	81.1	600
Total suspended solids	mg/l	392	62	84.18	800
Total dissolved solids	mg/l	72,390.8	72,390.0	–	–
Sett. solids 10′	ml/l	1.2	N.D	100	8
Sett. solids 30′	ml/l	3	N.D	100	15
Total Kjeldal Nitrogen	mg N/l	48.16	17.2	64.2	100
Ammonia	mg/l	11.2	1	91.07	–
Nitrite	mg N/l	0.04	5.5	–	–
Nitrate	mg N/l	2.9	20	–	–
Phosphorous	mg P/l	7.2	2	72.2	25
Total viable bacterial count at 37 °C	counts/ml	$8.8*10^3$	$3.2*10^2$	–	–

Salinity 72‰.
[a] Ministerial Decree for Wastewater Discharge into Public Sewage System.

Also, a case study using a vegetable pickled wastewater containing 72‰ salinity was treated using only the isolated *S. xylosus* culture. The results obtained depicted the followings:

- The adaptation time increased by increasing the NaCl contents and it was ranged from 20 to 60 days. Also, the same behavior was observed for the three cultures under investigation.
- The use of adapted activated sludge only as a microbial culture works efficiently up to 1% salinity. Further increase in salinity causes great deterioration of the system performance.
- The performance of the treatment process using a mixture of *S. xylosus* supplement by activated sludge culture was very efficient even by increasing the NaCl content up to 3%. The COD removal value reached 92.4%.
- By increasing the salinity to 3%, good results were obtained when *S. xylosus* was used alone; the COD removal values reached 95%.
- The use of isolated salt-tolerant microorganism "*S. xylosus*" resulted in a significant improvement in the efficiency of the biological treatment of a very high saline wastewater containing 72‰ salinity.

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