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NITRATE REMOVAL BY ANTARCTIC PSYCHROPHILIC YEAST CELLS UNDER HIGH SALT CONDITIONS

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Abstract: We researched nitrate removal in water with high salt concentrations at low temperature using *Candida* sp. which was isolated from the upper layer of Lake Vanda in the McMurdo Dry Valleys, Antarctica. The strain was cultured in a synthetic medium that contained nitrate as the sole nitrogen source The time course for the growth, and the nitrate and the total organic carbon (TOC) removals were examined aerobically in the presence of 0–20% NaCl at 5°C. The effects of pH and the C/N ratio on the removal of nitrate were studied

Candida sp. can remove more than 90% of nitrate up to an NaCl concentration of 10% in the medium As compared with 0% NaCl, 4% NaCl in the medium did not influence the growth rate and the removal of nitrate and TOC, but, at NaCl of 8 and 10%, the growth and removal of nitrate and TOC were decreased slightly. The yeast did not grow in the medium that contained NaCl of 15 and 20%. At pH of 3 to 7, yeast cells exhibited a similar removal nitrate level but it decreased at pH 1 and 2 The removal of nitrate increased with an increase in the C/N ratio and the nitrate in the medium was completely removed at a C/N ratio of 40. Nitrite was not observed in the cultured broth throughout the experiments The amino acid composition of the yeast cells did not change with nitrogen sources

key words: *Candida*, nitrate, psychrophilic, salt, waste water

Introduction

The increase of nitrate concentration in ground water is becoming an important problem in water supply. Anion exchange resins are widely used to remove nitrate in contaminated water. In this process nitrate is removed from contaminated water by ion exchange. The waste water that is left over the regeneration of the resins contains high concentrations of nitrate and NaCl. Re-use of this regenerant would reduce costs and environmental problems. VAN DER HOEK and KLAPWIJK (1987), VAN DER HOEK *et al.* (1987), NITISORAVUT and YANG (1992) and YANG *et al.* (1995) analyzed the use of mixed anaerobic denitrifying bacterial systems. Most biological treatment of waste water as well as the above experiments were performed at room temperatures (20–

28°C), however, little is known about the biological treatment of wastewater at low temperatures.

The use of yeast in biological treatment is advantageous because yeast grows well at low pH, reduces high organic matter, and may be able to perform biomass recovery. Several yeast strains has been researched in regards to the biological treatment of industrial and domestic waste water (THANH and SIMARD, 1973; OHNO *et al.*, 1991).

In the previous paper, we reported the treatment of dissolved organic matter at low temperatures by using the psychlophylic yeast *Candida* sp. which was isolated from water samples from Lake Vanda in Antarctica (KATAYAMA-HIRAYAMA *et al.*, 1997). Here we describe the results of a basal experiment about nitrate removal in the presence of NaCl and at low temperatures using *Candida* sp.

Materials and Methods

Microorganism and medium

Candida sp. which was isolated from the upper layer of Lake Vanda in the McMurdo Dry Valleys in Antarctica was used in this study. The physiological characteristics of this strain are as follows: DNase activity (+), nitrate utilization (+), sensitivity for cycloheximide (+), splitting of arbutin (−), starch test (−), vitamin requirement (−), acid formation (+), NaCl tolerance 0–5% (w/v) (+), 50% of glucose (+), splitting of fat (+), ester production (−), DBB reaction (−), gelatin liquefaction (−) and urease activity (−). It had fermentable activity and produced acid substances from various types of sugar, but produced none from lactose (NAGASHIMA *et al.*, 1990). The stock cultures of the strain were maintained on YM agar slants that contained 5 g of peptone, 3 g of yeast extract, 3 g of malt extract, 10 g of glucose and 20 g of agar in 1000 m*l* of water. The pH of the medium was adjusted to 6.5 before sterilization. The culture was prepared by transferring a loop of the stock culture and putting it into 50 m*l* of liquid YM medium in a 200-m*l* flask and incubated it at 5°C at 130 rpm on a rotary shaker. The effect of temperatures on the growth of the strain was examined at a range of 0 to 25°C using YM medium.

Nitrate removal in the presence of NaCl

Cultures were incubated at 5°C on a rotary shaker. The washed yeast cells (0.1 g, dry weight) were suspended in 100 m*l* of synthetic medium that contained nitrate as the sole nitrogen source (Table 1) and 0–20% NaCl. The effect of an initial pH of 1–7 on the removal of nitrate was examined using the synthetic medium. The pH of the medium was adjusted by 1M HCl or 1M NaOH. The effect of the C/N ratio on the removal of nitrate was also analyzed in regards to various C/N ratios of 5–40 of the synthetic medium. The C/N ratio of the medium changed with the addition of glucose. The supernatants that were filtered through a pore size of 0.45 μm were used for analysis.

Amino acid composition of the cells

The cells that were cultured on a synthetic medium that contained various nitrogen sources and YM medium were hydrolyzed by 6M HCl at 110°C for 22 hours (JSAC, 1985). The supernatant obtained by filtration with a 0.2 μm membrane filter was analyzed

Table 1 Composition of a synthetic medium containing nitrate as the sole nitrogen source

Constituent	
Glucose	10 g
KNO_3	780 mg
KH_2PO_4	1 g
$MgSO_4$ $7H_2O$	500 mg
$CaCl_2$ $2H_2O$	100 mg
NaCl	100 mg
Vitamin solution	1 ml
Distilled water	1000 ml

Vitamin solution (μg/ml) contains biotin 2, Ca-pantothenate 400, inositol 2000, thiamine-HCl 400, piridoxin-HCl 400, nicotinic acid 400 and *p*-aminobenzoic acid 200

by a HITACHI L-8500 amino acid analyzer. The crude protein content of the cells was calculated from the value for the Kjeldahl nitrogen.

Analytical methods

Total organic carbon (TOC) was analyzed by a SHIMADZU TOC Analyzer 10B. Nitrate, nitrite and Kjeldahl nitrogen were analyzed according to Standard Methods (APHA *et al.*, 1992).

Results and Discussion

Effects of the temperatures on the growth of *Candida* sp.

Figure 1 shows the effects of temperatures on the growth of the yeast cells. The optimum temperature of the strain was about 10–15°C. At 0 and 5°C, the growth rate slowed down, but the same growth yield was obtained at 0 to 15°C. The strain did not

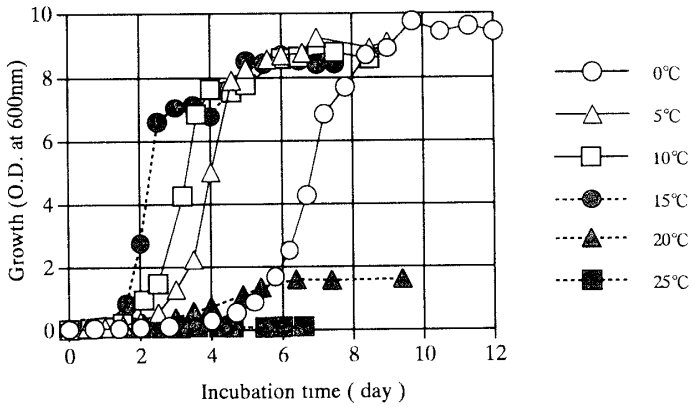

Fig. 1 Effect of temperature on the growth of *Candida* sp The strain was cultured on YM medium at various temperatures.

grow at 25°C. The following experiments were performed at 5°C for low temperature treatment.

Nitrate removal in the presence of NaCl

Figure 2 shows nitrate nitrogen (nitrate-N) and TOC removal and the growth under high salt conditions at 5°C. *Candida* sp. can remove more than 90% of nitrate-N up to an NaCl concentration of 10% in the medium. As compared with 0% NaCl, 4% NaCl in the medium did not influence the growth rate (data is not shown) and the removal of nitrate-N and TOC, but at 8 and 10% NaCl the growth and removal of nitrate-N and TOC decreased slightly. The yeast did not grow on the medium that contained 15 and 20% NaCl.

VAN DER HOEK and KLAPWIJEK (1987) and YANG *et al.* (1995) analyzed nitrate

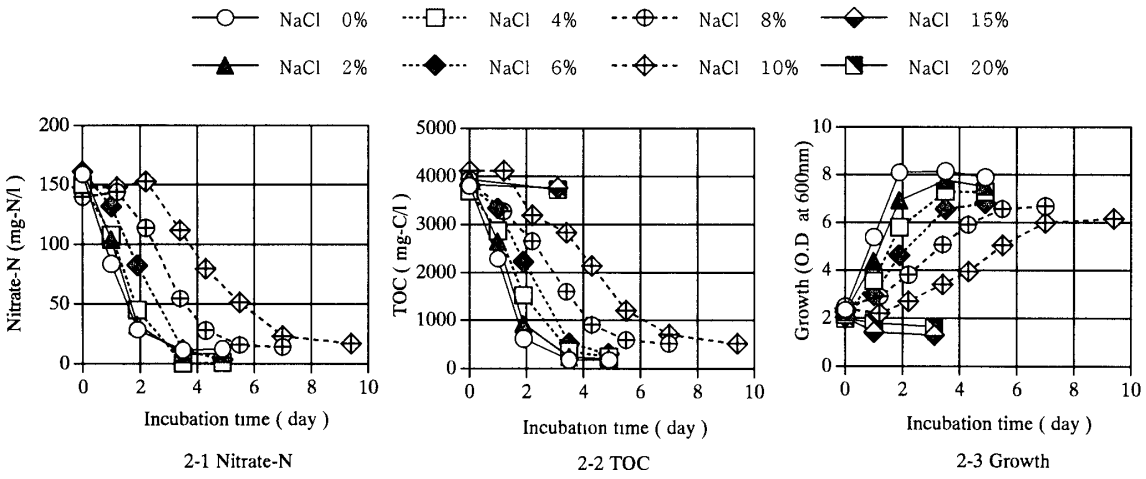

Fig. 2. Time course of nitrate removal, TOC and growth of *Candida* sp. under high salt conditions. The strain was cultured on the synthetic medium containing nitrate as the sole nitrogen source and various concentrations of NaCl at 5°C.

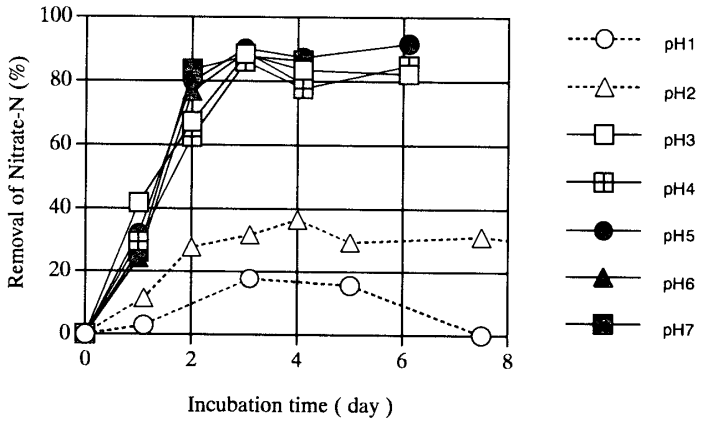

Fig. 3. Effect of initial pH on the removal (%) of nitrate by *Candida* sp. The strain was cultured on the synthetic medium containing nitrate as the sole nitrogen source at various pH at 5°C.

removal using mixed anaerobic denitrifying bacterial systems under high salt conditions. The nitrate removal was affected by 2% NaCl. Use of *Candida* sp. may be advantageous for the removal of nitrate under high NaCl conditions at low temperatures.

Figure 3 shows the effect of initial pH on the removal of nitrate-N. At pH of 3 to 7, the yeast cells grew well and showed a similar nitrate-N removal level. The pH of the medium increased to a range of 7.6–8.2 after incubation. The nitrate removal levels decreased largely at pH 1 and 2.

Figure 4 shows the effect of the C/N ratio on nitrate removal (%). Removal of nitrate (%) increased with an increase in the C/N ratio. The nitrate-N in the medium was completely removed at a C/N ratio of 40. Nitrite was not observed in the cultured broth throughout the experiments.

Amino acid composition of the cells

The amino acid composition of the cells that were cultured on different nitrogen sources is summarized in Table 2. The percentages of glutamic acid, glysine and alanine were relatively high. The amino acid composition of the yeast cells did not change with

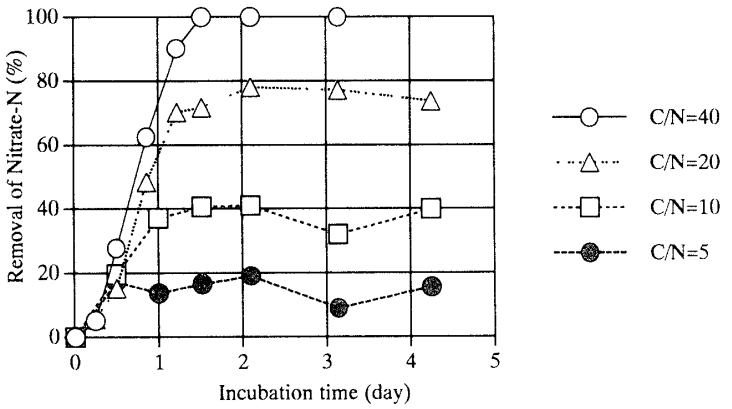

Fig 4 Effect of C/N ratio on the nitrate removal (%) by *Candida* sp The strain was cultured on the synthetic medium containing nitrate as the sole nitrogen source at various C/N ratios at 5°C The C/N ratio of the medium was changed with addition of glucose

Table 2. Amino acid composition of the cells of *Candida* sp cultured on various nitrogen sources

Medium	Nitrogen source	Amino acid (mol %)															
		Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Pro
YM medium	Peptone & yeast extract	7.9	5.1	8.3	12.5	10 8	13.8	3.1	0 9	2.4	7.6	2 3	3 4	8.7	2 3	6.0	5.0
Synthetic medium	NH_4-N	8 5	5 7	9 1	11 3	11 3	12 9	3.4	0 9	2 4	7 8	2.5	3 7	6 5	1.9	6 5	5 6
	NO_3-N	7 7	4 9	8.6	13 1	11 1	13.7	3 0	0 9	2.2	7 3	2 3	3 5	6 6	1 7	7 8	5 6

the nitrogen source. Biomass production per TOC was about 0.9 and the biomass contained about 42% crude protein.

Acknowledgments

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