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Enhancement Assimilation of Nitrite and Nitrate Containing Factory Effluents via Recombinants Induced in Yeast and Bacteria

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Abstract: This investigation aimed to improve the quality of drinking and irrigated water in industrial regions via apply microbial genetic techniques to induce recombinants in bacteria and yeast to be used for maximal assimilation of nitrite and nitrate from factory effluents. In this study ten bacterial strains and seven *Saccharomyces cerevisiae* strains were used. Bacterial strains were marking using 19 antibiotics to be use as a selectable marker in conjugation process. The available markers obtained were used in 14 mating, 10 of them were success, two transconjugants from each mating were selected to be use in biosorption experiments. Two from *Saccharomyces cerevisiae* strains were mated and the hybrids were isolated to be use in uptake experiments. Modern ecological biotechnology attempts to solve the problems of pollution by screening for and molecularly breeding microbial strains that are capable of degrading recalcitrant. This enhancement the biosorption which shall resulting in a decrease of environmental loading, i.e., in lesser contamination of groundwater and also receiving surface waters. The results appeared that some of bacterial strains expressed higher uptake of nitrite and nitrate such as; NRRL B-1584. In addition, transconjugants resulted from some matings appeared higher efficiency in nitrite and nitrate uptake above their parental strains. These including Tr1 and Tr2 resulted from the mating between NRRL B-571 X NRRL B-41228; Tr1 resulted from the mating between NRRL B-1584 X NRRL NRS-213, Tr1 and Tr2 resulted from the mating between NRRL B-642 X NRRL B-4375, and Tr2 resulted from the mating between NRRL B-642 X NRRL NRS-213. Most of bacterial strains and their transconjugants increased the removal of nitrite and nitrate more than 50%. *Saccharomyces cerevisiae* NRRL Y-12632 and *Saccharomyces cerevisiae* NRRL Y-136 plays an important role in uptake of nitrite and nitrate from wastewaters than the other yeast strains, as well as, hybrid No. 1 appeared the same trend in the uptake of nitrite and nitrate. This indicated that we can use these strains in improving effluent quality for potential reuse. A great improvements in the percentage of removal of nitrite and nitrate from factory effluents were achevied which increased more than 50%, this leading to improve effluent quality parameters.

Key words: Biosorption, factory effluents, hybrids, nitrite, nitrate, pollutants uptake, transconjugants

INTRODUCTION

Nitrate is an inorganic compound that occurs under a variety of conditions in the environment, both naturally and synthetically. Nitrate and nitrite are compounds that contain a nitrogen atom joined to oxygen atoms, with nitrate containing three oxygen atoms and nitrite containing two. In nature, nitrates are readily converted to nitrites and vice versa. Both are anions, or ions with a negative charge. They tend to associate with cations, or ions with a positive charge, to achieve a neutral charge balance. Nitrate is one of the most common groundwater contaminants in rural areas. It is regulated in drinking water primarily because excess levels can cause methemoglobinemia, or "blue baby" disease. Methemoglobinemia is the most significant health problem associated with nitrate in drinking water. Although nitrate levels that affect infants do not pose a direct threat to older children and adults, they do indicate the possible presence of other more serious residential or agricultural contaminants, such as bacteria or pesticides.

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Nitrate in groundwater originates primarily from fertilizers, septic systems, and manure storage or spreading operations. Fertilizer nitrogen that is not taken up by plants, volatilized, or carried away by surface runoff leaches to the groundwater in the form of nitrate. This not only makes the nitrogen unavailable to crops, but also can elevate the concentration in groundwater above the levels acceptable for drinking water quality. Nitrogen from manure similarly can be lost from fields, barnyards, or storage locations. Septic systems also can elevate groundwater nitrate concentrations because they remove only half of the nitrogen in wastewater, leaving the remaining half to percolate to groundwater.

Nitrate in drinking water is measured either in terms of the amount of nitrogen present or in terms of both nitrogen and oxygen. The federal standard for nitrate in drinking water is 10 milligrams per liter (10 mg/l) nitrate-N, or 45 mg/l nitrate-NO3. when the oxygen is measured as well as the nitrogen. Unless otherwise specified, nitrate levels usually refer only to the amount of nitrogen present, and the usual standard, therefore, is 10 mg/l.

Short-term exposure to drinking water with a nitrate level at or just above the health standard of 10 mg/l nitrate-N is a potential health problem primarily for infants. Babies consume large quantities of water relative to their body weight, especially if water is used to mix powdered or concentrated formulas or juices. Also, their immature digestive systems are more likely than adult digestive tracts to allow the reduction of nitrate to nitrite. In particular, the presence of nitrite in the digestive tract of newborns can lead to a disease called methemoglobinemia.

Nitrate in drinking water starts affecting the health of the general populace at levels in the range of 100 to 200 mg/l nitrate-N, but the effect on any given person depends on many factors, including other sources of nitrate and nitrite in the diet. Some of the nitrate consumed can be converted in the body to nitrite, which under appropriate circumstances can combine with amines (portions of protein molecules often found in foods, medications, cigarette smoke, decaying plants, soil, and sometimes water) to form nitrosamines, well-documented cancer-causing substances. So far, the only studies linking nitrate in drinking water with cancer have involved nitrate levels that are quite high (at or above 100-200 mg/l nitrate-N).

Nitrification, the microbiological process by which ammonia is converted to nitrate, is a major component of the global nitrogen cycle, plays a crucial role in transformation of fertilizer nitrogen in agricultural systems, and is a key component of nitrogen removal in wastewater treatment. Excess production of soluble nitrogen by nitrification results in the contamination of potable water and eutrophication of aquatic and terrestrial ecosystems, while the gaseous by-products of nitrification, nitric oxide and nitrous oxide, are two of the most potent greenhouse gases. As anthropogenic inputs of fixed nitrogen continue to expand to meet the demands of a growing global population, intimate knowledge of the nitrification process and the microorganisms that control this process will be necessary to address environmental nitrogen imbalances.

Therefore, both adsorption and desorption are independent of the total number of sites occupied. Adsorption is considered as a state of dynamic equilibrium, in which the rate at which metals are adsorbed equals the rate at which metals are desorbed. In the early stage, the rate of biosorption is fast since most of the binding sites on cell surface are freely available, whereas the rate of biosorption decreases when the cell surface is occupied with bound metal molecules. In other words, the rate of biosorption decreases with decreasing accessible surface area on the cell walls.

Nitrogen is the nutrient applied in the largest quantities for lawn and garden care and crop production. In addition to fertilizer, nitrogen occurs naturally in the soil in organic forms from decaying plant and animal residues. In the soil, bacteria convert various forms of nitrogen to nitrate, a nitrogen/oxygen ion (NO_3-). This is desirable as the majority of the nitrogen used by plants is absorbed in the nitrate form. However, nitrate is highly leachable and readily moves with water through the soil profile. If there is excessive rainfall or over-irrigation, nitrate will be leached below the plant's root zone and may eventually reach groundwater.

Nitrate-nitrogen (NO_3-N) in groundwater may result from point sources such as sewage disposal systems and livestock facilities, non-point sources such as fertilized cropland, parks, golf courses, lawns, and gardens, or naturally occurring sources of nitrogen. Proper site selection for the location of domestic water wells and proper well construction can reduce potential nitrate contamination of drinking water source.

Nitrate is a major source of nitrogen for most algae, bacteria, fungi, and higher plants, and it is the nutrient that most frequently limits their growth (Daniel-Vedele *et al* 1998, Crawford and Glass, 1998, Williams and Miller, 2001). The first step in the assimilation of nitrate is the influx of nitrate into cells, which is an active process, because it can occur against an electrochemical potential gradient (Vidmar *et al*, 2000) followed by the catalytic activities of nitrate reductase and nitrite reductase that sequentially produces nitrite and ammonium, the latter being converted to organic nitrogen for cellular growth.

Biosorption of Metal Ions Usually Can Be Classified as Two Types The Freundlich model, in which the amount of metal uptake by the biomass increases with time, and the Langmuir model, in which the amount of metal uptake by the biomass reaches equilibrium [Chang and Hong 1994]. Therefore, both adsorption and desorption are independent of the total number of sites occupied. Adsorption is considered as a state of dynamic equilibrium, in which the rate at which metals are adsorbed equals the rate at which metals are desorbed. In the early stage, the rate of biosorption is fast since most of the binding sites on cell surface are freely available, whereas the rate of biosorption decreases when the cell surface is occupied with bound metal molecules. In other words, the rate of biosorption decreases with decreasing accessible surface area on the cell walls. The present study aimed to apply biotechnology techniques for maximal reduction of nitrite and nitrate from factory effluents by genetically constructed bakers yeast and bacterial strains to improve the quality of drinking and irrigated water in industrial regions.

MATERIALS AND METHODS

Ten bacterial strains and seven *Saccharomyces cerevisiae* strains (Table 1) were used in this study, they are kindly obtained from National Center for Agriculture Utilization Research, USA. One of *Saccharomyces cerevisiae* strains (NBIMCC 82) was kindly obtained from National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria, Sofia. All strains used in this investigation are wild type strains.

Table 1: Bacterial and yeast strains used in this study .

No.	Strains	Designation	Origin
1	*Citrobacter amalonaticus*	NRRL B-41228	USA
2	*Citrobacter freundii*	NRRL B-2643	USA
3	*Bacillus subtilis* var *niger*	NRRL NRS-213	USA
4	*Bacillus subtilis*	NRRL B-642	USA
5	*Bacillus licheniformis*	NRRL B-571	USA
6	*Bacillus licheniformis*	NRRL B-1584	USA
7	*Bacillus licheniformis*	NRRL NRS-1264	USA
8	*Bacillus licheniformis*	NRRL B-358	USA
9	*Micrococcus luteus*	NRRL B-287	USA
10	*Kocuria rhizophila*	NRRL B-4375	USA
11	*Saccharomyces cerevisiae*	NRRL Y – 12632	USA
12	*Saccharomyces cerevisiae*	NRRL Y - 11562	USA
13	*Saccharomyces cerevisiae*	NBIMCC 82	Bulgaria (National Bank for industrial microorganisms and cell cultures) , sofia
14	*Saccharomyces cerevisiae*	NRRL Y - 12619	USA
15	*Saccharomyces cerevisiae*	NRRL Y – 136	USA
16	*Saccharomyces cerevisiae*	NRRL Y – 137	USA
17	*Saccharomyces cerevisiae*	NRRL Y - 1370	USA

Factory Effluents:

The present study was undertaken using the wastewaters resulted from ammonia unit of Fertilizer Factory (FF). Polluted water was collected from the main pipe of the factory before being mixed with water in the river. This collection was done in October 2007. A specific problem associated with nitrite and nitrate in the environment is accumulation in the food chain and persistence in the environment.

Media:

Bacterial strains were grown as described before previously by Horikoshi *et al.* (1981). However, yeast strains were grown on yeast extract peptone dextrose (YEPD) medium.

Ii. Methodology:

Antibiotic Susceptibility Assays:

Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne (1985) with cultures grown to logarithmic growth phase in nutrient agar medium for each microbe. All antibiotics were used at a concentration of 100 mg/ml, according to Roth and Sonti (1989). The selectable markers were identified as antibiotic resistance and or sensitive genes as listed in Table 3. Antibiotic designation was listed in Table 2.

Conjugation:

Nutrient broth cultures, in the late-exponential growth phase were used. Quantitative spot mating of conjugal transfer was carried out according to Lessel *et al.* (1993) by inoculating 10 ml samples of the donor culture onto the surface of selective medium, previously seeded with 100 ml of the recipient culture. A single

colony of transconjugants was picked up and transferred to slant nutrient agar medium. Conjugation was carried out between strains carrying the opposite genetic markers. From each mating, two different isolates were selected to be used in pollutants uptake experiments.

The genetic information transferred (Table 3) is often beneficial to the recipient cell. Benefits may include; antibiotic resistance, heavy metals uptake, other xenobiotic tolerance, or the ability to utilize a new metabolite (*Holmes and Jobling 1996).* Such beneficial plasmids may be considered bacterial endosymbionts. Some conjugative elements may also be viewed as genetic parasites on the bacterium, and conjugation as a mechanism was evolved by the mobile element to spread itself into new hosts. Five single colonies from that appeared in each conjugation were picked up and transferring to a nutrient agar slant, each colony may differ than other ones on the same plate resulted from the same mating in harboring genetic background. This because these are recombinations, each recombination resulted from the mating between two bacterial cells.

Table 2 : Antibiotics and their abbreviations used for genetic marking against different bacterial strains .

Antibiotics	Designation
Flucamox	*flu*
Streptomycin	*Str*
Tetracycline	*Tc*
Neomycinsulphate	*Nm*
Ampicillin	*Ap*
Erythromycin	*Erth*
Amoxycillin and flucloxacillin	*Am-Fluc*
Rifampicillin	*Rf*
Ibiamox	*Ibim*
Amoxycillin	*Amoxy*
Ibidroxil	*Ibid*
Haiconcil	*Hico*
Velosef	*Velo*
Epicocillin	*Epico*
Nystatin	*Nyst*
Epicocillin	*Epico*
Erythrocin	*Ery*
Duricef	*Duri*
Pencillin	*pen*

Table 3: Mating between bacterial strains that having the opposite genetic markers.

No. of mating	Mating	Revelant geneotype of mating
1	NRRL B-571 X NRRL B-1584	$Erth^{+}$, Ap^{+}, $Ibim^{+}$, $Amoxy^{+}$, *Hico+*, $Epico^{+}$,Cp^{-} X $Erth^{-}$, Ap^{+}, $Ibim^{-}$, $Amoxy^{-}$, $Hico^{-}$, $Epico^{-}$,*Cp+*
2	NRRL B-571 X NRRL B-358	$Erth^{+}$, flu^{+}, *Hico+* $Epico^{+}$,Cp^{-} X $Erth^{-}$, Flu^{-}, $Hico^{-}$, $Epico^{-}$,*Cp+*
3	NRRL B-571 X NRRL B-2643	$Erth^{+}$, flu^{+}, $Epico^{+}$, $Velo^{-}$, $Duri^{-}$, Cp^{-}, $Ibid^{-}$ X $Erth^{-}$, flu^{-} $Epico^{-}$, $Velo^{+}$, $Duri^{+}$, Cp^{+}, $Ibid^{+}$
4	NRRL B-571 X NRRL B-41228	$Erth^{+}$, flu^{+}, Ap^{+}, $Epico^{+}$,Cp^{-} X $Erth^{-}$, flu^{-}, Ap^{-}, $Epico^{-}$,Cp^{+}
5	NRRL B-1584 X NRRL B-41228	Ap^{+}, $Ibid^{-}$, $Amoxy^{-}$, $Ibim^{-}$ X Ap^{-}, $Ibid^{+}$, $Amoxy^{+}$, $Ibim^{+}$
6	NRRL B-1584 X NRRL B-642	Ap^{+}, Cp^{+}, $Am\text{-}Fluc^{+}$, pen^{+}, $Hico^{-}$, $Epico^{-}$ X Ap^{-}, Cp^{-}, $Am\text{-}Fluc^{-}$, pen^{-}, Hico^{+}, $Epico^{+}$
7	NRRL B-1584 X NRRL NRS-213	Ap^{+}, Cp^{+}, $Am\text{-}Fluc^{+}$, pen^{+}, $Amoxy^{-}$ X Ap^{-}, Cp^{-}, $Am\text{-}Fluc^{-}$, pen^{-}, $Amoxy^{+}$
8	NRRL NRS-1264 X NRRL B-2643	$Erth^{+}$, Tc^{+}, $Ibim^{+}$, flu^{+}, $Ibid^{-}$, $Velo^{-}$, $Duri^{-}$ *X* $Erth^{-}$, Tc^{-}, $Ibim^{-}$, flu^{-}, $Ibid^{+}$, $Velo^{+}$, $Duri^{+}$
9	NRRL B-358 X NRRL B-642	Ap^{+}, Cp^{+}, $Am\text{-}Fluc^{+}$, pen^{+}, $Ibim^{+}$, $Amoxy^{+}$, $Hico^{-}$, $Epico^{-}$ X Ap^{-}, Cp^{-}, $Am\text{-}Fluc^{-}$, pen^{-}, $Ibim^{-}$, $Amoxy^{-}$ $Hico^{+}$, $Epico^{+}$
10	NRRL B-2643 X NRRL B-642	Ap^{+}, Cp^{+}, $Am\text{-}Fluc^{+}$, pen^{+}, $Ibim^{+}$, $Amoxy^{+}$, $Ibid^{+}$, $Velo^{+}$, $Duri^{+}$, $Epico^{-}$ *X* Ap^{-}, Cp^{-}, $Am\text{-}Fluc^{-}$, pen^{-}, $Ibim^{-}$, $Amoxy^{-}$, $Ibid^{-}$, $Velo^{-}$, $Duri^{-}$, $Epico^{+}$
11	NRRL B-41228 X NRRL B-642	Cp^{+}, $Am\text{-}Fluc^{+}$, pen^{+}, $Ibim^{+}$, $Amoxy^{+}$, $Epico^{-}$ *X* Cp^{-}, $Am\text{-}Fluc^{-}$, pen^{-}, $Ibim^{-}$, $Amoxy^{-}$ $Epico^{+}$
12	NRRL B-642 X NRRL B-4375	*Hico+*, $Epico^{+}$, $Am\text{-}Fluc^{-}$, pen^{-} X *Hico-*, $Epico^{-}$, $Am\text{-}Fluc^{+}$, pen^{+}
13	NRRL B-642 X NRRL NRS-213	*Hico+*, $Epico^{+}$, $Amoxy^{-}$ *X* *Hico-*, $Epico^{-}$, $Amoxy^{+}$
14	NRRL B-4375 X NRRL NRS-213	$Am\text{-}Fluc^{+}$, pen^{+}, $Amoxy^{-}$ x $Am\text{-}Fluc^{-}$, pen^{-}, $Amoxy^{+}$

Uptake Experiments:

In the nitrite and nitrate uptake test, overnight cultures grown in in a 250 ml flask containing nutrient broth for bacteria and YEPD for yeast supplemented factory effluents without any dilution were harvested, washed twice with distilled water, and resuspended in 250 ml conical flasks each containing 150 ml factory effluents supplemented with 1 mg glucose / 10 ml wastewater, glucose was used as a sole source of carbon. The flasks were incubated under a static conditions at 30°C for 48 h, which was enough time to achieve steady state biosorption. The pH was uncontrolled throughout the experiment. Thereafter, the cells were collected by

filtration on membrane filter (pore size 0.45 mm). After the cells removed, the filtrate was used to determine the amount of nitrite and nitrate according to Nakajima and Sakaguchi (1986).

Dry Cell Weight:

Dry cell weight measurements were carried out by passing a volume of 50 ml cell culture through a previously weighted Millipore filters (Watman No. 1). Cell pellets were also washed twice with filtered deionized/distilled water to remove non-biomass ash. Filtered and collected cells were dried in an oven set at temperature 110 ◦C and weight for every 24 h until constant weight was obtained.

Nitrite and Nitrate Assays:

This step was done at fine analysis unit, Chemistry Department, Fac. Of Agric., Mansoura University. In this procedure, each water sample was divided into two equal aliquots. The first aliquot was analyzed for No^-_2 by a modification of the Griss II sovay method, which was described by Bremmer (1965). In this analysis, the aliquot was treated with diazotizing reagent (sulfanilamide) in Hcl solution to convert the No^-_2 to a diazonium salt, and it is subsequently treated with a coupling reagent (N - [1-naphthyl ethylene diamine di hydrochloric acid) to convert the diazonium salt to an azo compound. The intensity of the reddish purple color that develops as a result of these treatments is then measured. The second aliquot was analyzed for No^-_2 + No^-_3 by reducing the No^-_3 to No^-_2 using a Zn metal powder according to Heans (1975). Then it was determined by the previous method for the No^-_2 .The concentration of No^-_3 was calculated by difference.

Data Evaluation (Langmuir Isotherms):

The uptake of the nitrite and nitrate (in mg of metal/g of dry cell weight) was calculated according to Liu *et al.*, 2004 using the following formula:

$Q = v(C_i - C_f)/m$

Where Q is the metal uptake (mg metal per g biosorbent), v the liquid sample volume (ml), C_i the initial concentration of the metal in the solution (mg/L), C_f the final (equilibrium) concentration of the metal in the solution (mg/L) and m the amount of the added biosorbent on the dry basis (mg).

RESULTS AND DISCUSSION

Bacteria converting nitrite, the end product of ammonia oxidation, into nitrate according to the reaction; $NO2^- + H_2O \rightarrow NO3^- + 2H^+ + 2e^-$. Nitrite functions as an electron donor for the reduction of NAD via reverse electron flow and the generation of ATP by oxidative phosphorylation (Freitag and Bock. 1990). Nitrification, the microbiological process by which ammonia is converted to nitrate, is a major component of the global nitrogen cycle, plays a crucial role in transformation of fertilizer nitrogen in agricultural systems, and is a key component of nitrogen removal in wastewater treatment.

Nitrite and Nitate Uptake by Bacterial Cells Using Wastewaters Supplemented with 0.01% Glucose as a Carbon Source :

As shown from the results presented in Table 4 some of bacterial strains expressed higher uptake of nitrite and nitrate such as; NRRL B-1584, this indicated that this is a superior bacterial strain in nitrite and nitrate uptake. In addition, transconjugants resulted from some matings appeared higher efficiency in nitrite and nitrate uptake above their parental strains. These including Tr1 and Tr2 resulted from the mating between NRRL B-571 X NRRL B-41228; Tr1 resulted from the mating between NRRL B-1584 X NRRL NRS-213, Tr1 and Tr2 resulted from the mating between NRRL B-642 X NRRL B-4375, and Tr2 resulted from the mating between NRRL B-642 X NRRL NRS-213.

The results obtained in this study are in agreement with those obtained by Araki *et al.* (2005), who studied the genetic differences in nitrate uptake in two clones of the common reed, *Phragmites australis*, common reed, which could be useful in removing eutrophic substances from river and lake water. In their study, the genetic differences in nitrate uptake ability of the reed were investigated with a view to breeding a reed plant useful for phytoremediation. Two reed clones (W-6 and W-8) isolated from a reed community in the lakeside wetland along Lake Biwa, Japan, were used for a study on the physiological and molecular basis of nitrate uptake. Kms for nitrate uptake were 80.8 and 45.2 microM and Vmaxs for nitrate uptake were 10.62 and 2.37 micromol g-1 root f.w. h-1 in W-6 and W-8, respectively, suggested that there were critical differences in kinetic parameters for nitrate uptake. To investigate these differences at the molecular level, they are isolated a high-affinity nitrate transporter gene (NRT2) from the two reed clones and analyzed the reed NRT2 structure and expression.

Table 4: Nitrite and nitate uptake from wastewaters supplemented with 0.01 % glucose as a sole carbon soure treated by parental strains of bacteria and their transconjugants .

Biocontrol agents		ppm				
		Amonia	Nitrite	Available	Nitarte	Total
Control (Untreated) without glucose		23.8	16.8	40.6	6.72	47.32
Control (Untreated) with glucose		25.2	25.2	50.4	5.74	56.14
NRRL B-571 X NRRL B-1584	571	0	2	3	1	3
	1584	2809	4058	6867	1280	8147
	M.P.	1405	2030	3435	641	4075
	Tr1	0	1	1	0	1
	Tr2	1	3	4	-1	3
NRRL B-571 X NRRL B-2643	571	0	2	3	1	3
	2643	0	2	2	0	2
	M.P.	0	2	3	1	3
	Tr1	0	1	1	0	1
	Tr2	0	1	1	0	2
NRRL B-571 X NRRL B-41228	571	0	2	3	1	3
	41228	0	2	1	0	1
	M.P.	0	2	2	1	2
	Tr1	324	2590	2913	1327	4240
	Tr2	299	5373	5672	896	6567
NRRL B-1584 X NRRL B-642	1584	2809	4058	6867	1280	8147
	642	0	2	2	1	2
	M.P.	1405	2030	3435	641	4075
	Tr1	1193	1193	2386	924	3310
	Tr2	0	2	2	0	2
NRRL B-1584 X NRRL NRS-213	1584	2809	4058	6867	1280	8147
	213	0	2	2	0	2
	M.P.					
	Tr1	258	1288	1546	240	1787
	Tr2	0	1	1	0	2
NRRL NRS-1264 X NRRL B-2643	1264	1	1	3	0	3
	2643	0	2	2	0	2
	M.P.	1	2	3	0	3
	Tr1	1	1	2	0	3
	Tr2	0	2	2	1	3
NRRL B-358 X NRRL B-642	358	1	1	3	0	3
	642	0	2	2	1	2
	M.P.	1	2	3	1	3
	Tr1	1	3	3	0	3
	Tr2	0	2	2	1	3
NRRL B-2643 X NRRL B-642	2643	0	2	2	0	2
	642	0	2	2	1	2
	M.P.	0	2	2	1	2
	Tr1	0	2	2	0	2
	Tr2	0	0	0	0	0
NRRL B-41228 X NRRL B-642 41228	0	2	1	0	1	
	642	0	2	2	1	2
	M.P.	0	2	2	1	2
	Tr1	2	1	3	0	4
	Tr2	0	1	1	0	1
NRRL B-642 X NRRL B-4375	642	0	2	2	1	2
	4375	0	2	2	-1	2
	M.P.	0	2	2	0	2
	Tr1	885	2950	3836	1062	4898
	Tr2	-420	4204	3784	883	4667
NRRL B-642 X NRRL NRS-213	642	0	2	2	1	2
	213	0	2	2	0	2
	M.P.	0	2	2	1	2
	Tr1	0	2	2	0	2
	Tr2	854	5976	6829	1750	8579
NRRL B-4375 X NRRL NRS-213	4375	0	2	2	-1	2
	213	0	2	2	0	2
	M.P.	0	2	2	-1	2
	Tr1	1	1	2	0	2
	Tr2	0	1	1	0	2

Tr = Transconjugants.

Plant mutants with low levels of nitrate reductase in barley possess considerable nitrate transport activity, and nitrate reductase-defective mutants in tobacco accumulate high levels of nitrate indicative of functional uptake. However, these mutants still possess some nitrate reductase activity making the interpretation of the importance of an active nitrate reductase for nitrate uptake somewhat equivocal, as low nitrate reductase activity alone might be sufficient to allow substantial transport. NRT2 transcription were also observed between the two clones. It was not clear whether the difference in kinetic parameters for nitrate was due to the reed NRT2 structure or expression. The results obtained in this study indicate the possibility of selecting genotypes more useful for the removal of nitrate such as we seen herein by bacterial strain NRRL B-1584 other than all baterial strains used in this proposal and that also appeared by some of transconjugants other than their parental strains.

The results summarized in Table 5 appeared the percentages of nitrite and nitrate uptake from wastewaters by most of bacterial strains and their transconjugants which were increased than 50%.

Nitrate is a major source of nitrogen for most algae, bacteria, fungi, and higher plants, and it is the nutrient that most frequently limits their growth . The first step in the assimilation of nitrate is the influx of nitrate into cells, which is an active process, because it can occur against an electrochemical potential gradient followed by ammonium, the latter being converted to organic nitrogen for cellular growth. Although there is considerable biochemical, genetical, and molecular biological information about these systems and their regulation, it is still not clear whether nitrate reductase is required for nitrate transport activity or whether transport occurs quite independently and in the absence of nitrate reductase activity.

Table 5: Percentage of nitrite and nitrate uptake from wastewaters supplemented with 0.01 % glucose as a sole carbon soure treated by parental strains of bacteria and their transconjugants .

Biocontrol agents		ppm				
		Amonia	Nitrite	Available	Nitarte	Total
Control (Untreated) without glucose		23.8	16.8	40.6	6.72	47.32
NRRL B-571 X NRRL B-1584	571	6	56	31	76	35
	1584	50	72	61	100	65
	M.P.	28	64	46	88	50
	Tr1	6	56	31	2	28
	Tr2	17	72	44	-71	33
NRRL B-571 X NRRL B-2643	571	6	56	31	76	35
	2643	11	83	47	76	50
	M.P.	8	69	39	76	43
	Tr1	0	56	28	-22	23
	Tr2	6	61	33	27	33
NRRL B-571 X NRRL B-41228	571	6	56	31	76	35
	41228	-6	50	22	2	20
	M.P.	0	53	26	39	28
	Tr1	6	44	25	100	33
	Tr2	6	100	53	73	55
NRRL B-1584 X NRRL B-642	1584	50	72	61	100	65
	642	0	50	25	76	30
	M.P.	25	61	43	88	48
	Tr1	22	22	22	76	28
	Tr2	-6	56	25	2	23
NRRL B-1584 X NRRL NRS-213	1584	50	72	61	100	65
	213	-6	56	25	27	25
	M.P.	22	64	43	63	45
	Tr1	17	83	50	68	52
	Tr2	-6	56	25	76	30
NRRL NRS-1264 X NRRL B-2643	1264	72	78	75	100	78
	2643	11	83	47	76	50
	M.P.	42	81	61	88	64
	Tr1	39	39	39	46	40
	Tr2	11	44	28	76	33
NRRL B-358 X NRRL B-642	358	61	78	69	76	70
	642	0	50	25	76	30
	M.P.	31	64	47	76	50
	Tr1	11	56	33	41	34
	Tr2	-6	50	22	95	30
NRRL B-2643 X NRRL B-642	2643	11	83	47	76	50
	642	0	50	25	76	30
	M.P.	6	67	36	76	40
	Tr1	-2	63	31	71	35
	Tr2	17	67	42	2	38

Table 5: Continued.

NRRL B-41228 X NRRL B-642	41228	-6	50	22	2	20
	642	0	50	25	76	30
	M.P.	-3	50	24	39	25
	Tr1	67	56	61	76	63
	Tr2	11	33	22	88	29
NRRL B-642 X NRRL B-4375	642	0	50	25	76	30
	4375	6	89	47	-132	29
	M.P.	3	69	36	-28	30
	Tr1	17	56	36	88	41
	Tr2	-6	56	25	51	28
NRRL B-642 X NRRL NRS-213	642	0	50	25	76	30
	213	-6	56	25	27	25
	M.P.	-3	53	25	51	28
	Tr1	-11	67	28	-17	23
	Tr2	11	78	44	100	50
NRRL B-4375 X NRRL NRS-213	4375	6	89	47	-132	29
	213	-6	56	25	27	25
	M.P.	0	72	36	-52	27
	Tr1	33	56	44	83	48
	Tr2	0	39	19	76	25

Tr = Transconjugants .

Lack of nitrate uptake is not because of a down-regulation of transporter gene expression or a lack of translation in fungi. such biophysical removal of nitrate in plant cells would maintain an appropriate gradient necessary to sustain nitrate transport, even in the absence of nitrate reductase activity. In fungal cells, the nitrate concentration localized at the membrane could rise rapidly in the absence of nitrate reductase activity to a concentration which would preclude further transport. (Shiela *et al*, 2004).

The results obtained in this study are in harmony with Herrero *et al*, 2001, who reported that the related nitrate assimilatory genes, for nitrate reduction and nitrate uptake, in cyanobacteria are in general clustered as a *nirA* operon (*nirA-nrtABCD-narB*), which is transcriptionally activated by withdrawing the combined nitrogen source and by inhibiting ammonium assimilation. In addition, expression of the nitrate assimilatory operon is repressed by the presence of ammonium. In general, nitrate or nitrite is not required for transcriptional activation. Interestingly, induction of nitrate uptake activity in *Synechococcus* sp. strain RF-1 strictly requires nitrate or nitrite, which in turn could be repressed by ammonium (Frías *et al* , 1997). Regulation of *Synechococcus* sp. strain RF-1 nitrate uptake, in general, is reflected on expression of the *nrtC* transcripts . In addition, the expression of *nrtC* in nitrate treatment is higher than in nitrate-free treatment. However, the relatively more modest level of nitrate induction observed with *nrtC* transcripts compared to the high level of nitrate-induced nitrate uptake suggests a role of post transcriptional regulation.

The different regulation associated with nitrate uptake and nitrate reduction in *Synechococcus* sp. strain RF-1 can partly be explained by the dissociation of *narB* from *nrtABCD* gene cluster in the genome. For all cyanobacteria examined, *nirA* and *narB* are generally located upstream and downstream of the genes encoding nitrate transporter (*nrtABCD* or *nrtP*), respectively, within the *nirA* operon (Frías *et al* , 2000). *Synechocystis* sp. strain PCC 6803 is an exception, the *nirA* being found ~400 kb apart from the *nrtABCD-narB* operon (Kaneko *et al*, 1996). No sequences homologous to any of nitrate transporter genes were found in the examined flanking sequences of *narB* in *Synechococcus* sp. strain RF-1.

Expression of the ammonium-repressible genes commonly requires the NtcA protein, a Crp-type transcriptional regulator, which is a critical component of a global nitrogen control in cyanobacteria (Herrero *et al*, 2001). The strict requirement of nitrate or nitrite for induction of nitrate uptake in *Synechococcus* sp. indicates a possible difference in NtcA-mediated gene regulation. In *Synechococcus* sp. strain PCC 7942, nitrate and nitrite are also not essential for the expression of *nirA*-operon. However, NtcB (LysR family transcription factors), in cooperation with NtcA, is involved in nitrite-promoted regulation (Kikuchi *et al,* 1996). In contrast, in *Anabaena* sp. strain PCC 7120 and *Synechocystis* sp. strain PCC 6803, NtcB acts as a nitrite-independent enhancer and is required for high-level expression of the nitrate assimilatory genes (Herrero *et al*, 2001). However, the efficiency of the nitrite-induced nitrate uptake of *Synechococcus* sp. was lower than that of nitrate. This suggests that nitrate per se or nitrate reduction has additional positive effects on the promotion of nitrate uptake in *Synechococcus* sp.

Nitrite and Nitate Uptake by Saccharomyces Cerevisiae Using Wastewaters Supplemented with 0.01% Glucose as a Carbon Source:

The results sumarized in Table 6 appeared that *Saccharomyces cerevisiae* NRRL Y - 12632 and *Saccharomyces cerevisiae* NRRL Y - 136 plays an important role in uptake of nitrite and nitrate from wastewaters than the other strains, as well as, hybrid No. 1 appeared the same trend in the uptake of nitrite and nitrate. This indicated that we can use these strains in improving effluent quality for potential reuse.

Table 6: Nitrite and nitate uptake from wastewater suplemented with 0.01 % glucose as a sole carbon source treated by parental strains of *Saccharomyces cerevisiae* and their hybrids .

Biocontrol agents	ppm				
	Amonia	Nitrite	Avilable	Nitrate	Total
Control (Untreated) without glucose	23.8	16.8	40.6	6.72	47.32
Control (Untreated) with glucose	25.2	25.2	50.4	5.74	56.14
Saccharomyces cerevisiae NRRL Y -12632	8155	24466	32621	9786	42408
Saccharomyces cerevisiae NRRL Y - 11562	-284	2556	2272	880	3152
M.P. 3936	13511	17447	5333	22780	
Hybrid No. 1	838	4611	5449	-587	4862
Hybrid No. 2	1	3	3	1	4
Hybrid No. 3	0	2	2	1	3
Hybrid No. 4	1	2	3	1	3
Hybrid No. 5	0	2636	2636	-1142	1494
Saccharomyces cerevisiae NBIMCC 82	0	2	2	0	2
Saccharomyces cerevisiae NRRL Y - 12619	1	2	3	0	3
Saccharomyces cerevisiae NRRL Y- 136	2662	4881	7544	-2840	4704
Saccharomyces cerevisiae NRRL Y – 137	0	1	1	0	1
Saccharomyces cerevisiae NRRL Y – 1370	0	1	1	0	2

The results have shown a great improvements in the percentage of of nitrite and nitrate removal from factory effluents which increased more than 50%, this leading to improve effluent quality parameters (Table 7). The use of *Saccharomyces cerevisiae* NRRL Y - 12632 and their hybrids appeared higher percentage of nitrate removal. This indicated that these strain and its hybrids resulted in better results in the mode of removal which allows to improve water quality for resue in irrigation. This leading to apply microbial genetic techniques in wastewater treatment plants to improve the biosorption processes that occur in the environment. In this case, these techniques were used to adjust the biochemical processes of a wastewater treatment plant of a chemical fertilizer industry, which has a pond as biological treatment.

Table 7: Percentage of nitrite and nitrate uptake from wastewater suplemented with 0.01 % glucose as a sole carbon source treated by parental strains of *Saccharomyces cerevisiae* and their hybrids .

Biocontrol agents	ppm				
	Amonia	Nitrite	Avilable	Nitrate	Total
Control (Untreated) without glucose	23.8	16.8	40.6	6.72	47.32
Saccharomyces cerevisiae NRRL Y - 12632	17	50	33	88	39
Saccharomyces cerevisiae NRRL Y - 11562	-6	50	22	76	28
M.P. 6	50	28	82	33	
Hybrid No. 1	11	61	36	-34	29
Hybrid No. 2	22	67	44	88	49
Hybrid No. 3	-6	50	22	76	28
Hybrid No. 4	17	39	28	68	32
Hybrid No. 5	0	50	25	-95	13
Saccharomyces cerevisiae NBIMCC 82	6	39	22	51	25
Saccharomyces cerevisiae NRRL Y - 12619	22	67	44	2	40
Saccharomyces cerevisiae NRRL Y- 136	33	61	47	-156	26
Saccharomyces cerevisiae NRRL Y - 137	6	33	19	27	20
Saccharomyces cerevisiae NRRL Y - 1370	11	56	33	100	40

The river water nearing fertilizer industry is being highly polluted by letting out industrial effluents, industrial wastewater, agricultural run off and sewage into the stream. The presence of inorganic ions such as nitrite and nitrate ions, etc., has contributed to the pollution of the river water. As a result water borne diseases have become common in this industrial area and the raw water cannot be used as such for industrial purposes. These river water should be treated properly as seen in this Study and disinfected before being supplied for industrial purposes and human consumption. In relation to the effect of industrial effluents appeared some facts about the effects of hazardous fertilizer wastes on the environment.

The results obtained in this study are in harmony with those obtained by Samina and Pande 2001, who reported that several treatment processes for the removal of nitrates from drinking water have been studied. Every method have merits and demerits, however, the methods based on ion exchange for the removal of nitrates from drinking water and regeneration of resin by biological denitrification appear to have edge over other methods. This communication presents a review on existing denitrification processes and spells out needs for future research.

The results indicated that various yeast strains and hybrids process differ in their efficiency in the removal of nitrite and nitrate. The information on all these aspects are make the biological treatment are more effective to wastewaters suitable for safe reuse.

In conclusion, this study showed that yeast and bacteria can efficiently remove nitrite and nitrate from chemical fertilizer manufacturing industrial effluents. This study also emphasizes the importance and need for carrying out extended testing for the compatibility of biosorption to a specific industrial effluent. The findings of the study indicate that biosorption is a promising technology for removal of nitrite and nitrate in manufacturing effluent. From an overview of microbial sorbents and biowaste as sorbent candidate, it can be concluded that laboratory trials do show their potential for commercialization since it is technically feasible, ecofriendly with good removal capacity. Besides that, it helps in reduction of waste generation. The adsorbent can be regenerated using higher pH buffer and reused up to 8 times without any loss in ions binding capacity in wastewater stream.

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