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Biochemical aspects of water pollution

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Abstract

Water pollution is significant only when it influences living or biological systems either directly or indirectly. All modern activities of humanity increase environmental water pollution.

A Chinese proverb tells us: ***to pollute means to disperse and to disperse is immoral****. This problem is efficient solve with microorganism assistance, bacteria especially. From this point of view it well known that some reductase especially nitrate reductase are found in membrane fraction of bacteria. This scientific information is very important for study natural nitrogen cycle.*

Keywords: water pollution, denitrifying bacteria, specific enzymes.

Introduction

Most current waste treatment methods in use are biological processes which take advantage of the catalytic and metabolic activities of microorganisms for the purpose of converting pollutants to more highly oxidize and hence lees oxygen demanding forms.
A denitrifying bacteria exist is activated sludge and drinking water. These bacteria belong to two genuses. One genus (Nitrosamonas sp) of bacteria utilizes ammonia as the sole source of energy and oxygen is consumed by the respiration as follows

$$NH_3 + 1{,}5\ O_2 \xrightarrow{\text{Nitrosamonas sp}} NO^-_2 + H_2O + H^+$$

The other genus (Nitrobacter sp) of bacteria oxidizes nitrite to nitrate as follows

$$NO^-_2 + 0{,}5\ O_2 \xrightarrow{\text{Nitrobacter sp}} NO^-_3$$

These bacteria can tested for the synthesis of the dissimilatory nitrate reductase under aerobic conditions (dissolved oxygen concentration above 4 mg l^{-1}). The effect of the dissolved oxygen concentration during growth of the bacteria in the synthesis of the dissimilatory nitrate reductase in cells of bacterial strain was studied more extensively.

Enzymology of the processes

In a bacterial cell, nitrate may function as electron acceptor instead of oxygen. F. Pichinoty (1) demonstrated the existence of two types of nitrate reducing enzymes, nitrate reductase B, a soluble enzyme which has a nutritive rather than a respiratory function, and nitrate reductase A, a particle bound enzyme which functions mainly in nitrate respiration and which also has the capacity to reduce chlorate more nitrate reducing bacteria contain reductase A or nitrate reductase B (2). Some contains both types of reductase (3). It was shown, that the differentiation between the tow nitrate reductase with different functions as made by F. Pichinoty, cannot be applied to all nitrate reducing bacteria.

The dissimilatory nitrate reductase of most microorganisms can be induced by nitrate under anaerobic condition. However, sometimes nitrate needs not to be present to induce this enzyme (4). The mechanism by which oxygen and nitrate control the enzyme activities is not entirely clear. It has been found that oxygen repress the synthesis of the dissimilatory nitrate reductase (5).

A. Wirepenny and J.A. Cole (6) suggested that the redox potential of the medium is regulation factor rather than the concentration of oxygen.

In this study same preliminaries results has been given about the synthesis of the dissimilatory nitrate reductase under aerobic condition by a number of bacteria from activated sludge and drinking water.

Materials and Methods

Chemical composition of microbiological medium for activated sludge.

Denitrifying and aerobically grows activated sludge were produced in laboratory installations. The sludge was strongly aerated. The denitrifying sludge was stirred slowly thus favoring anaerobic condition. The load of installation was about 0,1 g COD g^{-1}.

Artificial sewage ($g.l^{-1}$)

Skim milk power 9; urea 0,04; gelatin 0,05; starch 0,10; Na_2HPO_4 2 H_2O 0,02; $MgSO_4$ 7 H_2O 0,002ş $FeCl_3$ 6 H_2O 0,002ş KCl 0,002; with the additional supply of 1,3 g. l^{-1} KNO_3 in the installation. A concentrated phosphate solution sterilized was added to media to a final concentration of 0,02 M pH 7.

Microbiological aspects and cultural production

Aerobic growth was obtained in 500 ml flasks filled with 100 ml medium. The cultures were vigorously aerated. Under these conditions the oxygen transfer rate (OTR) was found to be 10 – 12 mmol O_2 l^{-1} hr^{-1}. The dissolved oxygen concentration during cultivation was > 4 mgl^{-1} for bacteria from activated sludge. The flasks were closed with rubber stoppers and vigorously shaken at 200 rev / min/ 30 ^{0}C.

When the cell density of fee cultures was approx half of possible maximum, the flasks were flushed again with oxygen. During growth, the oxygen content of the medium in this case was maintained above approximately 15 mg l^{-1}. From the early logarithmic growth phase can observe the behaviour of bacterial cells.

Respiration experiments with cell material

The cells were harvested when the culture had reached the end of the logarithmic growth phase. Before experiments was added 20 µg ml^{-1} chloramphenicol to the culture cell to prevent protein synthesis. The culture cell were washed twice with 0,02 M phosphate buffer pH 7 when after they were re-suspended in the same buffer (containing chloramphenicol) supplied with 10 g $^{-1}$ glycerol. Oxygen and nitrate measurements were carried out in a respiromter containing an oxygen and a nitrate electrode as described by Kessel (7).

The oxygen uptake rate of the re-suspended cells was determined when the dissolved oxygen content of the suspension was zero; $NaNO_3$ 5 ml^{-1} was added. The nitrate electrode was also able to detect nitrite, but with much less sensitivity than nitrate was detected. Nitrate reduction rate cell suspensions which were unable to reduce nitrite was calculated by measuring the time necessary to reduce the added nitrate to nitrite assuming that the nitrate reduction proceeded linearly with time. Nitrite was determined chemically with the method of Gries – Ramiyn – van Fek (8). Protein was assayed by the Folin – Ciocalteu method as described Lowry (9) using bovine serum albumin as the standard.

Preliminary results and discussion

The effect of aerobic cultivation on the synthesis of the dissimilatory nitrate reductase is very important Denitrifying bacteria from aerobically grown activated sludge were cultivated in 500 ml flasks filled with 100 ml of medium were shaken vigorously. After centrifuging and washing the cells oxygen uptake rates and nitrate reduction rates of bacteria were determined. The results obtained are calculated as ratio: nitrate reduction rates (mg NO_3 g^{-1} min^{-1}) / oxygen uptake rate (mg O_2 g^{-1} min^{-1}).
The data in Tab 1 show that a number of bacteria from activated sludge possessed a considerable dissimilatory nitrate reductase activity when grown aerobically in a medium without nitrate.

Table. 1 The ratio of dissimilatory nitrate reduction and oxygen uptake rate of bacteria from drinking water and activated sludge.

Nr. Exp.	Bacterial cells from	Diss. Nitrate reduction rate / oxygen uptake rate mg NO^{-1}_3 $g^{-1}min^{-1}$ / mg O_2 g^{-1} min^{-1}
1	Drinking water	0,00
2	Artificial activated sludge	0,40 – 0,90
3	Natural activated sludge	0,90 – 1,50

The values of bacterial strains of 3 exp. (tab. 1) approximated the theoretical values obtained from the reactions (1) and (2) assuming that the respiration rates of glycerol with O_2 and NO_3 were equal

$$O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O \qquad (1)$$
$$NO_3^- + 5\ e^- + 6\ H^+ \rightarrow 3\ H_2O + ½\ N_2 \qquad (2)$$

(1 mg O_2 corresponds with 1,55 mg NO_3^-)

The effect of the oxygen concentration during the bacterial growth on the synthesis of the dissimilatory nitrate reductase.

Bacterial strain was cultivated at different dissolved oxygen concentration

a) above 15 mg l^{-1}
b) above 4 mg l^{-1}
c) above 0,1 mg l^{-1}

The growth of the cell at (a), (b) and (c) proceeded at similar rates.

The bacteria were harvested as soon as the cultures had reached the stationary growth phase. After washing the cells the oxygen uptake rate and dissimilatory nitrate reduction rate were measured in the respire-meter.

Table 2. The oxygen and nitrate rate in the solution.

Dissolved O_2 conc. mg O_2 l^{-1}	Oxygen uptake rate mg O_2 / 100 mg protein min^{-1}	Diss. nitrate reduction mg NO_3^{-1} / 100 mg protein min^{-1}
0	0,300	0,510
≥ 0,1	0,300	0,531
≥ 4,0	0,425	0,380
≥ 15,0	0,453	0,410

From the results obtained (Table. 2) it can be see that the oxygen uptake rates of cells derived from culture grown at a high dissolved oxygen concentration (≥ 15 mg l^{-1}) and grown at poor aeration (≥ 0,1 mg l^{-1}) were lower than those of cells above 4 mg l^{-1} dissolved oxygen. The synthesis of the dissimilatory nitrate reductase decreased with increasing dissolved oxygen concentration during growth of the cells.

The effect of chlorate concentration and nitrate on the synthesis of dissimilatory nitrate reductase of bacterial strains.

F. Pichinoty (3) reported that chlorate ClO_3^- can be used as substrate by the dissimilatory nitrate reductase. However the reduction product, chlorite (ClO_2^-) is very toxic to bacteria. Because of this feature chlorate is often used to obtain mutants which have lost the ability to synthesize the reductase.

The growth of the bacterial culture incubated above 4 mg l^{-1} dissolved O_2 was followed by measuring the cell densities (Fig 1).

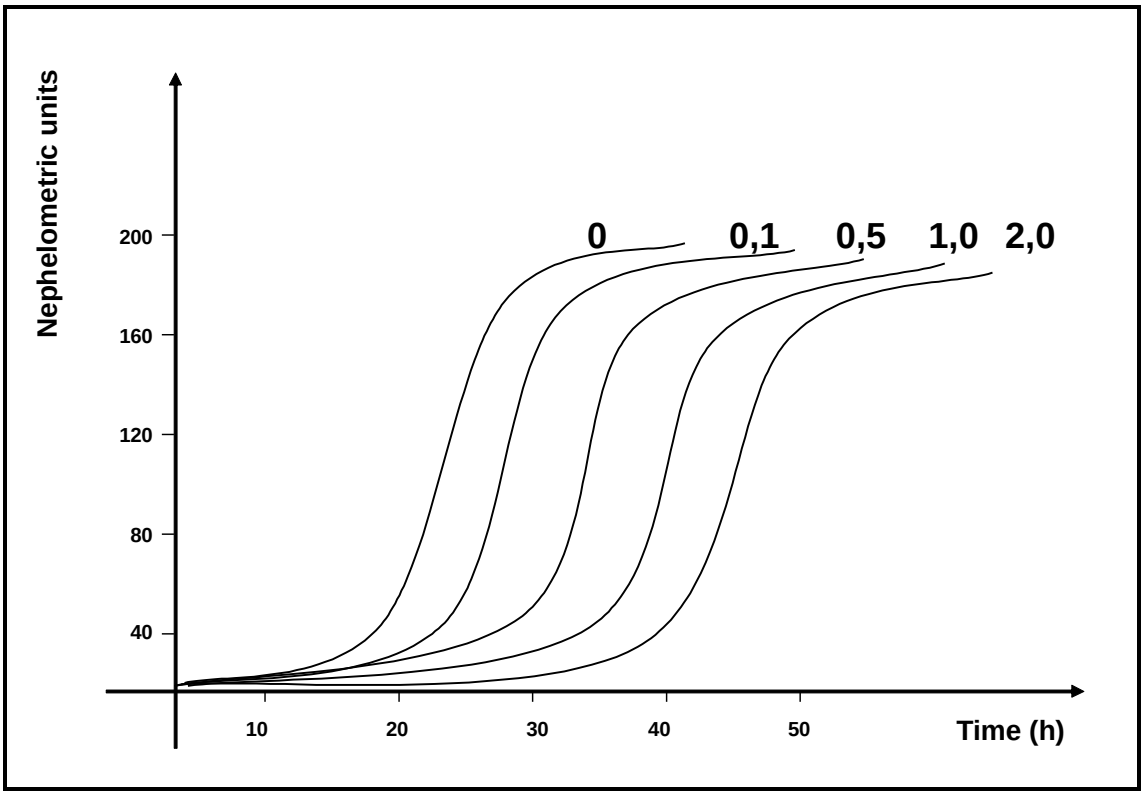

Fig. 1. The growth bacterial strains in microbiological medium supplied with 0; 0,1; 0,5; 1,0; 2,0 g l^{-1} $KClO_3$.

It was observed that under these conditions chlorate only retarded the growth of bacterial strain. Nitrate did not affect growth rate and cell yield.

The dissolved oxygen concentrations during cultivation were maintained above h mg l^{-1}.

Conclusions

From the results obtained in these experiments, it was clearly shown that a number of bacteria in activated sludge are able to synthesize dissimilatory nitrate reductase under aerobic conditions, independent of the N source present. This implies that as soon as the aeration of the sludge, nitrate reduction may be expected. To occur, resulting in a temporary accumulation of nitrite as the synthesis of dissimilatory nitrite reductase is strongly repressed by oxygen.

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