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ECOLOGICAL FACTORS INFLUENCING THE NUMBER OF IRON BACTERIA IN DRAINAGE PIPES

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Abstract. Factors influencing the number of iron bacteria in drainage pipes were examined in summer 1992 in the districts of Tartu and Jõgeva. The main aim of the study was to establish how the number of iron bacteria in the drainage water is influenced by oxygen regime and the type of organic cover material above the drainage pipe. It was found that the number of iron bacteria in drainage pipes depends mainly on the concentration of ferrous iron in the water. Its concentration has a strong relationship with the oxygen regime, which depends on the environmental conditions (moisture, carbon and nitrogen contents). The intensity and direction of the influence of organic cover materials on the count of iron bacteria depend presumably on the weather conditions, first of all on moisture.

Key words: iron bacteria, drainage systems, oxygen regime, organic cover materials, ferrous iron, carbon, nitrogen.

INTRODUCTION

One reason for the clogging of drainage pipes is the formation of ochreous deposits by iron bacteria. In agricultural landscapes the process is most probably carried out by the heterotrophic iron bacteria, which prefer neutral environment. The energy released in this process is probably not used by these bacteria (Дубинина, 1977). Deposition of iron(III) is a characteristic feature of 5—15% of soil microorganisms and of 6—60% of water microorganisms (Балашова & Дубинина, 1989).

Heterotrophic iron bacteria oxidize ferrous iron to decompose the toxic H_2O_2 arising in the aerobic catabolism of organic substrate, so it is obvious that the biological deposition of ochre is influenced by the number and activity of iron bacteria and it will be justified to prognosticate the probability of the clogging of drainage pipes by counting iron bacteria in the drainage water.

According to the literature the main factors determining the distribution of iron bacteria in the environment are the concentrations of oxygen, ferrous iron, and carbon (Дубинина, 1977; Кунце, 1986).

The aim of this study was to establish the effect of the oxygen regime and the type of organic cover material above the drainage pipe on the number of iron bacteria in the drainage water. Organic cover materials (straw, sawdust, etc.) are commonly used in order to inhibit the formation of ochreous deposits in the pipes, but the mechanisms for the inhibition are not clear yet. Presumably, compounds arising in the decomposition of organic cover materials can react with ferrous iron ions and form complexes that are inert and inaccessible to the microorganisms (Bloomfield, 1957). On the other hand, the oxygen-dependent decomposition of the cover materials may influence the number and activity of iron bacteria by depleting oxygen needed for microbial iron oxidation.

MATERIALS AND METHODS

Drainage systems affected by iron ochre deposition were examined in the spring and summer of 1992 in the districts of Tartu and Jõgeva, Estonia.

Descriptions of the sampling sites are presented in the Table. Samples were taken from different depths of the soil (0—15 cm, 15—30 cm, 30—45 cm, 45—60 cm) and from the layer of organic cover material. Oxygen concentration, the content of carbon and nitrogen, moisture, pH_{KCl}, bulk and specific density, and the specific surface of the soil were measured in every sample.

At every sampling site samples of water were taken from the drainage pipes to determine the number of single-cell and filamentous iron bacteria, pH of water, chemical oxygen demand (COD), and the contents of ferrous iron and nitrogen.

The consumption of oxygen by soil was measured by the manometric method of Warburg. A certain amount of soil was measured to the Warburg flask and moistened with distilled water to 60% of moisture; KOH (20%) was added to the internal vial in the flask to bind the arising CO_2; the soils were incubated at 27°C for two hours; results were registered every 20 minutes (Сеги, 1983).

The content of nitrogen was measured by Kjeldahl's method and that of carbon in the soil was established by the method of Tyrin (see Аринушкина, 1961).

Experimental sites

Experimental site	Year of foundation	Cover material	Soil	Use
Vara, Kitse system	1989*	a) Sawdust b) Straw	Sandy loam	Field of potatoes
Laeva	1984	Straw	Peaty sand clay	Hayfield
Verevi system No. 1	1978	a) Without cover b) Chips of wood c) Straw	Sandy loam	Pasture
Tamme	1984	Chips of wood	Wood & reed peat, decomp. degree 50%	Hayfield
Vaimastvere, Koiduküla system	1974	Straw	Wood & reed peat, decomp. degree 50%	Hayfield

* Year of reconstruction.

The bulk and specific density, specific surface, air-filled porosity, moisture and pH_{KCl} of the soil, and COD and concentration of ferrous iron in the water were found by standard methods described in manuals.

The number of single-cell and filamentous iron bacteria was established by filtering the water through a membrane to filters (pore size 0.33 μm) and counting the bacteria on the filters. The iron-containing capsules of the bacteria were stained with $K_3Fe(CN)_6$ (5%) and fixed by the solution of 1% HCl. Filters were dried at 60 °C for 48 h, cleared with immersion oil, and examined under a microscope (Кузнецов & Дубинина, 1989).

The oxygen content in the drainage water was measured with an oxygen meter (UT-8604, made at Tartu University). The oxygen content in the water is presented as the percentage of the maximum oxygen concentration under the temperature measured in the water.

The results were analysed by the correlation analysis by STATGRAFICS. The distribution of the values for carbon and nitrogen content and oxygen consumption of the soil was estimated to be log-normal. So the logarithmic values of these parameters were used to calculate the correlations between different characteristics in the soil.

RESULTS AND DISCUSSION

Factors influencing the number of iron bacteria in water

The number of single-cell iron bacteria varied from 1.1 to 8.1×10^5 cells·ml^{-1} (average $4.4 \pm 0.5 \times 10^5$ cells·ml^{-1}). The number of filamentous iron bacteria was smaller, varying from 0 to 0.9×10^5 filaments·ml^{-1} (average $0.2 \pm 0.04 \times 10^5$ filaments·ml^{-1}). In the collectors the counts of iron bacteria were always less than in the terminal pipes. The number of iron bacteria correlated neither with the type of the drainage field nor the age of the drainage.

The number of single-cell iron bacteria was in reliable correlation with the concentration of ferrous iron ($r=0.68$; $p=0.0004$; $n=22$). The number of filamentous iron bacteria correlated with the logarithmic content of nitrogen in the drainage water ($r=0.57$; $p=0.008$; $n=22$). Figs. 1 and 2 present relationships between the iron bacteria counts and the contents of the ferrous iron and nitrogen in the water.

The equations describing the numbers of single-cell and filamentous iron bacteria in the drainage water derived by the regression analysis are as follows:

$$x_s = 0.38(\pm 0.03) \times [Fe^{2+}], \quad (1)$$

where x_s — the number of single-cell iron bacteria$\times 10^5$, cells·ml^{-1};

$[Fe^{2+}]$ — concentration of ferrous iron in the drainage water, mg·l^{-1}.

The coefficient of determination (R^2) for Eq. (1) is 0.87.

$$x_f = 0.004(\pm 0.00007) \times [Fe^{2+}] \times \ln[N_w], \quad (2)$$

where x_f — number of filamentous iron bacteria$\times 10^5$, filaments·ml^{-1};

$\ln[N_w]$ — logarithmic content of nitrogen in the drainage water, ln mg·l^{-1}.

The coefficient of determination for Eq. (2) is 0.67.

Many researchers have pointed out the leading role of ferrous iron concentration in the environment in the formation of the community of iron bacteria (Дубинина, 1977; Wheatley, 1988). According to the literature the number of iron bacteria is also strongly influenced by the oxygen content of the environment. Some researchers (Seppänen, 1990; etc.) are convinced that the iron bacteria are microaerophilic and high concentrations of oxygen slow down their growth rate and lead to lysis. Some others (Дубинина, 1977) argue that the microaerophility or preference of a microaerobic environment by iron bacteria is connected with the low concentration of ferrous iron in oxygen-rich layers. They consider only *Siderocapsa limoniticus* and *Spirothrix pseudovacuolata* to be true microaerophiles and suggest that the majority of heterotrophic iron bacteria can successfully live both at the low and high concentration of oxygen in the drainage water. During our study period the

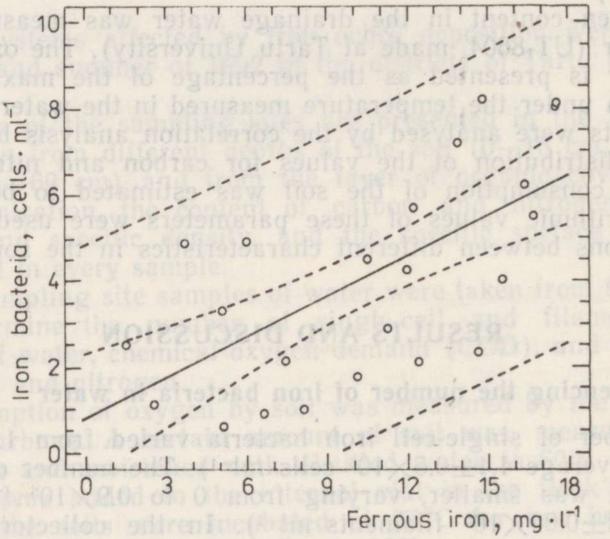

Fig. 1. Relationship between the number of single-cell iron bacteria (×10⁵) and the concentration of ferrous iron in the drainage water.

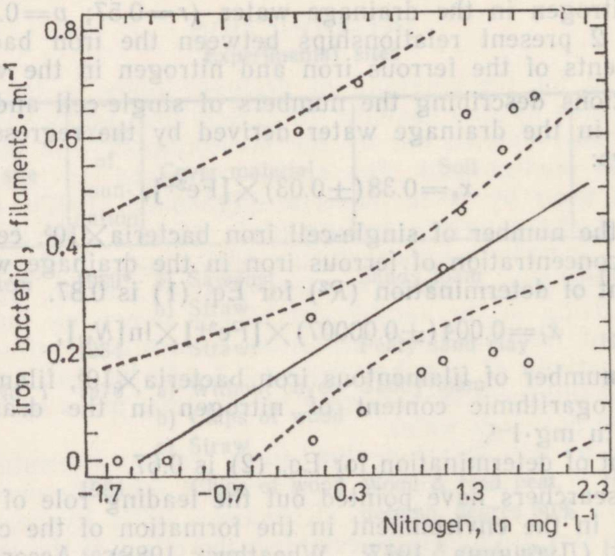

Fig. 2. Relationship between the number of filamentous iron bacteria (×10⁵) and the concentration of nitrogen in the drainage water.

concentration of oxygen was never so low that it might have inhibited the growth of iron bacteria, neither could we assert that high concentrations of oxygen might be unfavourable for the growth of iron bacteria. The fact that the concentration of oxygen of 79% of saturation did not inhibit the growth of iron bacteria may support the assumption of Dubinina that the majority of iron bacteria are not microaerophilic (Дубинина, 1977). Unfortunately, we have not yet enough experimental material to prove the assertion. In the near future additional experiments will be carried out to build up a curve describing the dependence between the oxygen content and the abundance of iron bacteria in the drainage water.

To characterize the content of organic matter in the water, COD was measured. Surprisingly, we could not find any correlation between COD and the number of iron bacteria in the water. The reason can be the evenly high COD in all the water samples studied.

Factors influencing the concentrations of oxygen and ferrous iron in the drainage water

The concentration of oxygen in the water correlated with the logarithmic content of nitrogen ($r=-0.57$; $p=0.03$; $n=12$) and the temperature of the water ($r=-0.5$; $p=0.05$; $n=12$). The concentration of ferrous iron was correlated with the logarithmic content of nitrogen in the water ($r=0.54$; $p=0.04$; $n=22$) and with the consumption of oxygen above the drainage pipes ($r=0.67$; $p=0.03$; $n=22$). The consumption of oxygen is expressed as the sum of the logarithmic numbers for oxygen consumption in different adjacent layers of the soil profile. The concentration of ferrous iron and oxygen in the drainage water showed also correlation ($r=-0.54$; $p=0.02$; $n=22$). Figs. 3 and 4 present relationships between the contents of ferrous iron and oxygen in the drainage water and the oxygen consumption above the pipes.

The equations describing the concentrations of ferrous iron and oxygen in the drainage water found by regression analysis are the following:

$$[O_w]=6.14(\pm0.56)\times T-2.35(\pm0.54)\times\ln[O_c], \qquad (3)$$

where $[O_w]$ — content of oxygen in the water, % of saturation;
T — temperature of the water, °C;
$\ln[O_c]$ — indicator of oxygen consumption, obtained by summing the logarithmic consumptions of oxygen in the adjacent layers of the soil profile, ln $\mu l \cdot g^{-1} \cdot h^{-1}$.

The coefficient of determination of Eq. (3) is 0.85.

$$[Fe^{2+}]=1.74(\pm0.21)\times\ln[O_c]. \qquad (4)$$

The coefficient of determination of Eq. (4) is 0.88.

Our results suggest that the consumption of oxygen above the drainage pipes is a very important factor influencing the concentration of both oxygen and ferrous iron in the drainage pipes.

Consumption of oxygen by the soil covering the drainage pipes

The consumption of oxygen in the soil correlated with the logarithmic contents of nitrogen ($r=0.75$; $p=0.0000$; $n=49$) and carbon in the soil ($r=0.70$; $p=0.0000$; $n=49$) and air-filled porosity of the soil ($r=0.66$; $p=0.0000$; $n=49$). Figs. 5 and 6 present relationships between the oxygen consumption and the contents of carbon and nitrogen in the soil.

The equation describing the consumption of oxygen by the soil was found by regression analysis as follows:

$$\ln[O_c] = 0.51(\pm 0.03) \times \ln[C] - 0.01(\pm 0.003) \times C/N, \quad (5)$$

where $\ln[O_c]$ — logarithmic consumption of oxygen in the soil layer, $\ln \mu l \cdot g^{-1} \cdot h^{-1}$;
$\ln[C]$ — logarithmic content of carbon in the soil, ln %;
C/N — ratio of the carbon and nitrogen contents in the soil.
The coefficient of determination for Eq. (5) is 0.81.

It is obvious from these correlations that the consumption of oxygen above the drainage pipe depends primarily on the contents of carbon

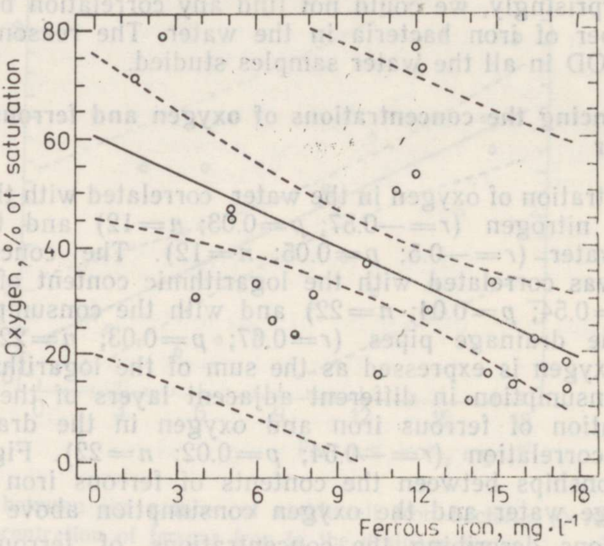

Fig. 3. Relationship between the oxygen content and the concentration of ferrous iron in the drainage water.

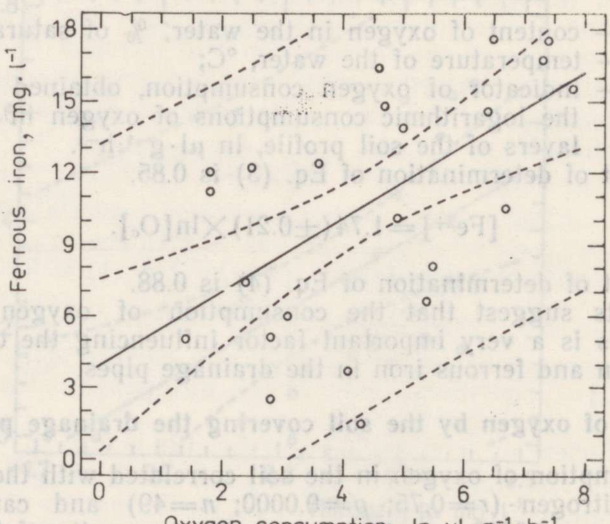

Fig. 4. Relationship between the ferrous iron concentration in the drainage water and the consumption of oxygen above the drainage pipes.

and nitrogen in the soil. According to the literature the temperature and moisture content of the soil are important factors influencing the oxygen consumption in the soil (Donnelly et al., 1990; Lindström, 1990). Unexpectedly, we could not find any reliable correlation between the consumption of oxygen and the temperature and moisture of the soil in our experiments.

In order to study the significance of the soil type the oxygen consumption was observed separately in peaty and mineral soils.

Factors influencing the oxygen consumption in the mineral soil were similar to those observed above (positive correlation with the content of

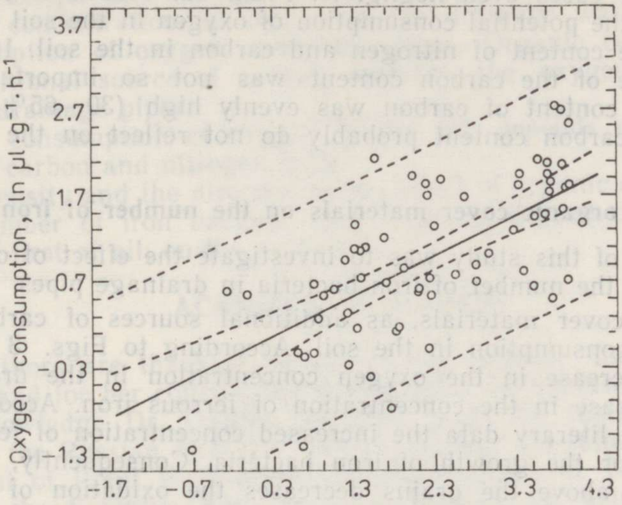

Fig. 5. Relationship between the consumption of oxygen and the concentration of carbon in the soil.

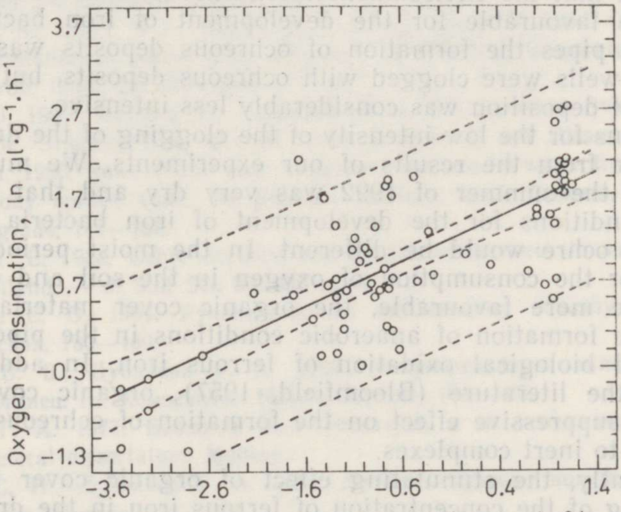

Fig. 6. Relationship between the consumption of oxygen and the concentration of nitrogen in the soil.

carbon and nitrogen). In the peaty soils the oxygen consumption correlated with the temperature ($r=0.68$; $p=0.0009$; $n=20$) and nitrogen content of the soil ($r=0.5$; $p=0.02$; $n=20$); no correlation was found between oxygen consumption and the content of carbon in the soil.

The fact that the consumption of oxygen in the soil seemed to be mainly affected by the concentrations of carbon and nitrogen but not the temperature and moisture of the soil, may be caused by the study methods we used. We incubated the samples and measured the oxygen consumption under optimal moisture and temperature conditions and so the results show the potential consumption of oxygen. The real *in situ* consumption of oxygen in the soil might have been much lower and in some cases even negligible. From our results it could be concluded that the potential consumption of oxygen in the soil depends first of all on the content of nitrogen and carbon in the soil. In peaty soils the influence of the carbon content was not so important, probably because the content of carbon was evenly high (30—65%) and minor changes in carbon content probably do not reflect on the oxygen consumption.

Influence of organic cover materials on the number of iron bacteria

One aim of this study was to investigate the effect of organic cover materials on the number of iron bacteria in drainage pipes.

Organic cover materials, as additional sources of carbon, increase the oxygen consumption in the soil. According to Figs. 3 and 4 this causes a decrease in the oxygen concentration in the drainage pipes and an increase in the concentration of ferrous iron. According to our findings and literary data the increased concentration of ferrous iron is favourable for the growth of iron bacteria. Consequently, high oxygen consumption above the drains decreases the oxidation of ferrous iron and may stimulate the growth of iron bacteria in the pipes.

Concluding the results of the experiments we can assert that the number of iron bacteria and thus the clogging of pipes with ochre are influenced by the environmental conditions above the pipes.

A comparison of the ochreous deposits in the pipes and in the settling wells showed that the environmental conditions in the drainage systems studied were favourable for the development of iron bacteria, but in the drainage pipes the formation of ochreous deposits was suppressed. The settling wells were clogged with ochreous deposits, but in the pipes the process of deposition was considerably less intensive.

The reasons for the low intensity of the clogging of the drainage pipes are not clear from the results of our experiments. We must take into account that the summer of 1992 was very dry and that in the moist years the conditions for the development of iron bacteria and for the formation of ochre would be different. In the moist periods, when the conditions for the consumption of oxygen in the soil and on the cover materials are more favourable, the organic cover materials may contribute to the formation of anaerobic conditions in the pipes, inhibiting chemical and biological oxidation of ferrous iron. In addition, as we know from the literature (Bloomfield, 1957), organic cover materials may have a suppressive effect on the formation of ochreous deposits by binding iron to inert complexes.

Consequently, the stimulating effect of organic cover materials on the increasing of the concentration of ferrous iron in the drainage pipes may be one of the effects of organic cover materials realized under concrete conditions.

For the investigation of the effect of organic cover materials on the number of iron bacteria additional experiments are needed.

CONCLUSIONS

One reason for the clogging of drainage pipes is the formation of ochreous deposits by iron bacteria. In summer 1992 the factors influencing the number of iron bacteria in the drainage pipes were investigated in drainage systems in the districts of Tartu and Jõgeva, Estonia.

According to the results the following conclusions can be drawn:

1. The number of iron bacteria in the drainage pipes depends mainly on the concentration of ferrous iron in the water.
2. The concentration of ferrous iron in the drainage water is correlated with the oxygen consumption above the pipes and the concentrations of oxygen and nitrogen in the drainage water.
3. The concentration of oxygen in the drainage water depends on the consumption of oxygen above the pipes. Organic cover materials, as an additional source of carbon, increase the oxygen consumption above the drainage pipes.
4. The consumption of oxygen in the soil depends on the concentrations of carbon and nitrogen in it.

The intensity and the direction of the effect of organic cover materials on the number of iron bacteria was found to depend on the weather conditions, first of all, on the moisture.

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