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# The Role of Biodiversity of Aquatic Organisms in Oil Biotransformation by Bacteria

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**Abstract:** Bioremediation of soils and natural waters polluted with hydrocarbons remains one of actual ecological problems. Many bacterial species were shown to be active in oil biodegradation. This work was devoted to investigation of oil-destructive bacteria grown in various cultivation media. It was found that biological components may influence the activity of the bacterial degraders.

**Key words:** Bacteria · Oil · Biotransformation · Aquatic organism

## INTRODUCTION

Bioremediation of soils and natural waters polluted with hydrocarbons (oil and other products) remains one of actual ecological problems [1,2]. In the past two decades, microbiological techniques developed for hydrocarbon degradation became promising [3]. Many bacterial species were shown to be active in oil biodegradation [4,5]. We previously reported on the oil-destructive activity of *Pseudomonas melochlora* [6]. However, it should be noted that some environmental factors may influence oil-destructive activity of bacterial degraders. This work was devoted to investigation of oil-destructive bacteria grown in various cultivation media.

## MATERIALS AND METHODS

10 unidentifiable bacterial strains were taken into this study to investigate their growth and oil-destructive activity. Bacteria were isolated from the root area of reed mace *Typha latifolia* L. Macrophytes were taken from Sredniy Kaban Lake (Kazan, Republic of Tatarstan, Russia). To cultivate the bacteria, we used the growth medium with the following content (g per L of water): $NH_4NO_3$ – 0.8, $NH_4H_2PO_4$ – 1.0, $KH_2PO_4$ – 0.2, $MgSO_4 \cdot 7H_2O$ – 0.1, NaCl – 0.1, desalinized oil (Romashkinskoe oil field (Tatarstan, Russia) - 600 mg/L, pH-7.0-7.2. For cultivation of bacteria, 2 mL of bacterial subculture was inoculated into 400 mL flasks. Growth rate of the bacteria was evaluated on Petri dishes with agar and the indicated supplements and without them (control). The amount of the used oil in growth medium was checked at the end of the experiment. The extraction of unoxidized oil was performed with the use of carbon tetrachloride (in proportion 1:20). To eliminate the dead bacteria, oil extract was centrifuged at 8000 *g*; then optical density of the solution was analyzed using infrared spectrophotometer at $\lambda$ = 3350 nm. The respiration of *P. melochlora* in a mixture of oil-oxidizing culture was analyzed by Warburg method [7]. To obtain root excreta of the macrophytes, each plant was carefully washed with water, parched and weighted. After that, plants were treated with 3% peroxide, washed twice with sterile water and put into 2 L flask with sterile distilled water (400 mL). Plants were incubated in this flask for 24 h at natural lighting. Solutions with macrophyte excreta were concentrated in rotary evaporator at 40-50°C.

To test the effect of different medium content, the following components were added – chlorella (*Chlorella vulgaris*) at concentration of $10^6$ cells per mL, daphnia (*Daphnia magna* Straus) – 10 species per 200 mL

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of medium, 10 oil-destructive bacterial strains (at concentration of 4.0-7.8x$10^6$ cells per mL, $N_1$). Spontaneous microflora of water with macrophyte excreta ($N_2$) as well as microflora from chlorella ($N_3$) and daphnia ($N_4$) were also assessed.

Experiments were performed in triplicate. The data on figures are presented as mean±standard deviation.

## RESULTS AND DISCUSSION

We investigated the possibility for increasing the intensity of oil biotransformation in the late autumn period (the 3rd decade of October) after amplification of the content of aquatic organisms in the medium. Figure 1 presents data on dynamics of number of the above-mentioned strains isolated from reed mace and concurrent microflora introduced with various hydrobionts. It is clear from Fig. 1 that maximal number of bacteria was detected in medium with the most complex content by the 3rd day (variant #5). In variant #5, we detected a rapid decrease in bacteria number by 5th day while in other variants a tendency to growth was still observed. It may be explained by accumulation of some toxic substances in the medium that may inhibit bacterial growth.

Figure 2 presents data on a number of chlorella cells in media with different content. It is clear that extension of the medium content resulted in inhibition of its growth, especially by 6th and 7th days. The observed phenomenon may be explained by competition for nutrient resources. It is likely that exometabolites of *T. latifolia* L. provoke decrease in chlorella number. The analogous effect was

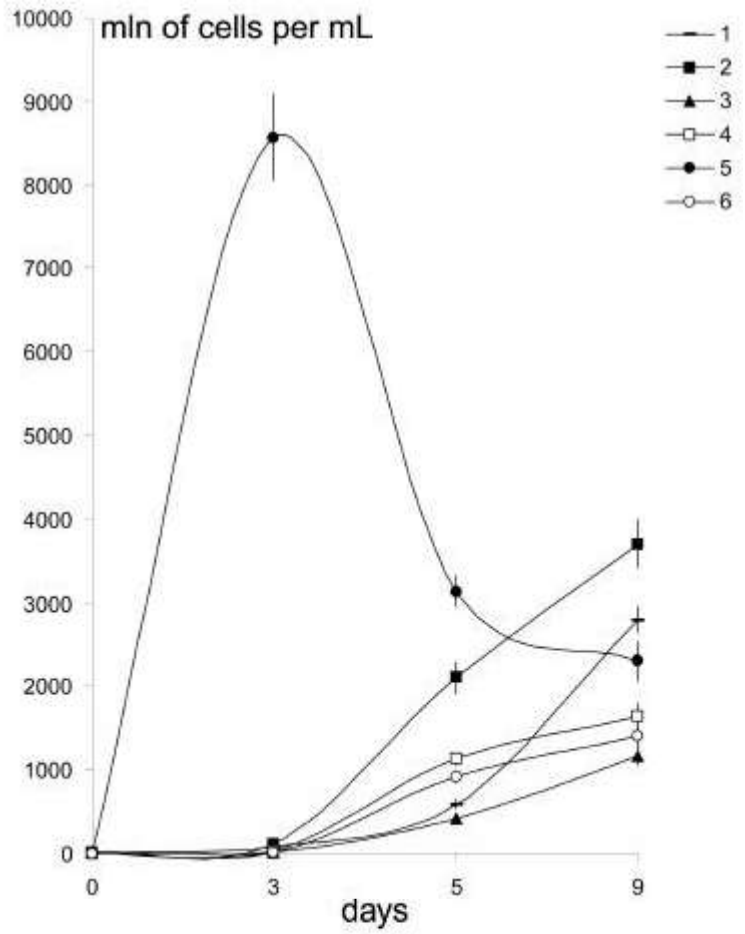


Fig. 1: Dynamics of microflora number in media with various biological and chemical content.
Note: 1 – sterile tap water + $N_1$; 2 - $N_1$ + $N_2$ + exometabolites of *T. latifolia* L.; 3 - $N_2$ + exometabolites of *T. latifolia* L.; 4 - $N_1$ + $N_3$ + $N_4$ + phyto- + zooplankton; 5 - $N_1$ + $N_2$ + $N_3$ + $N_4$ + phyto- + zooplankton + exometabolites of *T. latifolia* L.; 6 - $N_3$ + phytoplankton

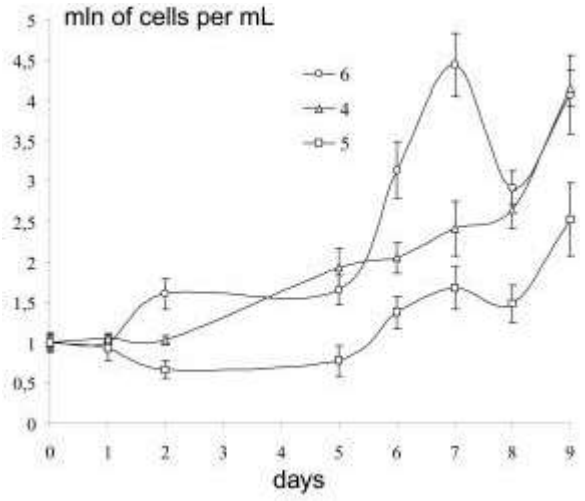


Fig. 2: Number of chlorella (mln of cells per mL) in media with different content.
Note: 6 - $N_3$ + phytoplankton; 4 - $N_1$ + $N_3$ + $N_4$ + phyto- + zooplankton; 5 - $N_1$ + $N_2$ + $N_3$ + $N_4$ + phyto- + zooplankton + exometabolites of *T. latifolia* L.

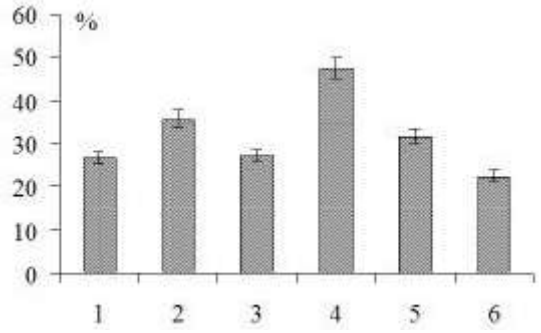


Fig. 3: Biotransformation of oil in media with various contents.
Note: 1 – sterile tap water + $N_1$; 2 - $N_1$ + $N_2$ + exometabolites of *T. latifolia* L; 3 - $N_2$ + exometabolites of *T. latifolia* L; 4 - $N_1$ + $N_3$ + $N_4$ + phyto- + zooplankton; 5 - $N_1$ + $N_2$ + $N_3$ + $N_4$ + phyto- + zooplankton + exometabolites of *T. latifolia* L; 6 - $N_3$ + phytoplankton

detected while daphnia were added to the medium. Synchronous introduction of the macrophyte exometabolites and daphnia to the medium caused a very prominent reduction of chlorella number.

Figure 3 reflects a rate of oil biotransformation in media with different content. It is clear that physiological activity of the macrophyte exometabolites in this period of a year is not significant towards oil-destructive and concurrent microflora. It is important to note that the maximal level of oil biotransformation was achieved in the case when the content of medium was not the most complex (47.2%, variant 4). Although a number of bacteria in this case are not too high, their physiologic activity is possibly more prominent. It is interesting to compare data of this study and previously reported data [6]. For example, in the absence of the macrophyte exometabolites we detected oil biotransformation at 26.7% in comparison with 15% in the case of bacterial monoculture *P. melochlora* [6]. It is interesting to note that oil oxidation was about equal during the present of plant exometabolites (35.8% and 38.7% for our strains and for *P. melochlora,* respectively). However, it is worth to note that the equal level of oil oxidation was detected at lower activity of the macrophyte exometabolites. It is clear from the Fig. 3 that complicating growth medium favors to oil oxidation even in the autumn period when the activity of all living processes are decreased. Changing growth medium, it is possible to achieve a level of oil oxidation that is observed in August [6].

The performed studies confirm that allelopathic interactions of the higher aquatic plants (owing to exometabolites and concurrent destruction microflora) mediate ecological-physiological mechanisms of water quality and biological diversity. Microphytes, heterotrophs and concurrent bacteria speed up the process of pollution transformation. Acting together, these representatives of phytohydrocoens take part in the formation of water quality via increasing xenobiotic's transformation (as it was shown with oil) and enhancing toxicoresistance of aquatic organisms to pollutants [8].

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