

## DEGRADATION OF AROMATIC COMPOUNDS BY THE OIL-OXIDIZING BACTERIA *NOCARDIA VACCINII* K-8 AND *ACENITOBACTER CALCOACETICUS* IMB B-7241

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**Abstract.** Nowadays the problem of environmental remediation of aromatic xenobiotics is rather actual because of their pronounced carcinogenic and mutagenic properties. Biotechnologies may be an alternative to physical and chemical methods of environmental remediation thanks for the high destructive potential of the microorganisms, ecological safety and cheapness. It was shown that the oil-oxidizing bacteria *Nocardia vaccinii* K-8 and *Acenitobacter calcoaceticus* IMB B-7241 assimilated substrates of aromatic nature and synthesized practically valuable surfactants. The growth of strains K-8 and IMV B-7241 on aromatic compounds was intensified in 1.5–2 times in the case of the consecutive cultivation of the inoculum of the studied strains on liquid medium containing aromatic compounds (0.1–0.25%) compared to using the inoculum cultivated on the meat infusion agar. These strains are promising for use in the remediation of water and soil polluted with aromatic xenobiotics.

**Keywords:** *Nocardia vaccinii* K-8, *Acenitobacter calcoaceticus* IMB B-7241, aromatic compounds.

**Introduction.** Up to 13 million lives could be saved each year by reducing environmental risks, according to the World Health Organization's first country-by-country analysis of the impact of environmental factors on human health. Environmental pollution is a great concern to all countries around the world. China has made great efforts in this area and plans to invest \$175 billion in environmental protection between 2006 and 2010, according to the National Development and Reform Commission of China [2].

Aromatic compounds are serious pollutants, being present mainly in industrial wastewater from chemical, petrochemical, pharmaceutical, textile and steel industries. Due to their ubiquitous occurrence, recalcitrance, bioaccumulation potential and carcinogenic activity, the aromatic compounds have gathered significant environmental concern [3]. They are widely distributed environmental contaminants that have detrimental biological effects, toxicity, mutagenicity and carcinogenicity [6]. Although aromatic hydrocarbons may undergo adsorption, volatilization, photolysis and chemical degradation, microbial degradation is the major degradation process. Bioremediation is the tool to transform the compounds to less hazardous or nonhazardous forms with less input of chemicals, energy and time [1]. Besides microbiological methods are economically advantageous, do not require large capital investments and operating costs, and local sewage treatment plants take low areas and very easy to maintain [3].

Numerous reports [1–6] have described the ability of bacteria species to degrade aromatic and hydrocarbons such as *Nocardia vaccinii* and *Acenitobacter calcoaceticus*. Previously it was shown that the bacteria *Nocardia vaccinii* K-8 and *Acenitobacter calcoaceticus* IMV B-7241 intensified the processes of oil degradation in contaminated sites. Since crude oil always contains aromatic hydrocarbons (10–50%), we assumed that the studied strains may be potential destructors of aromatic compounds. In this regard, the aim of our work was to study the ability of strains IMV B-7241 and K-8 to grow on nutrient media containing substrates of aromatic nature as a carbon and energy source.

**Materials and methods.** In the previous work the oil-oxidizing bacteria identified as *Nocardia vaccinii* K-8 and *Acenitobacter calcoaceticus* K-4 were isolated from the oil-polluted samples of soil. The ability of the strains to synthesize the metabolites with surface-active and emulsifying activity (biosurfactants) during their cultivation on different hydrophobic (*n*-hexadecane, liquid paraffin) and hydrophilic (glucose, ethanol) substrates was determined. The strain K-4 was deposited in the Depository of microorganisms of the Institute of Microbiology and Virology of National Academy of Sciences of Ukraine at the number of IMV B-7241.

*A. calcoaceticus* IMV B-7241 was cultivated on the nutrient medium of the following composition (g/L): NaCl – 1.0; Na<sub>2</sub>HPO<sub>4</sub> – 0.6; (NH<sub>2</sub>)<sub>2</sub>CO – 0.35; KH<sub>2</sub>PO<sub>4</sub> – 0.14; MgSO<sub>4</sub>×7H<sub>2</sub>O – 0.1; pH 6.8–7.0; the yeast autolysate – 0.5 % (v/v) and trace elements solution – 0.1 % (v/v) were also added.

*N. vaccinii* K-8 strain was grown on the synthetic nutrient medium containing (g/L): NaNO<sub>3</sub> – 0.5; MgSO<sub>4</sub>×7H<sub>2</sub>O – 0.1; CaCl<sub>2</sub>×2H<sub>2</sub>O – 0.1; KH<sub>2</sub>PO<sub>4</sub> – 0.1; FeSO<sub>4</sub>×7H<sub>2</sub>O – 0.1, yeast autolysate – 0.5 % (v/v).

Phenol, 4-chlorophenol, hexachlorobenzene, naphthalene, benzoic, sulfanilic and N-phenylantranilic acids 0.3–0.5 % (v/v), benzene and toluene 0.3–0.5% (w/v) were used as soul carbon and energy sources.

The variants of the inoculum were the following. Variant 1 – 24-hour culture cultivated on the meat infusion agar (MIA). Variant 2 – culture cultivated on the liquid mineral medium containing above-mentioned nutrients and aromatic compounds (0.1–0.25%) as soul carbon and energy sources. The inoculum preparation included the adaptation of bacteria to aromatic compounds by gradual increase of their concentration from 0.1 to 0.25 % in nutrient medium followed by bacteria inoculating on mineral medium with 0.3–0.5% of the substrate. For acclimatization a loopful of organisms cultivated on the MIA (24 h) was directly inoculated into flasks containing aromatic compounds (0.1%) and all the required nutrients. The culture was kept in a rotary shaker for 72 h. This formed the primary culture. The secondary acclimatized inoculum was prepared in the same way, wherein 10% (v/v) of primary culture was used instead of the subculture to inoculate the medium containing aromatic compounds (0.15%) and in this case the culture was incubated for 48 h. This was continued for the third and fourth acclimatization by gradual increase of aromatic compound concentration in nutrient medium to 0.2 and 0.25%, respectively. Variant 3 – 48-hour culture incubated in the liquid mineral medium containing above-mentioned nutrients and aromatic compounds (0.3–0.5%) as soul carbon and energy sources. The inoculum was used in a concentration of 10% (v/v).

The cultivation of bacteria took place in the 750 ml Erlenmeyer flasks with 100 ml of medium on rotor shaker (320 rpm) at 28–30 °C during 72–96 h.

The quantity of synthesized surfactant was evaluated by such indexes: conditional surfactant concentration (CSC\*) and emulsification index (E<sub>24</sub>, %) of the cultural liquid. The number of viable cells was determined by the Koch method on MIA, biomass – by the optical density of cultural liquid, followed by recalculation to absolutely dry biomass by calibration graph.

**Results and discussion.** It was determined that *N. vaccinii* K-8 and *A. calcoaceticus* IMV B-7241 intensively grew on phenol, hexachlorobenzene, naphthalene, N-phenylantranilic and benzoic acid, slightly worse on toluene, benzene and sulfanilic acid and died on 4-chlorophenol.

The utilization of aromatic compounds accompanied by the formation of extracellular metabolites with surface-active and emulsifying properties (Table 1).

Thus, during *A. calcoaceticus* IMV B-7241 cultivation on phenol (0.5%) the highest conditional surfactant concentration (CSC\*) and emulsification index ( $E_{24}$ , %) were 3.6 and 70%, respectively (while on ethanol CSC\* – 1.0 and  $E_{24}$  – 43%).

The maximum indexes of surfactant synthesis by *N. vaccinii* K-8 were observed as a result of strain growth on the media with naphthalene (0.5%): CSC\* – 2.6 and  $E_{24}$  – 70%, while on glycerol (0.5%) – 2.0 and 60%, respectively.

Similar results were described by Nitschke et al. [4] testing polycyclic aromatic hydrocarbon degradation by *Pseudomonas aeruginosa* strains. So, *P. aeruginosa* N43 synthesized extracellular metabolites with surface-active and emulsifying properties on phenantrene (0.5%): conditional surfactant concentration and emulsification index were 3,3 and 75%, respectively.

**Table 1.**  
**Surfactant synthesis during *Acenitobacter calcoaceticus* IMB B-7241 cultivating on aromatic compounds**

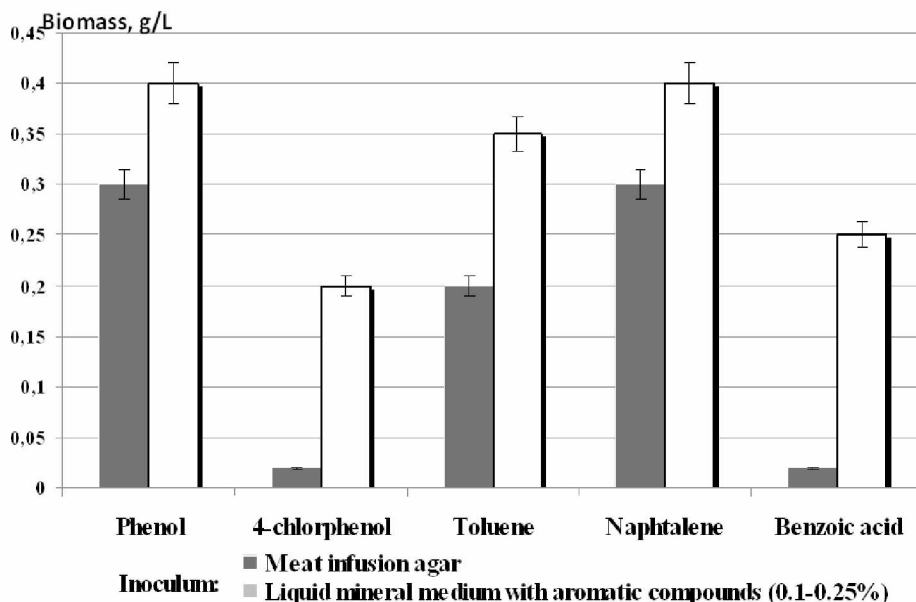
Substrate	Concentration, %	CSC*	$E_{24}$ , %
Phenol	0.3	3.2±0,16	65±3,2
	0.5	3.6±0,18	75±3,7
Benzene	0.3	1.6±0,08	50±2,5
	0.5	1.5±0,08	50±2,5
Toluene	0.3	1.7±0,09	55±2,7
	0.5	1.2±0,06	50±2,5
Benzoic acid	0.3	2.1±0,1	55±2,7
	0.5	2.8±0,14	52±2,6
N-phenylantranilic acid	0.3	1.9±0,09	45±2,2
	0.5	2.0±0,1	50±2,5
Hexachlorobenzene	0.3	1.5±0,08	45±2,2
	0.5	1.7±0,09	53±2,7
Ethanol (control)	0.3	0.8±0,04	40±2,0
	0.5	1.0±0,05	43±2,1

*Note.* The inoculum – 24-hour culture cultivated on the meat infusion agar, the cultivation took place during 96 h.

Moreover, it was shown that the increased concentrations of biomass (30–40 % of control) have been observed in the case of three consecutive inoculating of strains K-8 and IMV B-7241 on medium containing aromatic compounds (0.3–0.5%).

Based on this result, further the inoculum preparation included the adaptation of bacteria to aromatic compounds by gradual increase of their concentration from 0.1 to 0.25 % in nutrient medium followed by bacteria inoculating in mineral medium with 0.3–0.5% of the substrate. It was determined, that the process of cultivation was accompanied by 1.5–2.0-fold biomass increase compared to using the inoculum cultivated on the meat infusion agar (figure).

It should be noticed, that in the case of the consecutive cultivation of the inoculum in liquid medium containing aromatic compounds (0.1–0.25%) biomass concentration of the strain K-8 was 0.4 g/L while Shetty et al. [5] reported that biomass of *Nocardia hydrocarbonoxydans* on phenol (0.5%) was 0.25 g/L subject to use above-mentioned method.



*Effect of the inoculum quality on growth of N. vaccinii K-8 on aromatic compounds (0.3%)*

### Conclusions

1. It was determined, that *N. vaccinii* K-8 and *A. calcoaceticus* IMV B-7241 showed a particular ability to assimilate aromatic compounds as soul carbon and energy sources and to synthesize practically valuable surfactants.

2. The growth of strains K-8 and IMV B-7241 on aromatic compounds was intensified in 1.5–2.0 times in the case of the consecutive cultivation of the inoculum on liquid mineral medium with aromatic compounds (0.1–0.25%) compared to using the inoculum cultivated on the meat infusion agar.

3. These strains are promising for use in the remediation of water and soil polluted with crude oil and aromatic xenobiotics.

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