ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 2, pp. 158–163. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © D.A. Filatov, L.I. Svarovskaya, V.S. Ovsyannikova, L.K. Altunina, 2011, published in Mikrobiologiya, 2011, Vol. 80, No. 2, pp. 169–174.

EXPERIMENTAL ARTICLES

The Effect of Film-Corrected Light on Oxygenase Activity of Microorganisms of the Genus *Pseudomonas*

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Received January 19, 2010

Abstract—The influence of the light transformed by a light-correcting film on the growth dynamics and enzymatic activity of hydrocarbon-oxidizing microorganisms of the genus *Pseudomonas* (*P. stutzeri* and *P. putida*) was investigated under laboratory conditions in liquid medium with 2% oil. The numbers of investigated microorganisms increased by 2–2.5 orders of magnitude due to application of the light-correcting film as a cover material. The dehydrogenase and catalase activities increased by 2–2.5 times. The rate of accumulation of aldehydes (intermediate products of metabolism of oil hydrocarbons) increased by 2.5 times. According to the data of gas-liquid chromatography and elemental analysis, the processes of microbial oxidation of oil hydrocarbons proceeded much faster than in the control variants.

Keywords: hydrocarbon-oxidizing microorganisms, light-correcting films, photoluminophore, dehydrogenase, catalase, red light, photoluminescent activation.

DOI: 10.1134/S0026261711020056

The light sensitivity of microorganisms is presently attracting increasing attention. Significant results have been achieved in studies of a number of photobiological processes [1]. All plants and algae were found to possess specific photoregulatory systems that efficiently control the growth and development of these organisms [2]. Its presence in a wide variety of microorganisms suggests considering the photoregulatory system as an ancient light-sensitive regulatory system. Few data are available in the literature concerning the effects and mechanisms of the action of light on the metabolism of heterotrophic microorganisms. Investigation of the regulatory effects of light on microbial activity is one of the important problems of modern photobiology [3–5].

Light-correcting films used to increase the productivity of agricultural crops have recently become the subject of particular attention [6, 7]. The effect of these films on plant development is ascribed to modifications in the quantitative and qualitative characteristics of the passing solar electromagnetic radiation, namely, absorption of UV radiation and its transformation into the red part of the spectrum [8]. Earlier, we have shown that, after passing through the light-correcting film and transformation by the film photoluminophores, solar radiation stimulates the growth and oxygenase activity of aboriginal soil microflora during oil biodegradation [9, 10].

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It is not completely clear by which mechanism the transformation of solar radiation affects the growth and biochemical activity of microflora. An increase in bacteria numbers and changes in DNA, RNA, and protein synthesis have been detected [2].

Aerobic bacteria of the genus *Pseudomonas* are a scientifically and practically important group of microorganisms widespread in the biosphere and contributing actively to the processes of organic matter decomposition [12]. This was the reason they were used as the subjects for our study.

The goal of the present work was to investigate the stimulating effect of the light transformed by a light-correcting film on the population dynamics and biochemical activity of *Pseudomonas stutzeri* and *P. putida* during oxidation of oil hydrocarbons in a liquid medium.

MATERIALS AND METHODS

Strains *Pseudomonas stutzeri* and *P. putida* isolated from the formation water of an oilfield were used in this work.

Species identification of the strains was performed at TsKP Sekvenirovanie, Novosibirsk. DNA was isolated using a Medigen kit. The sequences were compared to the sequences in the nr NCBI database.

The organisms were grown in batch culture in a Raymond liquid mineral medium with 2% oil from the

Las-Egan deposit as the sole source of carbon and energy.

The experiment was performed in three modes.

(1) Under light that has passed through a light-correcting FE film (the luminophore embedded in the film is an organic complex of europium nitrate with phenanthroline, with the main peak in the luminescence spectrum at 615 nm).

(2) Under light that has passed through a film made of high-pressure polyethylene (HPPE).

(3) In darkness under a glass cover.

The two latter modes were used as the controls.

A combined spectrum of two lamps, LB-40 (Russia) and LH9-UV BLACK LIGHT (Nantong Sampfier Lighting Electrical Co., People's Republic of China), was used as a lighting source. The LB-40 lamp imitates the solar radiation spectrum within the region of photosynthetically active radiation (PAR, 400–710 nm) with irradiation intensity of 29 W/m^2. The UV lamp imitates the UV region of the solar radiation spectrum within the range of 320–400 nm with a maximum at 365 nm, power of 9 W, and irradiation intensity of 4 W/m^2. Irradiation was carried out for 6 h a day. Light transmittance of the HPPE film was 65% in the UV range (320–400 nm) and 59%, in the PAR range (400–710 nm). The relevant values for the FE film were 76 and 73%, respectively.

The duration of the experiment was 15 days at 18–20°C. During the experiment, the enzyme activity, number of microorganisms, and content of aldehydes as the intermediate products of hydrocarbon (HC) oxidation were monitored.

Cell numbers were determined by plating on agar media [13]. Catalase activity was determined in a gasometric assay by the rate of hydrogen peroxide decomposition expressed as ml O_2 per 1 ml of the medium for 1 min [14]. Dehydrogenase activity was determined in a reaction with 2,3,5-triphenyltetrazolium chloride (TTC) reduced to 2,3,5-triphenylformazan (TPF). The amount of TPF was determined by spectrophotometry at 540 nm. The activity was expressed as milligrams TPF formed upon incubation of 1 ml of the culture with 1 ml of 1% TTC solution for 24 h [14]. Aldehyde content was measured according to Faigle using the fuchsin-based reagent. Aliquots of the culture fluid (2 ml) were treated with equal volumes of sulfurous and fuchsin sulfurous acids. After 20–30 min, purple to blue coloration developed and the optical density was measured at 570 nm [15].

Alterations in the hydrocarbon composition of oil were determined at the end of the experiment by gas–liquid chromatography on a Chromos GKh-1000 (ZAO Khimanalitservis, Dzerzhinsk, Russia) equipped with a flame ionization detector and a 25-m capillary column filled with SE 54 stationary phase (Sigma-Aldrich, United States).

Oil elemental composition was determined by combustion of C, H, and N in a Pokrovsky reactor with subsequent gas chromatography of destruction products; S, by the Schoeniger flask method; and O, by the difference in the content of the elements [16, 17].

The measurements were performed in triplicate in three independent series of experiments. Statistics processing of the data was performed with Statistica for Windows using a 0.95 confidence interval. The data in the figures show the average values with two-sided confidence intervals.

RESULTS AND DISCUSSION

Irradiation with light transformed by a light-correcting film resulted in an increase in the numbers of hydrocarbon-oxidizing microorganisms *P. stutzeri* and *P. putida* by 2–2.5 orders of magnitude compared to the control variants; i.e., photostimulation of microbial growth occurred.

The maximum numbers of *P. stutzeri* and *P. putida* were observed on day 9 of the experiment and were 1.19–1.20 × 10^9 CFU/ml, respectively. The bacterial population in the controls did not exceed 6 × 10^6 CFU/ml (Figs. 1a, 1b).

As was previously demonstrated in a number of works [2, 3], in *P. fluorescens* irradiated with red light, prior to growth stimulation, intensified nucleic acid synthesis and cell respiration were observed. The structure of light action spectra was similar for different microorganisms. In all known cases, irradiation resulted in decreased duration of the lag phase and generation time, i.e., in photostimulation of microbial growth [11].

There are several hypotheses concerning the mechanisms of the stimulatory action of light on nonphotosynthesizing microorganisms.

(1) Supposedly, light affects the key segment of the regulation of cellular metabolism, since such fundamental processes as protein synthesis and cell division are modified. At the biochemical level, accelerated RNA synthesis was found to be the primary response of the microorganisms to exposure to light. Subsequently, irradiated cells were exhibited increased proliferation rate and more rapid arrival at the stationary growth phase [2, 5].

(2) One of the central elements of the microbial regulatory system, which normally functions independently of the presence of light quanta, is probably photosensitive and belongs to photochromes, which change their spatial configuration upon absorption of

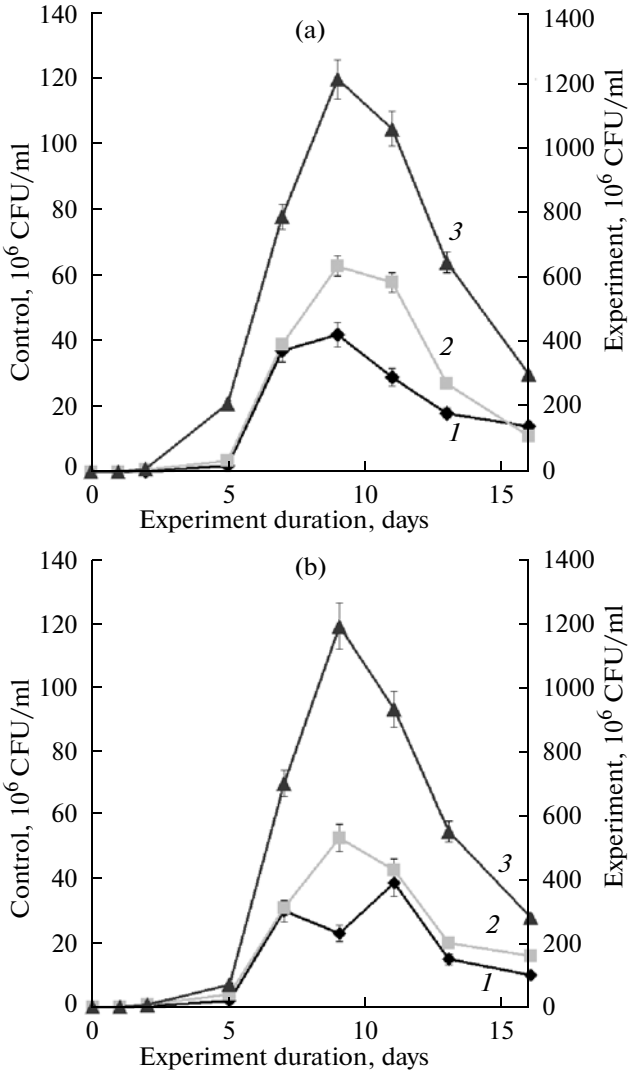

Fig. 1. Number of *P. stutzeri* (a) and *P. putida* (b) bacteria under film-corrected light (experiment *3*). Controls: HPPE film (*2*) and without irradiation (*1*).

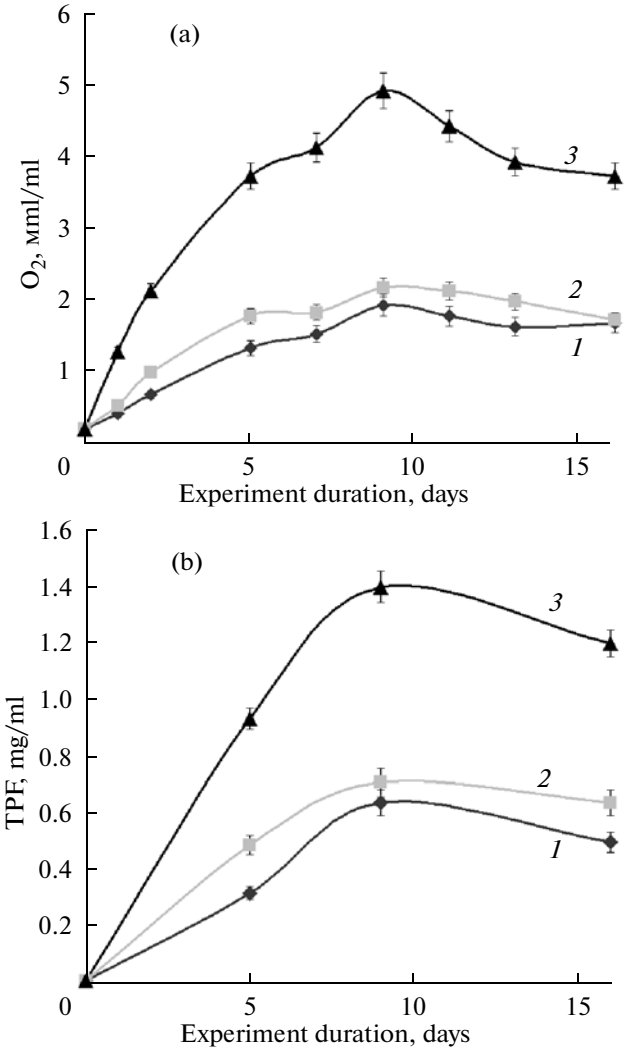

Fig. 2. Changes in catalase (a) and dehydrogenase (b) activity under light-correcting film (*3*). Controls: HPPE film (*2*) and without irradiation (*1*).

a light quantum. In this case, light causes certain changes in the metabolic regulatory program [4].

For example, there is evidence that, upon irradiation of prokaryotes (*Escherichia coli*) with red monochromatic light, the terminal enzymes of respiratory chains (for example, cytochrome *c* oxidase) act as photoreceptors, probably due to a change in the electron flow rate inside the oxidase complex, which in turn results in significant alterations in the properties of the cell membrane and the parameters of gene expression. Red light absorbed by the chromophores of the respiratory chain probably intensifies the respiratory metabolism and affects the electrogenic properties of the cell membrane [1].

In our experiment, the effect of the film-corrected light on the activity of catalase and dehydrogenase, enzymes known to catalyze reduction–oxidation reactions, was studied.

In experimental samples, the catalase activity increased by 2–2.5 times (Fig. 2a) and total hydrogenase activity by 2–2.3 times (Fig. 2b).

The increase in the enzymatic activity indicates an increase in the total metabolism rate in the microorganisms under study.

Accumulation of the products of metabolism was studied for aldehydes produced during oxidation of *n*-alkanes of oil. Aldehyde formation was shown to be 2.5–3 times higher under the light-correcting film than under HPPE film or without light (Fig. 3).

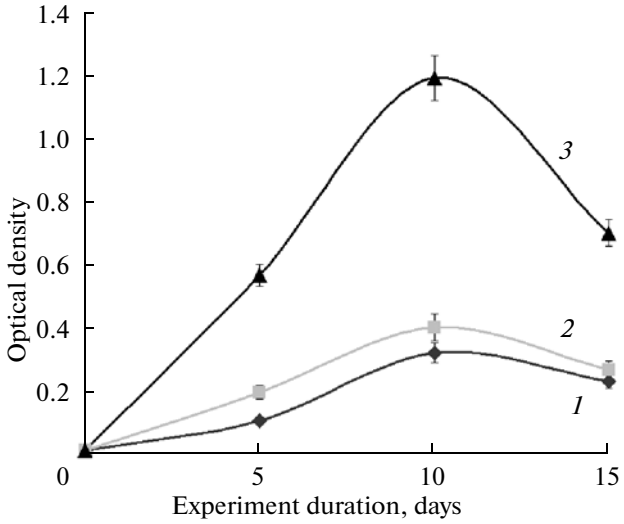

Fig. 3. Aldehyde accumulation in the culture liquid under light-correcting film (*3*). Controls: HPPE film (*2*) and without irradiation (*1*).

According to the elemental analysis, C and H content decreased (table) in the biodegraded oil samples, indicating the predominant destruction of aliphatic hydrocarbons or alkyl groups in aromatic and naphthene rings. The reason for decreased nitrogen content in the biodegraded samples may be that microorganisms use nitrogen-containing compounds as a source of biogenic elements. Increased sulfur content may evidence the relative accumulation of heterocyclic compounds, since sulfur is mainly found in resins and pyrobitumens that are difficult-to-degrade substrates for microorganisms. The content of oxygen-containing compounds increased. The most pronounced changes the elemental composition and, consequently, the most intense processes of biochemical oxidation were observed in the samples cultured under the FE film (table).

Elemental composition of oil before and after biodegradation for 15 days

Sample	Content, %				
	C	H	N	S	O
Initial	86.55	12.37	0.25	0.64	0.19
Without irradiation	86.22	12.01	0.20	0.95	0.75
HPPE	85.96	11.95	0.17	1.03	0.96
FE	84.30	10.06	0.01	2.38	3.06

Chromatographic analysis of the residual hydrocarbons revealed that, in the control under HPPE film, C_{10}–C_{16} *n*-alkanes were preferentially utilized. In the experimental variant, while C_{10}–C_{16} *n*-alkanes were significantly eliminated, heavier C_{17}–C_{28} hydrocarbons were also oxidized (Fig. 4). The results suggest intensified metabolic activity of *P. stutzeri* and *P. putida* microbial associations under irradiation with light transformed by a light-correcting film.

Thus, the data obtained evidence the intensification of metabolic processes in bacteria of the genus *Pseudomonas* treated with light transformed by the light-correcting film, which was manifested in intensified cell proliferation, activation of the enzymes of oxidative exchange, and intensified decomposition of organic matter.

The established response of nonphotosynthesizing heterotrophic bacteria toward light stimulation [9, 10] allows the conclusion to be drawn that a wide variety of microorganisms possess photosensitive regulatory systems that trigger a number of biochemical processes. The authors of [3] suggested terming this system the "photochrome regulatory system" and hypothesized that the system is similar to the phytochrome system of green plants. The photochrome regulatory system is considered the most ancient light-sensitive regulatory system, which, as developed and perfected in the course of evolution, resulted in modern photoregulatory systems and mechanisms [3]. The establishment of the evolutionary bond opens wide prospects for further studying of photobiological processes.

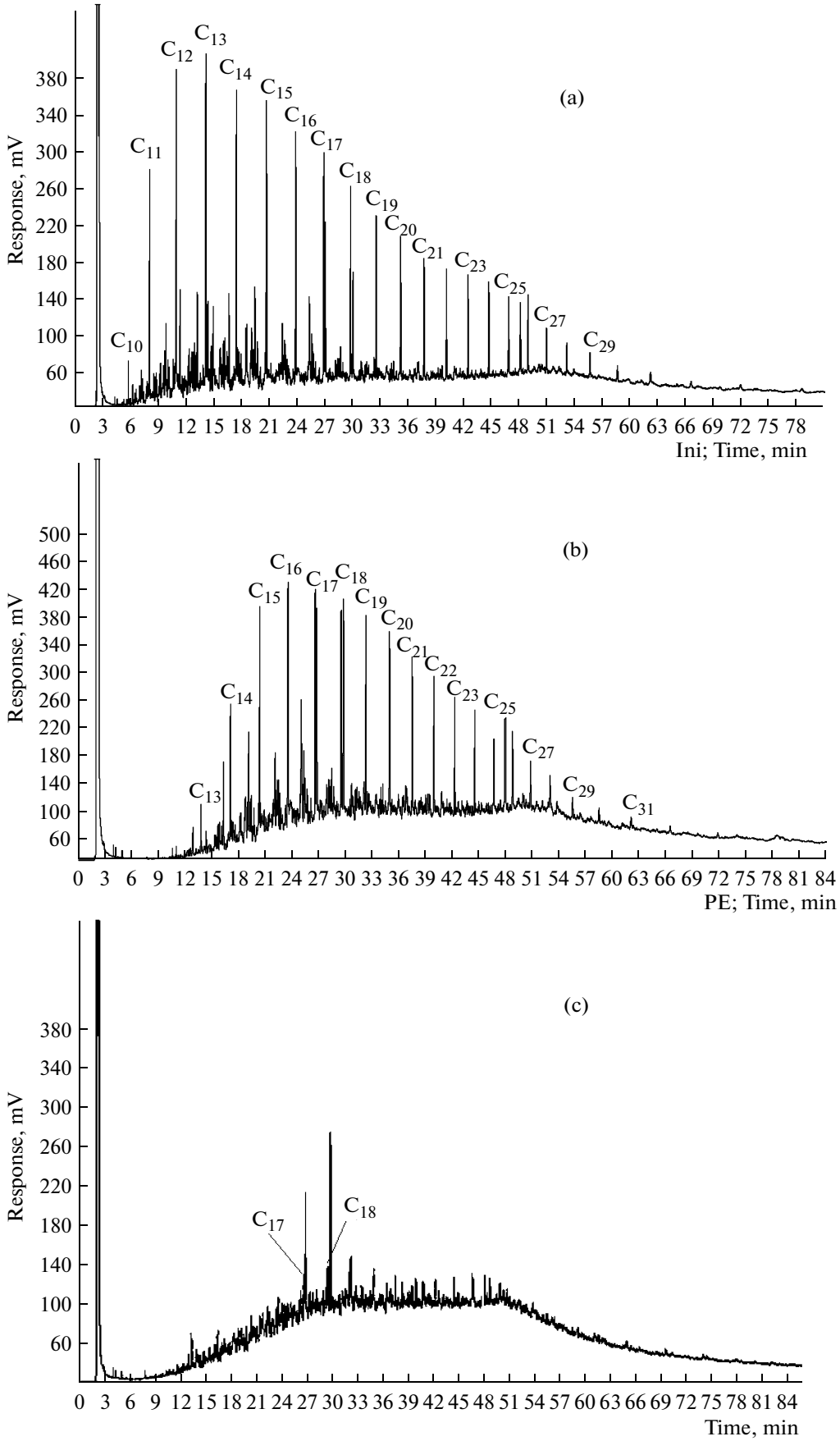

Fig. 4. Chromatograms of the initial oil (a) and after oxidation with the association of *P. stutzeri* and *P. putida* under HPPE film (b) and light-correcting film (c).

ACKNOWLEDGMENTS

This work was supported by a grant by Bortnik's Foundation, project no. 5208r/7638.

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