

INTRODUCTION

We have isolated a strain of oil-oxidizing bacteria from oil-polluted soil samples, which was identified as *Rhodococcus erythropolis* EK-1 [1]. The possibility of cleaning water from oil (100 mg/l) by clayite-immobilized cells of *R. erythropolis* EK-1 [1], as well as of intensification of the process of oil degrading by the accumulative culture of bacterial oil-oxidizers at the inoculation of the active hydrocarbon-oxidizing strain of EK-1 in this culture [2], was revealed. It has been shown that, during growth on hydrophobic (hexadecane and liquid paraffins) and hydrophilic (glucose and ethanol) substrates, *R. erythropolis* EK-1 forms surface-active substances (SAS) which, in chemical terms, are complexes of neutral lipids and glycolipids (trehalosomono and dimycolates) [3]. The study of peculiarities of n-hexadecane metabolism in the strain EK-1 allowed us to establish conditions for the cultivation of bacteria, ensuring an increase in SAS synthesis [4]. Thus, the drop of concentration of potassium cations (inhibitors of paraffin hydroxylase and NADP⁺-dependent aldehyde dehydrogenase) in the cultivation culture for *R. erythropolis* EK-1 and increase in the content of sodium cations (activators of these enzymes) and iron, which is necessary for functioning of paraffin hydroxylase, was accompanied with the rise of activity of enzymes involved in n-hexadecane metabolism, as well as fourfold increase in the amount of the synthesized surfactants.

Indicators of SAS synthesis on hexadecane, revealed for *R. erythropolis* EK-1, are comparable with the synthesizing capacity of rhodococci described in literature [5–7]. However, compared to other specimens of the *Rhodococcus* genus, the selected strain has the following advantages: (1) it is able to synthesize SAS on medium with the total content of salts of 3.15 g/l (for other rhodococci, up to 10 g/l); (2) it does not require the presence, in the medium, of microelements and yeast extract; and (3) it synthesizes SAS with a higher yield per substrate.

The necessary stage of the development of the technology for microbial synthesis is the scaling of the process in the enzymatic equipment. Cultivation of a producer in the fermenter allows for the study of impact on biosynthesis of such important parameters as aeration, rate of mixing, mode of introduction of the substrate, pH, etc. that permits an increase in the technology's efficiency. Despite a substantial number of publications devoted to research in microbial surfactants [5–7, 8–10], data on scaling technologies of their biosynthesis or on peculiarities of forming these products of microbial synthesis during cultivation of producer microorganisms in laboratory bioreactors are scarce [5, 11–15]. The first such data appeared in the late 1970s–mid 1980s and concerned scaling processing of biosynthesis of surface-active trehalosolipids [16] and rhamnolipids [17, 18]. Later, the data on cultivating bacteria of the *Rhodococcus* genus—SAS producers—in bioreactors were summarized in [5]. Note that today, numerous technologies of obtaining microbial SAS on various industrial wastes or products of processing vegetation raw materials are already scaled [11, 15, 19, 20]. Thus, during cultivation of *Bacillus subtilis* LB5a in the 40 l fermenter on waste of wheat production from manioc, the amount of the synthesized lipopeptides reached 10 g/l in 12 h [15]. Growth of *Pseudozuma* (earlier *Candida*)

antarctica ATCC 20509, the producer of mannosyl erythritol lipids, in a bioreactor (2 l) on medium containing 80 g/l of soya oil was accompanied with synthesis of 46 g/l glycolipids [11]. The yield of sophorolipids from the substrate at periodical cultivation of *Candida bombicola* ATCC 22214 on medium containing maize wheat (100 g/l) and glucose (100 g/l) in the fermenter (3 l) was 60%. Growth of *Pseudomonas* sp. DSM 2874 in the 2 l reactor with fractional introduction of the substrate (rapeseed oil) enabled the researchers to increase the amount of synthesized rhamnolipids up to 45 g/l [20].

As of today, there is little evidence in literature about the peculiarities of cultivating, in bioreactors, bacteria of the *Rhodococcus* genus which are surface_tant producers. Probably, one of reasons of this is the fact that SAS_synthesizing capacity of rhodococci is somewhat lower than that of the producers of other glycolipids (rhamno- and sophoro-, mannosyl erythritol lipids). Apart from this, a flaw of representatives of the *Rhodococcus* genus as SAS producers is their slow growth, and, as a consequence, high duration of the process of biosynthesis of the target product. Thus, at cultivation of *R. erythropolis* ATCC 4277 for 51 h in the fermenter with 1.5 l volume on the glycerin-containing medium (15 g/l), the amount of the synthesized SAS constituted only 1.7 g/l [13]. With the use of n_paraffins as carbon source (20 g/l), the amount of SAS synthesized by *R. erythropolis* DSM 43215 in the bioreactor (50 l) by 36–38 h of growth, reached 2 g/l [16], whereas cultivation of this strain in the 20 l fermenter during 160 h on the medium containing 100 g/l n-paraffins resulted in 32 g/l [5]. After cultivation in 5 l bioreactor for 240 h, the *R. erythropolis* SD-74 strain synthesized from 80 g/l hexadecane up to 40 g/l of surface_active lipids [5]. In most cases, high concentration of SAS was achieved as a result of synthesis of these compounds by resting or immobilized bacterial cells of the *Rhodococcus* genus.

Note that reports about rapidly growing strain *Rhodococcus* sp. Moj-3449 have recently appeared. This strain is characterized with a high growth rate (up to 0.2 h⁻¹) on media containing up to 180 g/l paraffins or crude oil [21]. However, this report did not provide data about the SAS-synthesizing capacity of the MoJ-3449 strain.

The goal of the present paper is to establish optimal conditions for the synthesis of surfactants at periodical cultivation of *R. erythropolis* EK-1 in the AK-210 laboratory fermenter.

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