Investigation of the bacterial adhesion process by infrared thermography

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Abstract

Presented work is devoted to the investigation of thermodynamic aspects of bacterial adhesion and demonstrating of the infrared thermography applicability for studying of this process. Bacterial adhesion plays an important role in construction of stable biocatalysts for targeted biotransformations and environmental protection. The heat dissipation rate was calculated by the data obtained from precise thermal detectors and from infrared camera and it was shown a good agreement between these values. As a result infrared thermography could be applied for investigation of the dynamics of weak heat sources as a precise express method.

1. Introduction

In modern conditions of manufacturing activity growth and increased risk associated with environmental contamination especially topical question is the "smart" and safe utilization of organic pollutants. One of the perspective directions here is application of biocatalysts based on living bacterial cells. Hydrocarbon oxidizing *Rhodococcus* actinobacteria assimilate a wide range of organic compounds (*n*-alkanes C_3-C_{37}, branched and cyclic paraffins, benzene, toluene, polycyclic aromatic hydrocarbons, olefins, organic sulfides, pentacyclic triterpenes, steroids, aromatic organic acids, heterocyclic nitrogen-containing compounds, quinolines and its derivatives) and can be used for targeted transformations and xenobiotic degradation [1, 2]. To stabilize functional activities of *Rhodococcus* cells, the cells are immobilized onto solid carriers. Presented work is devoted to the investigation of thermodynamics regularity of *Rhodococcus* cell adhesion to a solid surface. It is known that the bacterial adhesion is the exothermic process. This fact allows us to use the thermal methods to investigate the evolution of bacterial suspensions and to predict more suitable conditions for adhesion process. There are some techniques to measure effectiveness of bacterial adhesion such as radioactive labelling, fluorescence tagging, and staining of bacteria [3]. These methods allow only determining the number of attached cells after the event that is not permit to see the process step by step. IR scanning is the enough easy way to test the adhesion process towards native cells. The main problem here is to register of the weak heat sources by the infrared scanning. Temperature change during adhesion is about tenths of a degree that is close to the sensitivity of IR camera. So, the main goal of this work was to verify applicability of IR measurements for investigation of weak heat sources. To reach of this goal the special thermal detectors were used to find out the small changing of temperature. As a result, data obtained by both methods were processed and compared.

2. Experimental conditions

Experiments were carried out with *Rhodococcus ruber* strain IEGM 342 from the IEGM Specialized Collection of Alkanotrophic Microorganisms (acronym IEGM, WFCC # 768, www.iegm.ru/iegmcol). *R. ruber* IEGM 342 was selected for experiments because of its high (47%) adhesive activity towards solid surfaces, particularly polystyrene. This is a relevant condition for detection of total thermal radiation in infrared thermography experiments without any losses. Rhodococci were grown in Erlenmeyer flasks containing 100 ml of nutrient broth (FGUN State Research Center for Applied Microbiology and Biotechnology, Obolensk) in an orbital shaker (150 rpm) at 28°C for 28 hours. Then cells were washed twice with a phosphate buffer (pH 7.0) and resuspended in the buffer to achieve a cell concentration of 10^8-10^9 colony forming units (CFU)/ml. The composition of the buffer included, g/l: Na_2HPO_4 – 3.53, KH_2PO_4 – 3.39 [4].

The goal of the experiment was to measure the heat dissipated during contact of *Rhodococcus* cells with solid surface by infrared camera and by thermal detectors. The scheme of the experiment is presented in figure 1. To measure the temperature the thermal detectors (platinum thermistor with size 1,2x1,7 mm (figure 2-B)) and infrared (IR) camera FLIR SC5000 were used. Resistance of the platinum thermistor is 100 Om, temperature range is 0-150 °C. Spectral range of the infrared camera is 3-5 μm, maximum frame size is 320×256 pixels, a spatial resolution is 10^{-4} m and temperature sensitivity is 25 mK at 300 K.
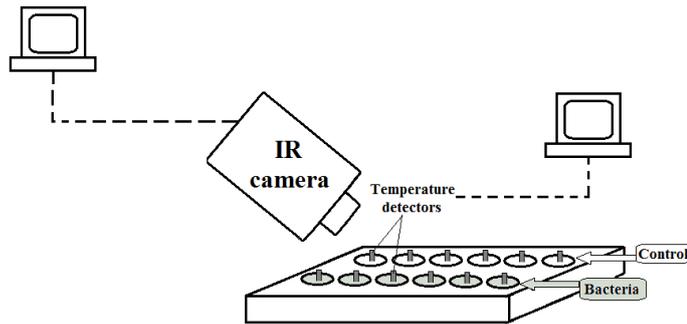
Fig. 1. *Scheme of the experiment.*

IR camera and temperature detectors were recording the signals independently. After experiment the signals were processed and synchronized. Figure 2-A shows an infrared image obtained during experiment. The temperature detectors have been immersed into the wells and they wrote the temperature inside of the wells while the IR camera recorded the temperature on the surface of liquids in the wells.

A   **B**
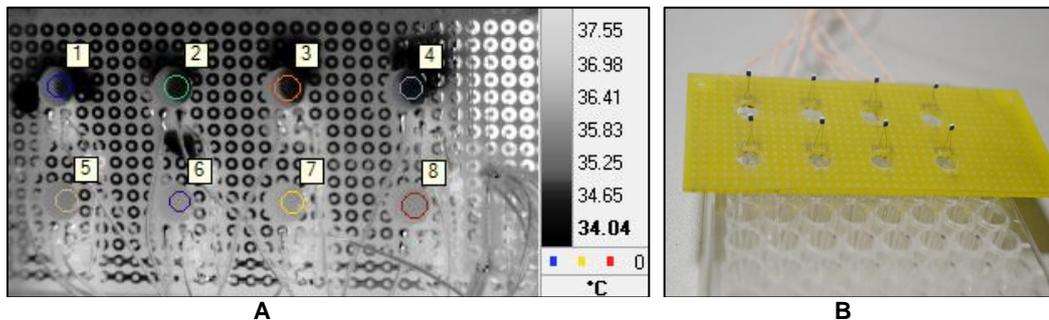
Fig. 2. A *- infrared images of polystyrene microplates with investigated liquids (points 1-4 indicate wells without cells, points 5-8 indicate wells with cells).* **B** *– photo of plate with temperature detectors.*

The 96-wells polystyrene microplates (Medpolymer, Russia) were used for immobilization of *Rhodococcus* cells. Two types of liquids, suspension of *R. ruber* IEGM 342 cells in a phosphate buffer and phosphate buffer without cells (abiotic control) were used in our experiments. Liquids were added into non-sterile polystyrene microplates in a volume of 0.1 ml=10^{-7} m^3 per well.The thermal detectors were inside of the wells and it allows one to measure local temperature inside of the wells at each time step. The temperature values from infrared images were averaged over the wells area to obtain the temperature signal depending on the time for bacterial suspension and abiotic control respectively. Then values of the temperature were averaged over the line of control wells (points 1-4 in figure 2-A) and over the line of wells with bacterial cells (points 5-8 in figure 2-A) for infrared data and for detectors data. Two experiments with different concentration of bacterial cells were carried out. We used 10^8 CFU/ml of concentration in experiment 1 and 10^9 CFU/ml of concentration in experiment 2. The experiment was carried out for 30-35 minutes. As a result in figure 3A and 3B the time dependence of the temperatures of abiotic control and bacterial suspension from IR camera and from detectors is presented.
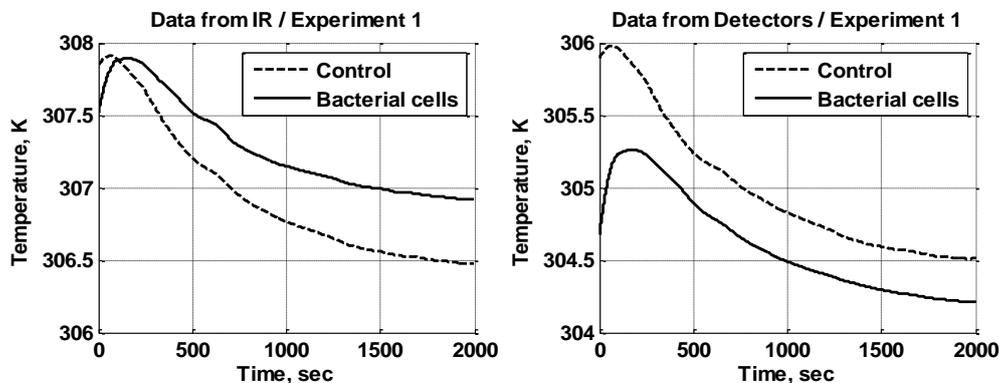
Fig. 3A. *The temperature evolution of Rhodococcus cell suspension at different cell concentrations and buffer without cells (concentration of cells is* 10^8 *CFU/ml – experiment 1).* *Infrared data are on the left; data from detectors are on the right.*
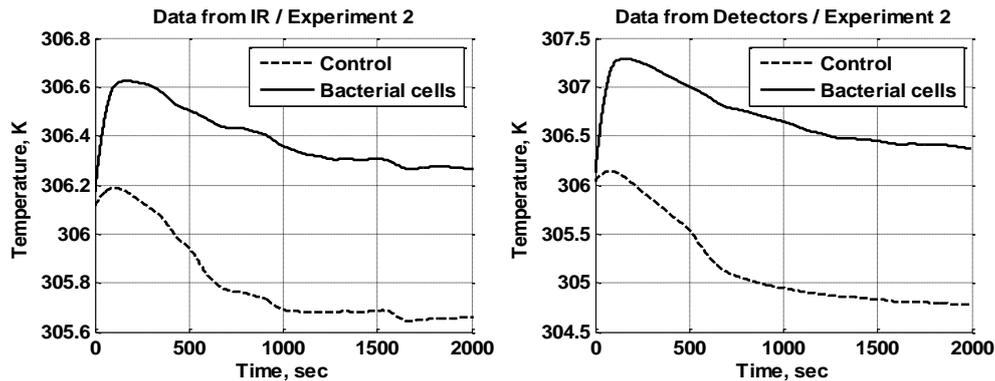
Fig. 3B. *The temperature evolution of Rhodococcus cell suspension at different cell concentrations and buffer without cells (concentration of cells is 10^9 CFU/ml – experiment 2). Infrared data are on the left; data from detectors are on the right.*

Data from IR camera and from detectors shows qualitatively similar evolution of the temperature. The process of adhesion appears during first 100-200 seconds after injection of bacterial cells and temperature rises about on 1 degree. After of it the bacterial suspension cools down. It should be noted that the temperature of control decreases also. It could be connected with the general lowering of the temperature in the experimental room. By this reason the temperature fluctuations need to be taken into account in heat sources calculations.

3. Heat dissipation rate calculation

To obtain the correct value of the heat source the fluctuations of the environment temperature have to be taken into account. The heat dissipation could be determined by the thermal conductivity equation (Eq. (1)).

$$Q(t) = (\rho V)c\frac{\partial T_b(t)}{\partial t} + \beta\left(T_b(t) - T_{env}(t)\right) \tag{1}$$

where T_{env} – environment temperature, T_b – temperature of bacterial suspension, ϱ – density of water (1000 kg/m^3), c – heat capacity of water (4183 J/(kg·K)), β – coefficient related to the heat losses, it was calculated from cooling curve of water (4·10^{-4} W/K), t – the time, V – volume of liquid in a well (10^{-7} m^3).
The control is non-inoculated buffer without heat sources inside. So, we can obtain the temperature of environment using control temperature as it is shown in equation 3:

$$0 = (\rho V)c\frac{\partial T_c(t)}{\partial t} + \beta\left(T_c(t) - T_{env}(t)\right) \quad \Rightarrow \quad T_{env}(t) = T_c(t) + \frac{(\rho V)c}{\beta} \cdot \frac{\partial T_c(t)}{\partial t} \tag{2}$$

where T_c – control temperature.
If we put in equation (1) the equation (2) for environment temperature, we can obtain the heat sources equation for determination of heat dissipation rate of bacterial adhesion as following:

$$Q(t) = (\rho V)c\frac{\partial(T_b(t) - T_c(t))}{\partial t} + \beta\left(T_b(t) - T_c(t)\right) \tag{3}$$

To calculate the heat sources from experimental time dependence of temperature, the forward finite difference scheme was used to evaluate the time derivative in equation (3).

Figure 4A and 4B illustrate the results reflecting the time dependence of heat dissipation rate of bacterial suspension and abiotic control. The heat dissipation rate was calculated for both investigated liquids from two types of the data, infrared data and data from thermal detectors.

In all cases heat dissipation curve could be divided into three parts. First part lasts about 100 seconds and corresponds to the fast decreasing of dissipation rate of sources. It means that the significant source exists in bacterial suspension at the beginning of experiment. The second part is about 100-500 seconds. This period is characterized of the slow decreasing of heat dissipation rate. Smaller slope of the heat dissipation curve indicates the existence of more weak heat source than on the first part. After 500 sec all processes finished, heat dissipation rate reaches the zero and system goes to the thermal equilibrium. Different concentrations of bacterial suspensions have to influence on the view of heat dissipation curve. As it is shown on the graphs (figure 4A and 4B) increasing of concentration from 10^8 to the 10^9

CFU/ml gives increment of heat dissipation rate about 0.5 J/sec from the IR data and about 3.5 J/sec from detectors data. Obtained results allow us to conclude that detectors are more sensitive to the changing of concentration of bacterial cells than IR technique. This result is enough clear because IR data represent integral value of heat dissipation on the surface while data from detectors shows local heat dissipation inside of bacterial suspension. It means that infrared technique could be used as a fast testing method to obtain the information about availability of heat sources and its integral value.
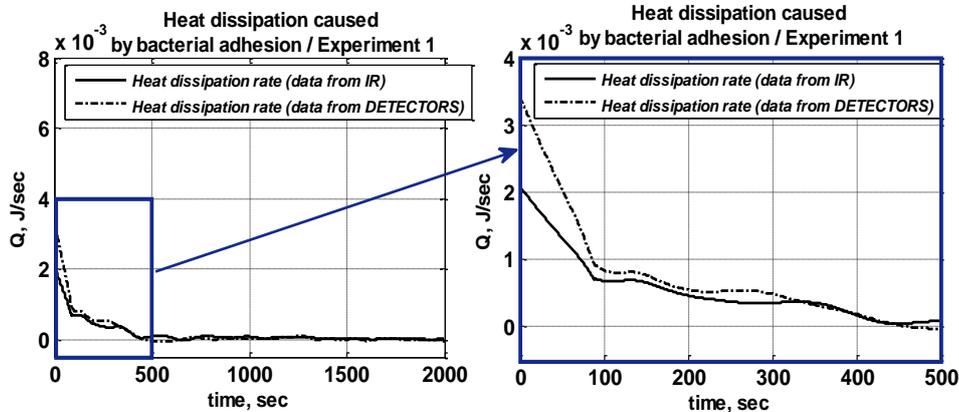
Fig. 4A. *The heat dissipation rate of the R. ruber IEGM 342 suspension based on the data from detectors and infrared data (concentration of cells is* 10^8 *CFU/ml – experiment 1).*
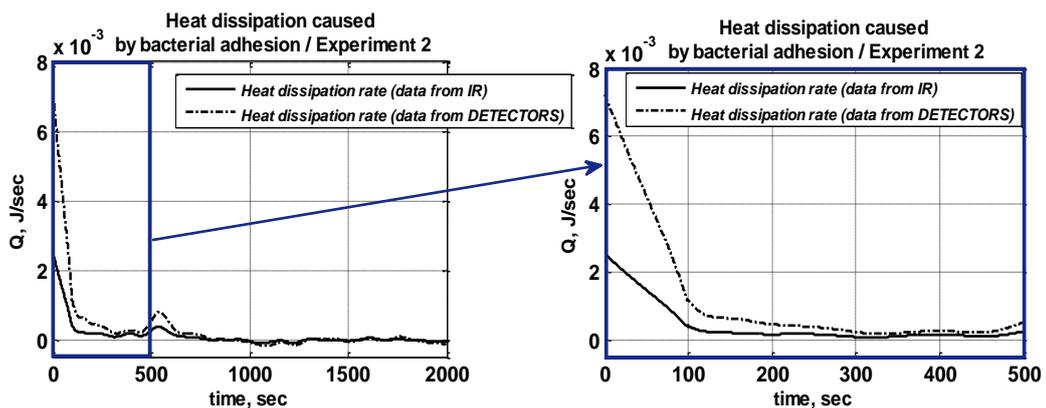
Fig. 4B. *The heat dissipation rate of the R. ruber IEGM 342 suspension based on the data from detectors and infrared data (concentration of cells is* 10^9 *CFU/ml – experiment 2).*

The thermal detectors are more precise for identification of the heat dissipation, but, in real conditions, the bacterial cells are attached to the enough big solid surface on which is not possible to use detectors. In this case IR technique shows a good result because it can show information about the space distribution of heat sources caused by adhesion what gives possibility to find the best conditions for intensification of this process.

Conclusion

In this work, we experimentally investigated the thermodynamics aspects of bacterial adhesion process by infrared technique and by direct method of temperature measurements by thermo detectors. The good qualitative correlation between data of temperature fluctuations from IR measurements and from detector's measurements was shown.

As a result it could be noted that the value of heat dissipation rate obtained by IR camera and from temperature detectors have a good qualitative agreement in general. Quantitative variance can be explained by two different ways of temperature measurements: thermal detectors measure the temperature inside the volume while infrared camera measures the integral value of the temperature on the surface of the well. So, in presented work it is shown that infrared thermography is enough sensitive method which allows one to measure small fluctuations of the temperature and this technique could be applied to study of weak heat sources (about 2-5 mJ/sec).

The future development of this work will be devoted to the investigation of influence of different concentrations of bacterial cells and adhesion conditions (modification of a solid surface, external temperature and etc.) and, on the base of this information, the construction of biocatalysts working in real conditions.

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