

Cultivated Meat Bioprocess Optimization

O₂, pH and CO₂ Monitoring in *E. coli* Culture with PreSens SensorPlugs

Vasa Radonic, Ivana Podunavac, Mila Djisalo, Teodora Knezic, Ivana Gadjanski
BioSense Institute, Novi Sad, Serbia

.....

We tested the efficacy of PreSens SensorPlugs for monitoring bacterial cultures in microfluidic bioreactors (MBs), which is important in the context of monitoring potential contamination in the culture meat bioprocess. *Escherichia coli* (ATCC® 25922™) was cultivated inside a custom-made MB with integrated SensorPlugs. The optical measurements showed the characteristic course of *E. coli* growth, and allowed precise assessment of current culture status at all times.

The cell culture medium is the most significant cost driver for cultivated meat production. Optimizing bioreactors' design and instrumentation, through additional monitoring features via sensors can help in decreasing these costs and achieving maximum cell production capacity per unit medium volume.

Scale-down approaches have long been applied in bioprocessing to resolve scale-up problems. Miniaturized bioreactors have thrived as a tool to obtain process-relevant data during early stage process development. We applied this principle i.e., the 'lab-on-a-chip' (LoC) approach when developing a new generation of low-cost sensors for monitoring of various cell culture parameters such as biomass and ammonia and glutamine in cell culturing media.

We used our custom-made microfluidic bioreactors complemented with our impedimetric sensor and an optical sensor in combination with the SensorPlugs for O₂, CO₂ and pH, provided by PreSens. The main idea of our project was to try to identify which processes are going on inside the microfluidic bioreactor, as well as to be able to quantify the effect on cell behavior, cell viability, and reagents effectiveness. We tested the efficacy of PreSens SensorPlugs for monitoring bacterial and mammalian cell cultures in microfluidic bioreactors. In addition, we performed a set of pilot experiments with human saliva, as a potential further expansion of the sensors' application for taste/flavor evaluation of the final product of the cultivated meat bioprocess. Here our results for bacterial culture monitoring are shown.

Materials & Methods

The multilayered MB chips were manufactured using the transparent and biocompatible

materials glass and PMMA. The top and the middle layer were manufactured in PMMA and the interconnecting layers were made using 3M double-sided adhesive tapes (3M™ GPT-020F, St. Paul, MN 55144-1000, Minneapolis, USA). The bottom layer of the chip for cell culture was made of glass where the impedance sensor was printed with the commercial Agfa-Gevaert N.V. nano silver ink with 15 % of Ag nanoparticles and the conductive ink using piezocontrolled inkjet printer Fuji Dimatix DMP-3000. The sensor was covered with a thin layer of SU-8 3000 Microchem resist. The top layer contained inlet/outlet holes whose diameters were adapted for pipetting the sample while filling the chip. In addition, three holes with a diameter of 2.1 mm were made in the top layer for mounting the PreSens SensorPlugs. The sensors were in direct contact with the sample through the holes which enabled real-time measuring of the parameters in the sample. The reservoir, made in the middle layer of the chip, had a volume of 1.8 mm for the sample. An overnight culture of *E. coli* was prepared by the seeding of bacteria in Tryptone Soya Broth. After that, overnight culture was used for the preparation of 0.5 McFarlan *E. coli* culture (1.5×10^8 CFU/mL), which served for cultivation in the MB. We left the chip together with the PreSens sensors inside the microbiological incubator at 37 °C (Fig. 1).

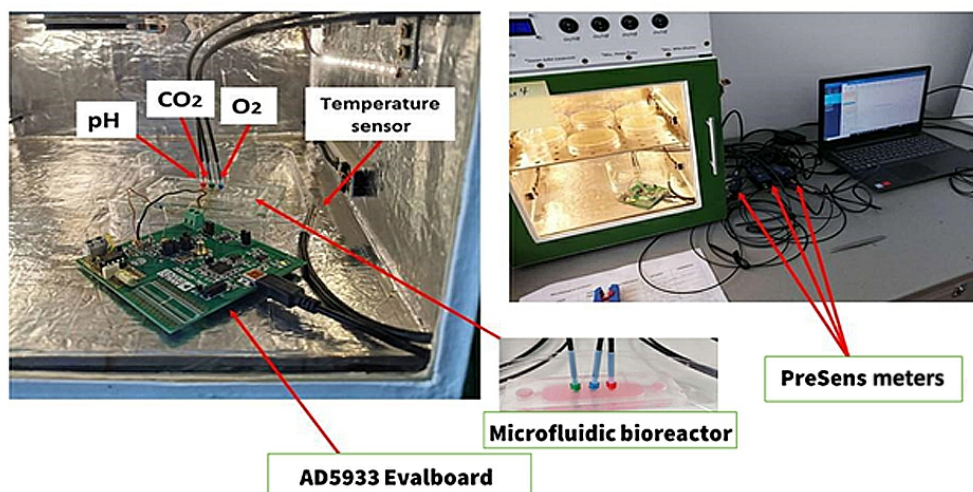


Fig. 1: Experimental setup for *E. coli* culturing. PreSens meters and electronics were placed inside the microbiological incubator.

Monitoring O₂, CO₂ and pH during Cultivation

The measurement results of O₂, CO₂ and pH during the cultivation of *E. coli* in the microfluidic chip are presented in figure 2. pH measurements (Fig. 2a) gave expected results during cultivating *E. coli* for 3 h. After the initial lag phase, bacteria enter exponential growth phase, characterized by the cell doubling rate i.e., generation time. Generation times for *E. coli* bacteria vary from about 15 - 20 minutes to 40 minutes in the laboratory. Therefore, the concentration of *E. coli* bacteria was determined using a hemocytometer, at the beginning and

at the end of the experiments. Figure 3 shows the enlarged part of the hemocytometer, the square in which the cell units are counted. 10 μL were weighed with a micropipette and placed on a hemocytometer. When the total number of cell units in all squares was counted, the mean value was calculated and the total concentration on the chip was determined. The process of counting bacteria was performed using an optical microscope. The number of bacteria increased almost 13 times. Figure 2 shows that the initial pH value (when we seeded bacterial culture inside the microfluidic chip) at $t = 0$ min was about 7.3. After three hours, we observed acidification of the bacterial culture (pH ~ 6.8) and an increase in % CO_2 , and a simultaneous decrease in % O_2 during incubation. The graphs show the expected, characteristic course of *E. coli* growth. In the first lag growth phase (~ 1.5 h), there were no significant changes in pH and CO_2 . During this phase, oxygen was consumed. After ~ 1.5 h, the CO_2 level rose exponentially, and O_2 decreased. In this phase, the TBS media was consumed by bacteria, and the cells entered the log growth phase. Oxygen decreased with increasing cell numbers and dropped to zero levels during the experiment. That also is displayed in an increase of CO_2 . Because of limited oxygen, *E. coli* cells could no longer grow inside the microfluidic chip and the process went into the stationary phase. After 3 h, O_2 levels dropped to 3 % while CO_2 increased to 4 %. The sensors for microbial culture monitoring showed comprehensive results and allowed precise assessment of current culture status.

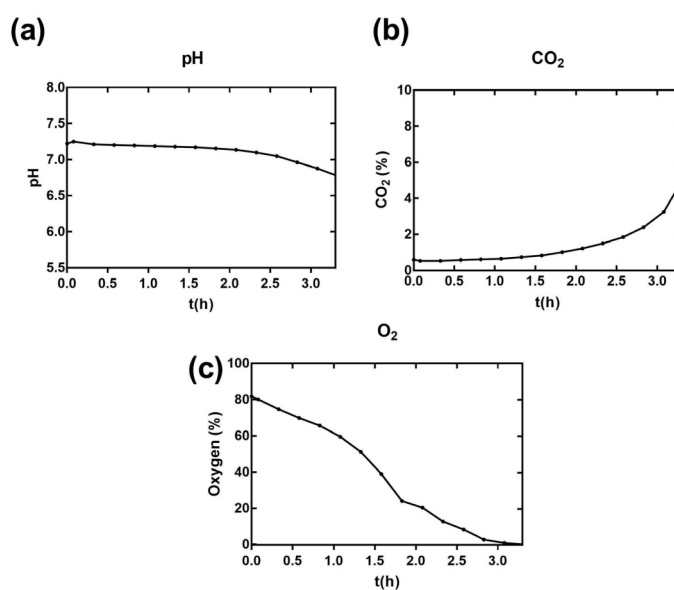


Fig. 2: Results of *E. coli* culturing inside the microfluidic chip: (a) pH; (b) CO_2 ; (c) O_2 .

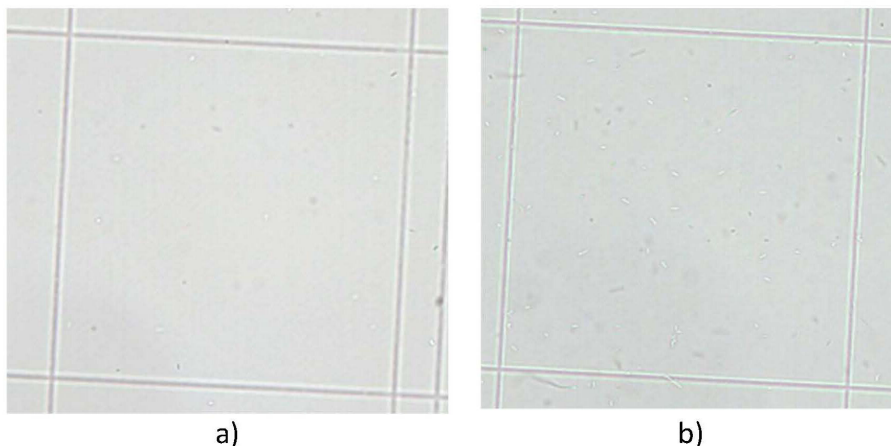





Fig. 3: *E. coli* bacteria in a square of hemocytometer for two tested samples: a) at $t = 0$ h, and b) after cultivation $t = 3$ h.

Conclusion

Based on the presented results, we can conclude that the PreSens SensorPlugs are a convenient way of monitoring basic parameters (O_2 , pH, CO_2) in the microfluidic bacterial culture. PreSens SensorPlugs present an important for scale-down approach analysis and process monitoring in cultivated bioprocess optimization. Future experiments will be directed toward continuous monitoring and optimization of culturing process, examining the effects of essential oils and antibiotics on different antibacterial and antifungal activity inside microbioreactors. The research will be focused on finding the correlation between readings of pH, O_2 , and CO_2 sensors with additional sensors (impedimetric, optical, and electrochemical) integrated into microfluidic bioreactors that we developed at the Institute for the measurement of conductivity of the medium, metabolite or biomass.

Get in contact

-  [Request more info](#)
-  [Request a quote](#)
-  [Rent-a-meter](#)

PreSens Precision Sensing GmbH
Am Biopark 11,
D-93053 Regensburg
Phone +49 941 942 72 100
Fax +49 941 942 72 111
info@PreSens.de