In-situ, real-time measurement of glucose during mammalian cell culture

Sarvani Manne, Shriram Kaliannan Chandramohan, Jean-François P. Hamel
(Contact: Dr. Hamel, jhamel@mit.edu)

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

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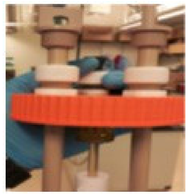
ABSTRACT

A novel online glucose sensor (C-CIT Sensors AG) was employed for monitoring glucose levels in microcarrier cell culture, and compared to offline analysis with a biochemical analyzer (BioProfile® Flex). The C-CIT glucose sensor is based on immobilized enzyme technology and is connected to a wireless transmitter. The format of the sensor enables its inclusion into the T-flask, spinner flask and bioreactor. In this study, FRhK-4 cells were grown over Cytodex-1 micro carriers in OptiMEM medium with 7.5% v/v Fetal Clone-2 serum. The cells were grown in 500-mL spinner flask for 23 days. Glucose trends generated from the online sensor were found to be consistent with punctual offline analysis.

MATERIALS AND METHODS

CITSens Bio Glucose Sensor, PG13.5 was integrated in glass spinner flask (500 mL Corning Proculture®).

Spinner Flask



Modified spinner flask cap to fit C-CIT glucose sensors (CITSens Bio Glucose Sensor, PG13.5)

Beamer

CytodexTM-1 microcarrier (2 g/L) in OptiMEM medium with 7.5% Fetal clone-II was inoculated with FRhK-4 cells at a seeding density of $1*10^8$ cells/g CytodexTM-1

Data collection

Instrument Set-up:

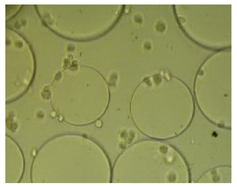
- Sensor was conditioned for 8 hours before calibration
- *In-situ* sensor was calibrated for 1 h before inoculation

Agitation: 45 RPM (increased to 60 RPM as the micro carriers started settling down

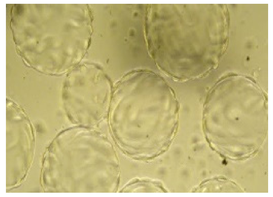
Incubation: 37ºC with 5% CO_2 v/v

The spinner flask was fed when the concentration of glucose dropped below 0.5 g/L by allowing the microcarriers to settle down

FRhK-4 cells on microcarriers



Day 0



Day 7

RESULTS

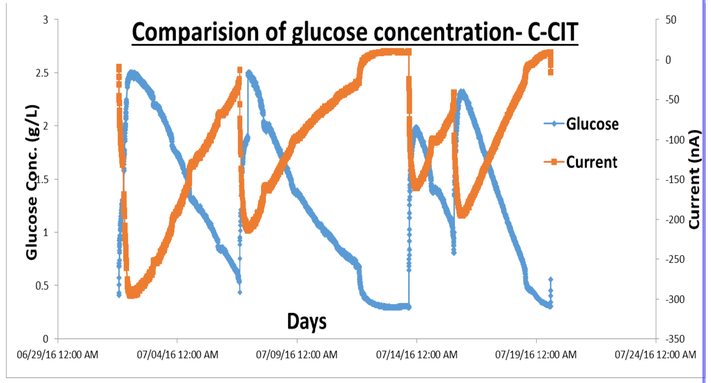


***Figure 1*: In-situ glucose measurement was performed for 22 days in the spinner flask with FRhK-4 cells. (data collected before and after feeding)**

Notes:

- Current data (nA) in the above Fig.1, is a measurement of electrons produced during the oxidation process (glucose with immobilized glucose oxidase on sensor)
- C-CIT software acts as an interface to convert the current (nA) to glucose concentration (g/ L)

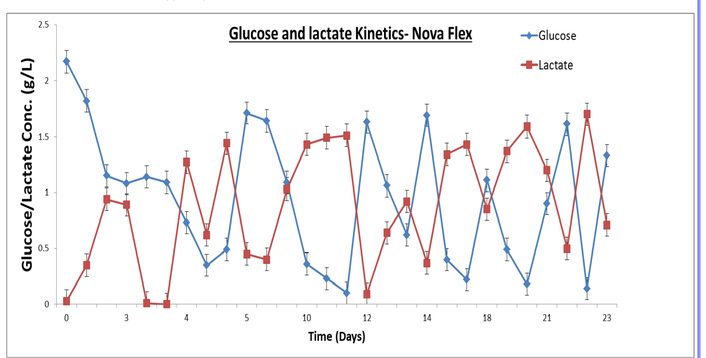


***Figure 2*: Comparison of glucose and lactate concentrations with offline analyzer Nova-FLEX (feeding was carried out at different time points over 22 days)**

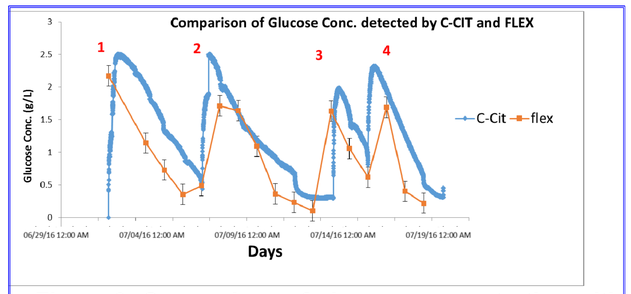


***Figure 3*: Comparison of glucose concentration with offline analyzer (FLEX) and online sensor (C-CIT) (data collected during 22 days culture process with four feeding points)**

CONCLUSION

- Decrease and increase in glucose levels were correctly detected before and after feeding, during the entire cell culture process
- Glucose trends with the online sensor and offline analyzer were similar
- *In situ* online sensor lasts long enough to support both batch and fed-batch processes

FUTURE WORK

- Include HPLC as offline method for comparing glucose concentration data with that from the online sensor
- Optimize protocol: shorten conditioning and calibration processes (automatically by software) to facilitate combining medium, cells and sensor, in one step
- Study the effect of current (nA) vs. agitation temperature and magnetic field on glucose concentration
- Assess contamination risks to cell culture when using online sensor technology
- Integrate (C-CIT Sensors) technology into microbial culture processes

ACKNOWLEDGEMENT
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