Efficient Bioethanol production - *S. cerevisiae*

Biofuels are in increasing demand to combat the well known issues of fossil fuels and their association with negative environmental impacts; as such, there is a need for more efficient production of alternatives. Bioethanol is a sustainable alternative fuel produced by *S. cerevisiae* during glucose fermentation. The aim of this study was to improve productivity and yield of ethanol from *S. cerevisiae* fermentation through on-line dynamic regulation using the Aber capacitance technology to recognize the growth and metabolic state of the yeast. This allowed for real-time acquisition of biomass to guide supplementation of nutrients during fermentation. On-line feeding strategies were based upon capacitance calibrated to read viable cell density whilst using an electric nose to measure ethanol concentrations.

5L bioreactors were initiated with differing glucose concentrations. Aber capacitance sensors were inserted into the bioreactors and offline methods such as dry cell weight (DCW), optical density (OD) and colony forming units (CFU) were used to evaluate cell growth throughout. To establish the effects of variable initial glucose concentration on ethanol yield, two parallel bioreactors were used: the first being used as the control batch and the other a supplemented batch. The control batch had a high initial glucose feed concentration whereas, the supplemented batch had a lower initial glucose concentration, however, following a 60-minute trend of continuous depletion in the viable biomass of the suspension, a glucose supplement was added. The supplementation was guided by real time viable cell density using the Aber capacitance technology and ethanol concentration throughout.

During the fermentation the capacitance readings showed close association with offline counts in initial stages although, as viability dropped, deviations were identified between online capacitance measurements and offline counts, specifically DCW and OD. This was to be expected as offline counts such as OD and DCW are unable to distinguish viable cell concentration hence, cannot reflect living cell state in real-time. Offline counts in CFU remained consistent with capacitance readings as this is also a measure of viable biomass. Aber capacitance was able to identify a reduction of viable cell concentration in real-time and was used effectively to identify the point of glucose supplementation to control and improve productivity. Offline measurements of glucose concentration also verified that glucose was exhausted at the point of supplementation, explaining the continuous reduction in viable cell concentration due to the depletion of nutrients.



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There was an increase in ethanol concentration, productivity and yield of 15.4%, 15.9% and 9.0%, respectively in the supplemented batch, showing that reduced initial glucose concentration could alleviate substrate inhibition. The Aber capacitance technology was able to establish optimum supplementation time to improve ethanol production and yield. Summary of benefits for using Aber capacitance sensors to monitor ethanol fermentation:

- Obtain fingerprint of the process in real-time
- Monitor and control yeast growth online and non-disruptively
- Real-time acquisition of the viable biomass
- Improved product concentration, yield and productivity.
- Real-time profile was used to optimize nutrient feed and control supplementation during the process.

References:

Feng, Y., Tian, X., Chen, Y., Wang, Z., Xia, J., Qian, J., Zhuang, Y. and Chu, J., (2021) Real-time and on-line monitoring of ethanol fermentation process by viable cell sensor and electronic nose. *Bioresources and Bioprocessing*. (Version 1) available at Research Square [https://doi. org/10.21203/rs.3.rs-234697/v1] [Preprint]

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