A novel online sensor unmasks the real specific metabolic quotient for glucose, in anthrax vaccine fermentation

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INTRODUCTION

At present, offline measurements of glucose predominate the biotech industry. However, material wastage and cross-contamination are some of the limitations of this method. Also, in fast-growing microbial cultures, if the sampling process time is considerable, the actual glucose value may be compromised.

The search for an online glucose sensor, which ideally should be accurate, robust, economical and have a minimal footprint is of importance. This is especially true for the future of biopharmaceuticals, as more regulatory authorities adopt the Quality by Design (QbD) concept then a higher penalty will be placed on process deviations.

Here, during the development of an improved anthrax vaccine, an online glucose monitoring system was compared to a typical offline analyser.

METHODS

The *B. anthracis* Sterne, used in all fermentations, was the attenuated strain which lacked the pXO2 plasmid (HSE 2007). The basal medium for cell growth had an initial glucose concentration of ≈10mM. The Wave™ 25 bioreactor system (GE Healthcare, USA) used was fitted with a 20L (working volume 10L) disposable bag (Figure 1). It had a volumetric oxygen mass transfer coefficient and a mixing time of 2.1 h^{-1} and 150s respectively.

The 2950 D biochemistry analyser (YSI, USA) was used offline, while the CIT Sens Bio (C-CIT Sensors, Switzerland) quantified the real-time glucose concentration (Figure 2). Both instruments were based on the enzymatic glucose oxidase (GOx) reaction with glucose (Equation 1), which is known to be reliable (Gopalan et al. 2016).

$$\beta\text{-D-glucose} + O_2 \xrightarrow{GO_x} \text{glucono-}\delta\text{-lactone} + H_2O_2 \quad \text{----} \quad 1$$

However, the CIT Sens Bio slightly differs in principle because it has a ferrocene derivative, which minimises the production of $H_2O_2$and O_2 interference (Wilson & Turner 1992).

These fermentations and cell manipulations were contained in a class III Microbiological Safety Cabinet (fabricated in-house). All chemicals used were of a reagent grade. The conditions of the two batches are shown in Table 1and were part of a larger investigation.

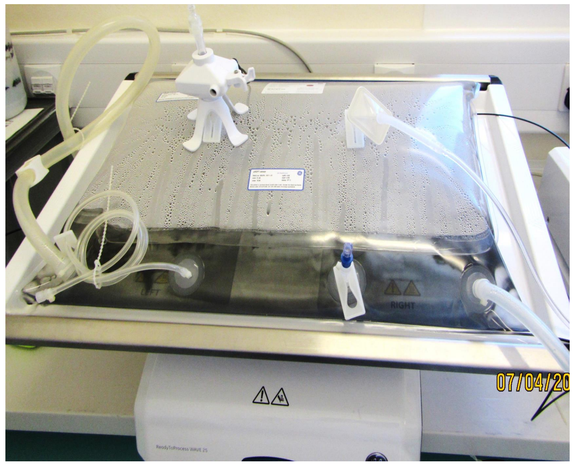

Figure 1, the Wave™ 25 bioreactor

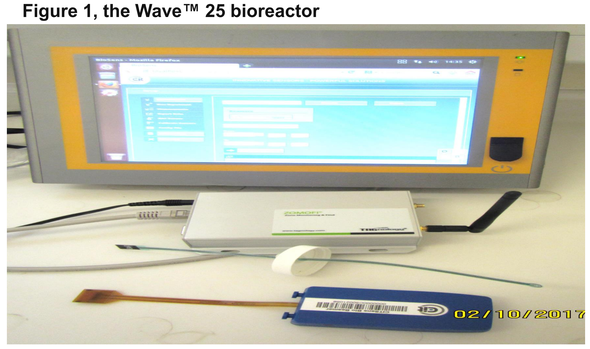

Figure 2, CIT Sens Bio glucose online system

Conditions	Batch 1	Batch 2
Temperature	38 ℃	34 ℃
Rocking speed	10 rpm	10 rpm
pH	7.3	7.8
Ax	Yes	No

Table 1, *B. anthracis* Sterne varying fermentation conditions of two batches

RESULTS AND DISCUSSION

The bacterial cell growth profiles for batch 1 and 2 were quantified by the optical density (OD) (Figure 4a). The specific growth rates (μ) were derived from a nonlinear regression fit. This analysis assumed that after the initial lag phase (0 – 16h), the rapid growth phase followed an exponential profile till termination (Equation 2).

$$OD_0 = OD * e^{-\mu t} \quad \text{----------} \quad 2$$

OD_0 = value at start fermentation, OD = value at time t

During the lag phase, the dissolved oxygen (DO) consumption was negligible. However, as the cell biomass and metabolic activity increased towards the end of these fermentations the conditions in the bioreactor became oxygen limited (Figure 4b). Also, Figure 4 a & b indicate that both batches reached the exponential growth phase at a similar time (≈16 h), but their growth rates were different.

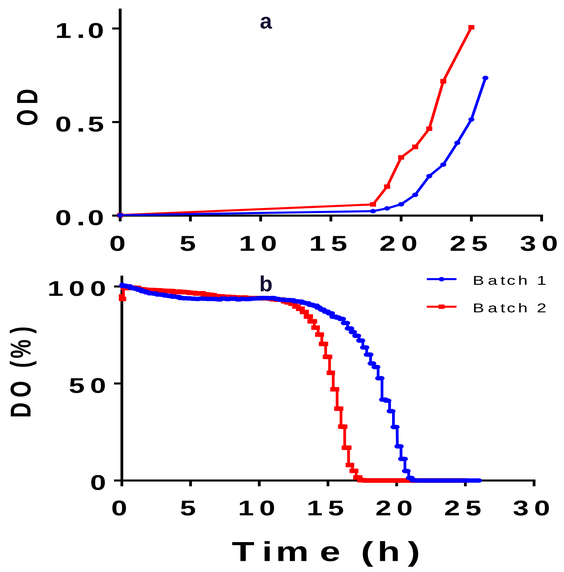

Figure 4 (a – b), *B. anthracis* Sterne growth and dissolved oxygen consumption profile

The μ for batch 1 and 2 was 0.20 h^{-1} and 0.23 h^{-1} respectively derived from Equation 2. This physiological response confirmed that the batch 2 conditions were more favourable for cell growth. The cell doubling time (t_d) calculated from Equation 3 also indicated that batch 2 was 17% faster than batch 1.

$$t_d = \frac{\ln 2}{\mu} \quad \text{----------} \quad 3$$

Figure 5 (a – b) show the glucose consumption profile for both batch 1 & 2 respectively. The graphs of Figure 5 (a – b) highlight both online and offline glucose measurements.

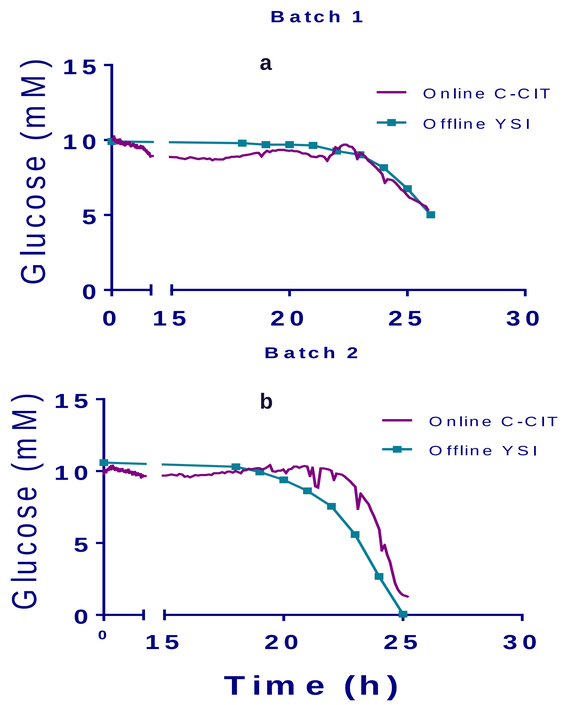

Figure 5 (a – b), C-CIT online and YSI offline glucose results for batch 1 and 2

The online C-CIT glucose measurements overall agreed with the offline results for batch 1. However, this was not the case for batch 2. Both fermentations started out similarly during the lag phase, but from the early exponential phase, the difference between the online and offline glucose measurements was significant for batch 2. The higher μ and cell-specific metabolic quotient for glucose (q_s) may have contributed to the discrepancy observed (Table 2).

Process run	Yield on glucose (Online C-CIT)	Yield on glucose (Offline YSI)	q_s (h^{-1}) Online derived	$q_s(h^{-1})$ Offline derived
Batch 1	0.17	0.15	1.18	1.33
Batch 2	0.12	0.10	1.92	2.30

Table 2, cell yield on glucose and q_s values for batch 1 and 2

The cell yield on 1g of glucose was lower in batch 2, which was contrary to the q_s values. This signified that there was a consistently higher consumption of glucose per cell in batch 2. From the online measurement, the q_s for batch 2 was 63% more than batch 1, while the offline value indicated a 72% increase. The online q_s result was 17% and 13% less than the offline q_s for batch 1 and 2 respectively. This suggests that the sampling time (5 to 10 min) became a factor as the q_s increased. Thus, the high cell glucose consumption rate resulted in the glucose measurement at the point of sampling differing from the measurement taken ≥ 5 min. Interestingly, towards the end of batch 2, the low glucose concentration which limited growth also reduced the difference between the online and offline measurements (due to the decreased glucose consumption rate).

CONCLUSIONS

In general, the online CIT Sens Bio and the offline YSI 2950D analyser glucose values were similar, although discrepancies resulted when q_s increased. Thus, in fast-growing fermentations, offline measurements will tend to overestimate q_s.

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