

On-line Metabolic Event Sensing & Prediction

The use of a Tandem gas analyser to predict metabolic events
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Escherichia coli is an attractive host for recombinant protein production because it can be grown to high cell densities, is amenable to genetic manipulation and has the ability to secrete and fold recombinant protein (Better et al., 1988; Pluckthun and Skerra, 1989; Skerra and Pluckthun, 1988; Missiakas and Raina, 1997)

There are wide ranges of promoters available for the induction of recombinant proteins. One of these is the *E.coli* alkaline phosphatase (*phoA*) promoter, where transcription of the recombinant protein starts when phosphate depletes (Carter et al., 1992).

In microbial fermentations it is essential to measure both on and off line parameters to monitor growth and to evaluate the physiological state of the cultured organism. Analysis of fermentation exhaust gas composition offers a convenient and sensitive way of following physiological events in close to a real time manor. In particular, derivations of O₂ and CO₂ concentrations, such as respiratory quotient and specific oxygen utilization rates, provide a means to evaluate physiological phenomena which cannot readily be examined by biochemical analysis of off line samples.

The following example shows how the Tandem gas analyzer was used to determine the point of phosphate depletion. There was a sharp fall in carbon dioxide and a rise in oxygen, in the exhaust gas, corresponding to a metabolic event (Figure 1). Confirmation that this metabolic event was phosphate depletion was provided by a western blot targeting a protein that is induced under phosphate depleted conditions, PHO-S (Figure 2).

Figure 1: Exhaust gas data for a fermentation producing a recombinant antibody fragment. The point of phosphate depletion is marked as sample 13. There is a sharp fall in carbon dioxide together with a sharp rise in oxygen concentration within the exhaust gas. It is also possible to calculate carbon dioxide evolution rate (CER) and Oxygen utilization rate (OUR) online using these data.

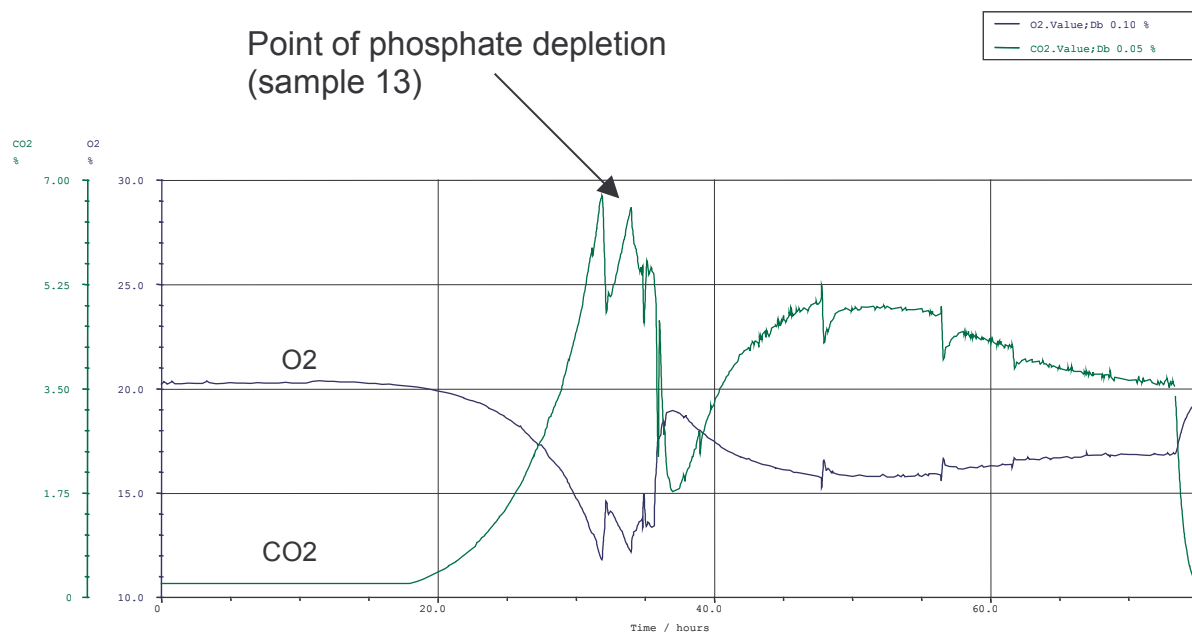
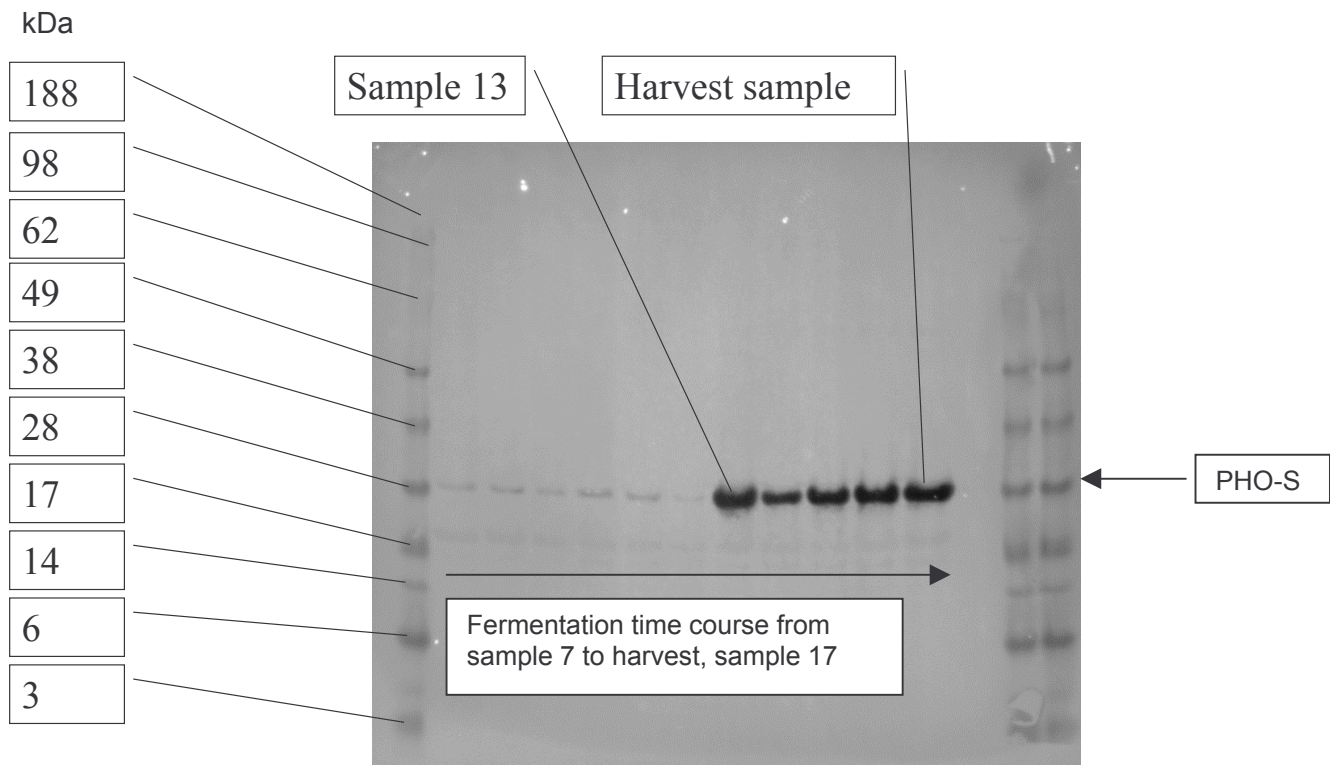


Figure 2: A western blot using an anti-PHO S antibody to reveal the point of PHO S induction which indicates phosphate depletion. Fermentation samples were taken every fifteen minutes before, during and after the PHO S induction period. These samples were centrifuged to a pellet. The pelleted samples were re-suspended in an osmotic shock buffer to a normalized OD at 600nm of 7, extracted overnight, and centrifuged to a pellet and a clarified extract. The clarified extracts were treated with Novex NuPAGE non-reducing loading buffers and ran on a 4-12% NuPAGE bis-tris gel. The marker used was See-blue plus pre-stained marker obtained from Novex. The gel was run and blotted, onto PVDF membrane, following the Novex Nu-PAGE standard protocols. The anti-PHO-S antibody was a gift from Humphreys et.al. (manuscript in preparation). The Blot was revealed using a metal enhanced DAB substrate kit (Pierce).



References

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