Biotechnology Techniques Vol 6 No 1 1-4 (1992)
Received 1st October

SUGAR ANALYSIS OF INDUSTRIAL PENICILLIN FERMENTATION BROTHS CONTAINING COMPLEX NUTRIENT SOURCES

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Summary

Four methods for sugar analysis in industrial penicillin-G fermentation broths are compared. It is concluded that volumetric and colorimetric assays have little value in specific analyses of complex industrial fermentation media. The combined use of HPLC and enzymatic methods constitutes an efficient and simple approach to the problem of mono- and polisaccharide on-line monitoring in the fermentation process.

Introduction

Bioreactor modelling, optimization and control requires the availability of on-line measurements to adequately characterize the bioreactor state.

In industrial fermentation processes the use of complex nutrient media further complicates the state estimation problem. Industrial fermentation media are made up of complex nutrient sources and of other double-purpose substances. The former - e.g. corn steep liquor (CSL) - are added to satisfy metabolic requirements for components such as amino-acids, vitamins and oligoelements. The latter - e.g. lard oil - serve as secondary carbon sources and antifoaming agents. In addition, the primary carbon source, usually a reducing sugar, is charged in and/or fed to the fermenter together with other slow metabolizable substrates.

Here we report on industrial penicillin-G fermentation data obtained from pilot plant runs. Complex chemical composition of these fermentations renders the results by chemical analysis useless for specific carbohydrate assay. An HPLC set-up and an enzyme based auto-analyzer were used that can overcome the difficulties encountered. The first and, to our best knowledge, the only time they were applied together to the study of penicillin fermentation, was in the late seventies when Hospodka (1980) used them for total hexose assaying in laboratory-scale defined-medium fermentations.

Carbohydrate Analysis

Chemical Analysis by either colorimetric - e.g. dinitrosalycilic acid method (Miller 1959) and phenol-sulfuric acid assay (Dubois 1956) - or volumetric methods - e.g. Somogyi's (1952) - consitute the standard assays for determining carbohydrates such as reducing sugars in fermentation media. All these methods suffer from lack of specificity and to some extent of reproducibility. They respond not only to any kind of reducing sugar and derivatives but also to other reducing substances, such as fats, containing reducing groups. Therefore, determination of a specific reducing sugar by classical chemical analysis of complex fermentation broths can only be achieved after thoroughly fractioning the sample in its constituents.

Enzymatic methods and high performance liquid chromatography (HPLC) can overcome these difficulties. Their application to fermentation monitoring has recently been reviewed (Schügerl 1988).

Enzymatic analyzers are very specific, fast, accurate and reproducible over short periods of time. Sample preparation is simple - viz. centrifuging and/or filtration - but frequent calibration is needed due to adherence of extraneous substances onto the enzyme support.

HPLC is more robust and general-purpose than enzymatic methods. Both HPLC and enzymatic analyzers offer a further advantage over the classical chemical methods in use by antibiotics industries: they can be moved from the laboratory to the process and put to work on-line with it (Schügerl 1988).

Materials and Methods

Penicillin-G fermentation data was obtained from pilot plant runs in 400 and 1000 liter bioreactors. The initial charge was made up of CSL and glucose syrup. Several additions are made continuously to the broth viz. pH controlling substances such as ammonium sulfate, antifoaming substances, and a penicillin-G precursor liquid feed. Glucose is also continuously fed at a convenient variable rate.

HPLC analyses were carried out with a BIORAD Aminex HPX 87H column connected to a Waters 410 differential refractometer (HPLC-DR) (P=85 bar; T=20°C; injected volume 20 μl, mobile phase 0.01N sulfuric acid; flow rate=0.5 ml/min). The detection limit achieved was 100-200 ppm. In order to measure concentrations below such limit an enzyme-based automatic analyzer (Y27) was also employed, giving low readings down to 5-10 ppm. This combination of techniques therefore overcomes the low sensitivity of detectors commonly used in HPLC carbohydrate analyses - e.g. diferential refractometers (Chaplin 1986).

The glucose auto-analyzer Y27 (Yellow Springs Instruments Co.) was operated as suggested by the manufacturer. Both the Somogyi and phenol-sulfuric acid assays were implemented as described in the literature (Somogyi 1952, Dubois 1956).

Results and Discussion

Carbohydrates in the Broth. The HPLC broth composition on carbohydrates was found to be glucose, fructose and lactose, their relative importance depending on fermentation age (Fig. 1). Appreaciable amounts of lactic acid were also found by HPLC (Fig. 1). Smaller amounts of other sugars such as sucrose and maltose, and some acids were detected but they account for less than 10% of all carbohydrates present in the samples. HPLC analysis of both glucose syrup and CSL have shown the sources of these substances. Glucose and fructose are the main constituents of the glucose syrup, while lactose, lactic acid and minor quantities of other acids are present due to the initial charge of CSL. These results are consistent with the syrup and CSL specifications.

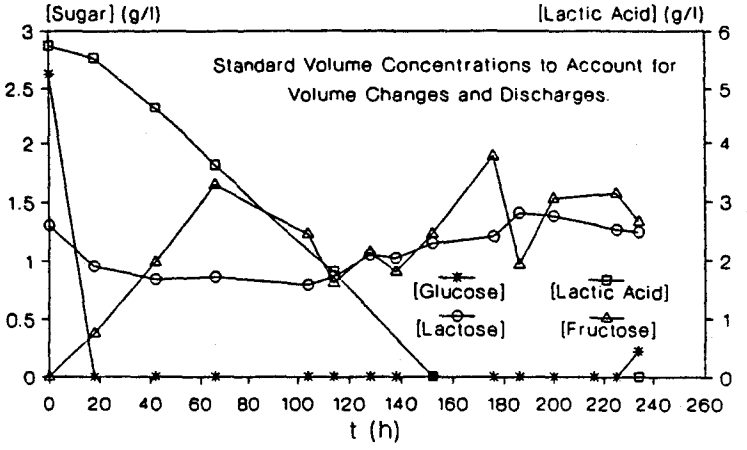

Figure 1 - HPLC Fermentation Monitoring.

Somogyi and Phenol-Sulfuric Acid Assays. The two methods indicate increasing concentration of total sugars and reducing substances in the broth along a fermentation run (Fig. 2). This increase cannot be assigned to glucose accumulation in the media. The HPLC (Fig. 1) and Y27 (Fig.2) results clearly rule this out. Other reducing sugars and reducing substances accumulate in the broth. The higher values obtained with Somogyi's method with respect to those of phenol-sulfuric acid, can satisfactorily be accounted for by the amount of lard oil that continuously accumulates in the fermenter by adding an antifoaming mixture. In the phenol-sulfuric acid method the less pronounced increasing trend can be explained by accumulation of small amounts of sugars other than glucose.

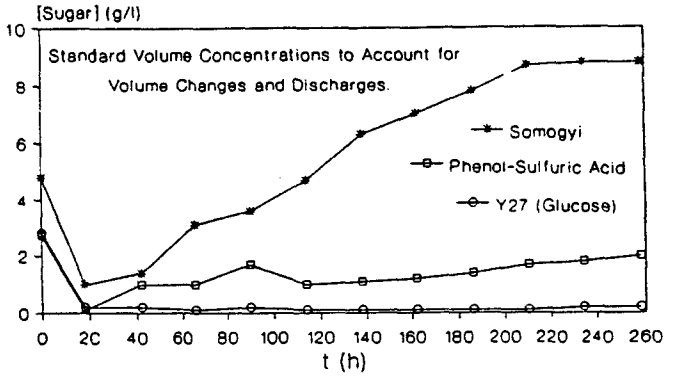

Figure 2 - Chemical Analysis Vs. Enzymatic Analysis.

Glucose Auto-Analyzer Results. The Y27 glucose analyzer was required to overcome the lack of sensitivity of the HPLC-DR set-up used. Glucose peaks are absent of all HPLC chromatograms after 20-40 h of fermentation. Residual Y27 glucose concentrations subsequently determined were in the range 100-400 ppm, thus supporting the evidence obtained by HPLC that the broth is depleted of most glucose (Fig. 2). Control tests with synthetic samples containing various sugars, lactic acid and fats have shown that interferences due to these compounds can be ruled out and that Y27 specificity and accuracy are very high.

Fermentation Monitoring. The combined use of Y27 glucose auto-analyzer and an HPLC-DR set-up can help to understand some characteristics of the operation. Firstly, it is observed that there is no glucose acccumulation (Figs. 1 and 2), i.e. glucose added is rapidly consumed. Secondly, the ability of *Penicillium chrysogenum* to consume substrates other than glucose - e.g., fructose, lactose and lactic acid - is shown by HPLC (Fig. 1). As glucose concentration goes down to 400 ppm fructose catabolism begins, cf. Fig. 2 and 3.

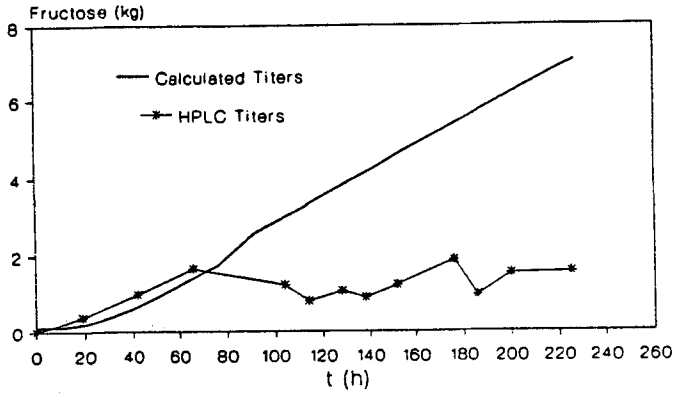

Figure 3 - Fructose Depletion. HPLC Titers Vs. Zero-Consumption Calculated Titers.

Concluding Remarks

Having identified several matabolizable substrates in the broth, one should conclude that several substrates other than glucose must be simultaneously monitored in industrial penicillin fermentation media containing complex nutrient sources; modelling fermentation growth and production phases on the basis of a single substrate is rather inadequate.

The Somogyi and phenol-sulfuric acid assays are non-specific, therefore of little value in complex fermentation media sugar analysis. HPLC produces the necessary fractioning but the general carbohydrate analytical set-up with a refractive index detector may have sensitivity limitations.

The combination of an enzyme-based auto-analyzer and of an HPLC-differential refractometer set-up constitutes a relatively low cost and simple alternative to more elaborate HPLC techniques. It allows the on-line characterization of reactor state, thus providing the way for the implementation of on-line and control strategies.

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Acknowledgments. This work was partially supported by CIPAN (Companhia Industrial Portuguesa de Antibióticos S.A.), JNICT (Junta Nacional de Investigação Científica e Tecnológica) under contract No. PMCT/C/BIO/904/90, and INIC (Instituto Nacional de Investigação Científica).