Physical and chemical optimization of a synthetic medium for *Pichia pastoris* growth

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Introduction

The methylotrophic yeast *Pichia pastoris* is widely used as a host strain for the production of a variety of heterologous proteins.
The basal salt medium (BSM) described by Invitrogen (2000) is one of the most broadly used media for this expression system, although it possesses many problems, namely unbalanced composition, salt precipitation and undesirable ionic strength. This medium also uses NH_4OH simultaneously, as the nitrogen source and the pH corrector, fact that may be responsible for several growth constrains, namely nitrogen limitation and low protein productivity.

Objective

Design of an alternative culture medium with balanced composition and pH correction independent from the nitrogen source.

Results

New Culture Medium Formulation

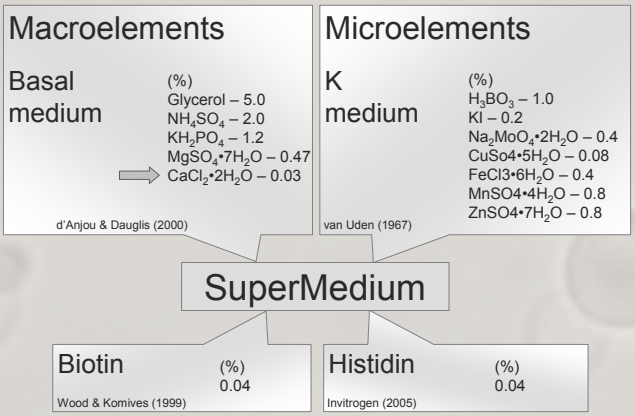

Figure 1 – Conjugation of components from four different media (the arrow indicates a adjustment) to design a new fermentation medium – SuperMedium (SMg).

Specific Growth Rate Achieved in Shaken Flask Cultures

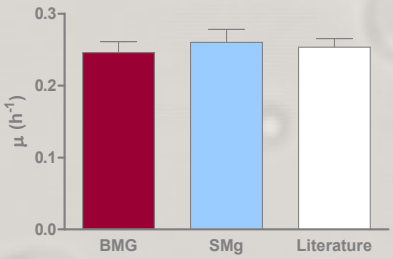

Figure 2 – Comparison between specific growth rates obtained in shaken flask cultures using the BMG (Invitrogen, 2005), SMg and values found in literature (d'Anjou & Daugulis, 2000) for the same conditions (30ºC, 200 rpm).

Growth Profile in Shaken Flask Cultures

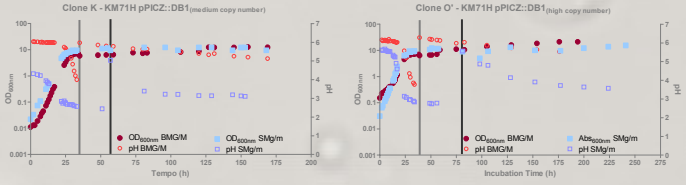

Figure 3 – Variation of the cultures optical density (DO) and pH along time. The bars indicate the beginning of the methanol induction phase (I) for the BMG/M, and (I) for the SMg/m (30ºC, 200 rpm).

Growth Profile in BioFlo110 Fermentor

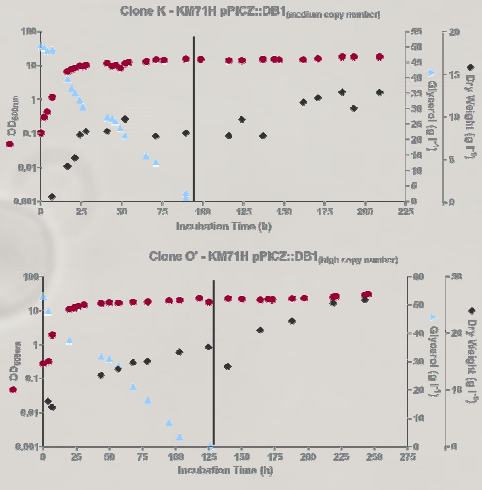

Figure 4 – Variation of optical density (OD), glycerol consumption and dry weight of *P. pastoris* along time in fermentor with SMg/m. (I) represents the beginning of the methanol induction phase (30ºC, dO_2 30% cascade with agitation 300-1100 rpm, 2 vvm and pH 5.5)

Conclusions

The designed medium was shown to be balanced, and allowed *Pichia pastoris* to achieve μ values similar to those referred in literature, with the further advantage of being less expensive.

Acknowledgements

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