Microbial Cell Factories

Poster Presentation

Set up and optimization of a fermentation protocol for the production of a human antibody fragment (Fab') express in *E. coli*. Pre-pilot and cGMP pilot scale studies

E Riscaldati, A Ciabini, A Baccante, D Moscatelli, MF Errichetti, A Colagrande, S Cencioni, F Marcocci, V Di Cioccio, L Di Ciccio, M Allegretti, F Martin and G Maurizi*

Address: Biotechnology Department and Biotech Pilot Plant – Dompé pha.r.ma s.p.a., Via Campo Di Pile, 67100 L'Aquila, Italy
* Corresponding author

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Background

The efficacy of mAb and their fragments in cancer therapy depends on their capacity to recognize tumor associated antigens. From a clinical point of view, intact mAb are employed in the treatment of lymphoid tumors whereas antibody fragments such as Fabs or $F(ab')_2$ can be the therapeutic of choice for solid tumors due to shorter circulating half life and a higher tumor penetration, both characteristics well suited in a radio immunotherapy (RIT) approach.

Ovarian cancer is the leading cause of death among gynecological tumors. Standard first line therapy consists of surgery followed by chemotherapy cycles. About 90% of patients relapse with a 5-year survival rate of 5–20% [1]. So far, second line chemotherapy after clinical relapse has shown limited efficacy. With the advancement of the recombinant biotechnology, a novel approach based on human monoclonal antibody immunotherapy has began to be investigated. The alfa folate receptor (αFR), which is a 38000 Dalton anchored membrane protein, is over expressed in more than 90% of ovarian carcinoma cells, and in 60% of other gynecological carcinomas [2]. In contrast to normal epithelial cells, ovarian carcinoma cells express this receptor on their external surface making it accessible for binding to monoclonal antibodies.

The basic concept of our project is to produce a full human $F(ab')_2$ fragment for radio immunotherapy to specifically target the αFR. The candidate Fab fragment was isolated and cloned into an *Escherichia coli* expression vector. After classical shaking flask cultures expression the recombinant strain was taken to lab scale fermentation plant where different feeding strategies were designed to increase the production yield [3].

Results

Fermentation scale up is aimed at the manufacture of large quantities of product while maintaining the specific yields and quality. We report the development of a Fed-batch fermentation process evaluated at 10 and 300 liters scale, for the production of functional Fab' monomer in *E. coli*, at high cell density fermentation, under DO-stat control strategy using a synthetic medium. The construction of a stable bicistronic expression vector and a proprietary host-plasmid pair allows us to obtain both high level expression (up to 100 mg/L) and correctly folded protein expressed in the periplasm but straightforwardly in the broth supernatant were the Fab' accumulate. To identify process-specific stress factors and to understand the physiological responses to the vessel specific physical conditions, the process was evaluated at 10 liters scale for animal grade production and with comparable results, at

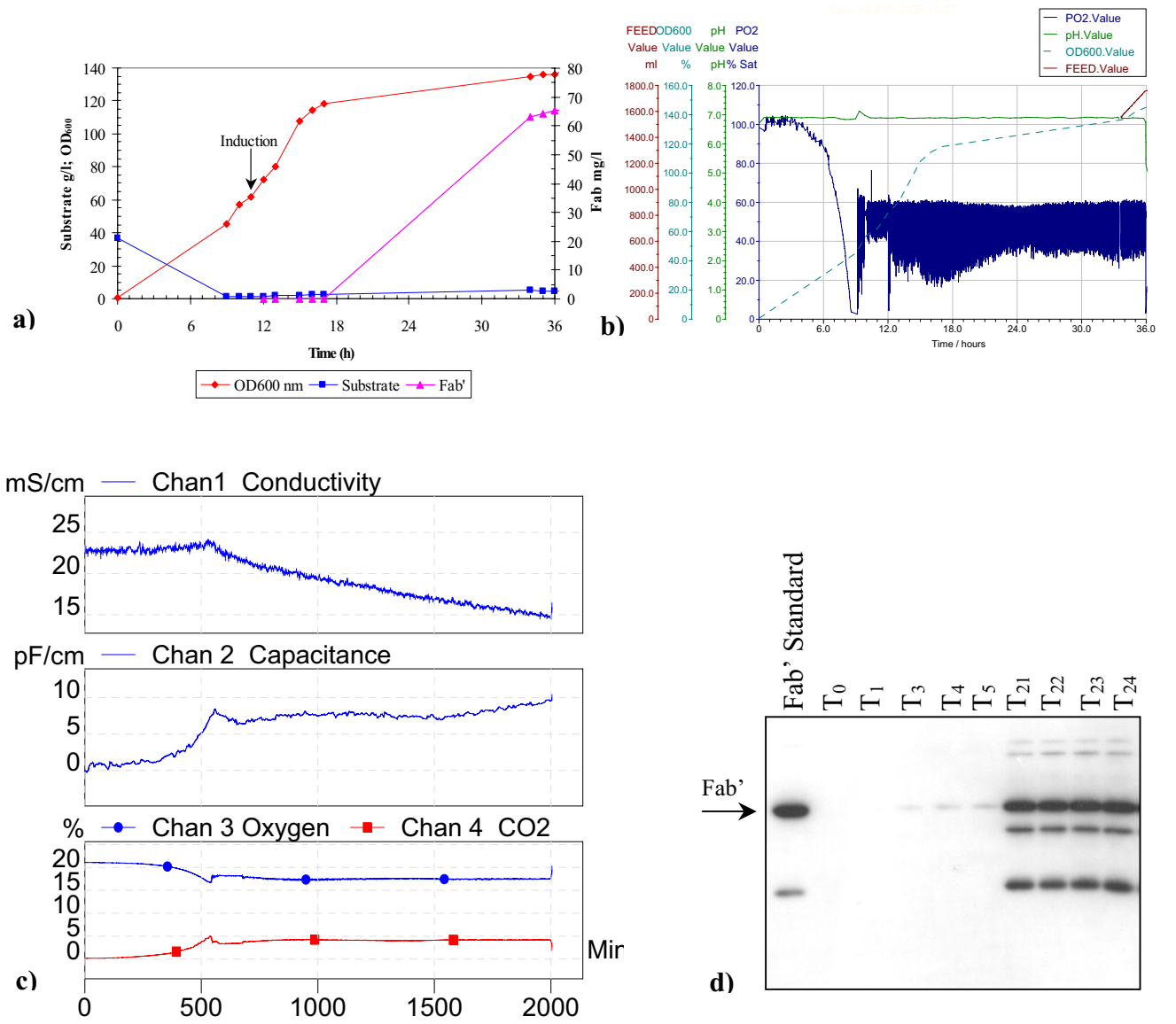

Figure 1

a)Effect of DO-Stat strategy on: (♦) biomass level (OD_{600}), (■) residual substrate concentration and (▲) Fab' accumulation at 10 liters scale fermentation (HPLC analysis); **b** typical curve of (–) DO, (–) pH, (**---**) OD_{600} and (**---**) Inductor (FEED) during DO-Stat fermentation; **c** representative curves of (–) conductivity and (–) capacitance in fermentation broth and Oxygen and CO_2 in exhausted air; **d** Western blot of samples taken throughout the induction phase

300 L scale in a clinical grade cGMP plant. Despite substrate oscillation due to the DO-stat mechanism, whitch final results is a linear feeding, at both scale the plasmid stability and cell vitality, measured by means of a biomass monitor (Aber instrument) were maintained. Neither lysis nor protease production were detected during the fermentation course.

Typical process parameter and analytical data coming from 10 and 300 L are reported in figure 1 (a-d) and in Figure 2 (a-b). In Table 1 mean yield coefficient at both scales are calculated.

All activities carried out for 300 L production scale are developed in a GMP compliance pilot plant. At the end of

Figure 2

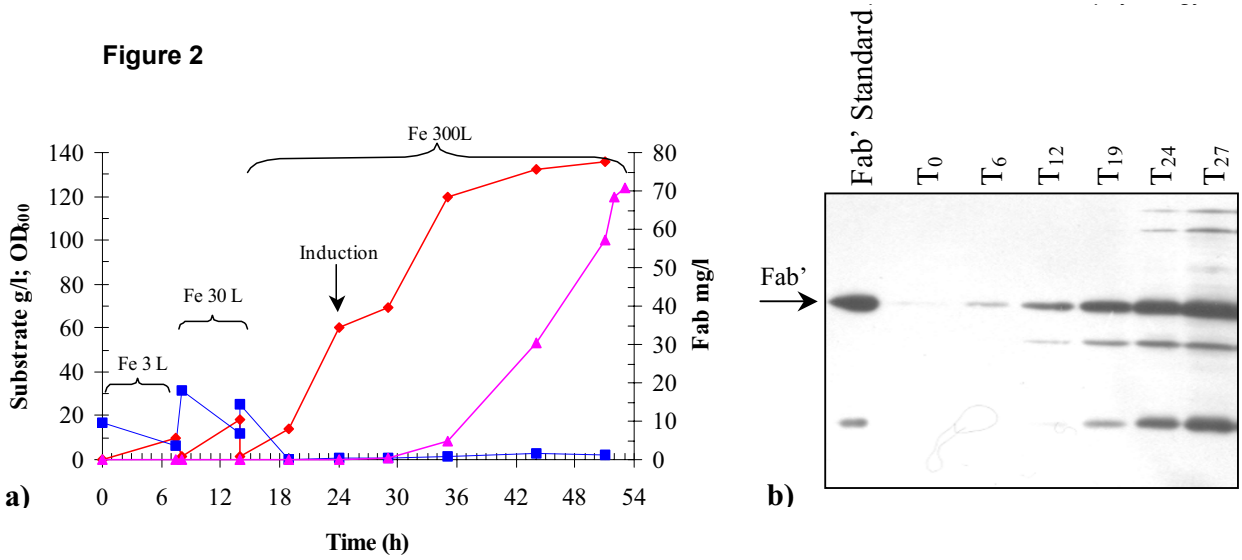

Figure 2
a Effect of DO-Stat strategy on: (♦) biomass level (OD_{600}), (■) residual substrate concentration and (▲) Fab' accumulation at 300 liters scale fermentation (HPLC analysis); **b**Western blot of different samples taken throughout the induction phase.

Table 1: Parameters for mean yield coefficient calculation at 10 and 300 L scale fermentation.

Fermentation scale (l)	Time (h)	^{OD}600	DCW g.l^{-1} (X)	Substrate g.l^{-1} (S)	Fab g.l^{-1} (P)	Fab g.l^{-1}.h (rP)	DCW g.l^{-1}.h (rX)	Substrate g.l^{-1}.h (-rS)	^{Y}XS g.g^{-1}	^{Y}PX g.g^{-1}	^{Y}PS g.g^{-1}
10	36	137	36.4	186	0.0642	0.00178	1.01	5.16	0.196	0.0018	0.00035
300											
3	8	10	2.9	15			0.36				
30	6	18	5.8	30			0.96				
300	37	136	36.2	190	0.0685	0.00185	0.98	5.13	0.190	0.0019	0.00036

fermentation both pH and temperature are adjusted to trigger contaminant proteins precipitation. The remaining soluble proteins were recovered from the biomass by tangenzial flow filtrations (TFF) and purified to homogeneity. Fab biological activity was monitored by BIACORE, FACS and ELISA analysis.

Conclusion

Altogether the reported data confirmed the technical feasibility of 10 to 300 L scale-up along with the process robustness and recombinant Fab' production consistency. Several fed-batch methods for carbon source feeding maintaining low by-products levels were developed. Most of these are based on mathematical models that foresee growth patterns and expected demand for nutrients. Our results suggest that a DO-stat feeding strategy is an efficient, straightforward scalable, physiological and elegant way of carrying out fermentation process to obtain both high expression level and properly folded protein. The Fab' quantity and quality produced by the described process were adequate to justify the production of clinical trials material. Moreover some critical parameters for scale up were identified.

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