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### ► **To cite this version:**

Kraïem Hazar, Manon Yannick, Hmila Issam, Ben Abderrazek Rahma, Susana María Alonso Villela, et al.. Expression of the recombinant NbF12-10 and its chimeric antibody format in Escherichia Coli: investigation of fed-batch bioprocess on minimal media. 2nd Mediterranean Congress on Biotechnology, Mar 2019, Hammamet, Tunisia. <hal-04982256>

**HAL Id: hal-04982256**

**<https://hal.science/hal-04982256v1>**

Submitted on 7 Mar 2025

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# Expression of the recombinant *NbF12-10* and its chimeric antibody format in *Escherichia Coli*: investigation of fed-batch bioprocess on minimal media.

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**Background and aim:** The unique recombinant clone expressing bispecific NbF12-10 nanobody was distinctly developed to remediate the shortcomings of the current polyclonal antivenom therapy (Hmila et al., 2010; Hmila et al., 2012). Recently, its humanized CH10-12 format-expressing clone was constructed (Institut Pasteur de Tunis). Both antibody formats display good neutralizing capacity toward AahII and AahI' toxins of *Androctonus* sp. scorpion venom. This work aimed to conduct fed-batch fermentative expression of the recombinant nanobodies (Nbs) by optimizing bacteria fermentation variables (Tp, pH, pO<sub>2</sub>, carbon feed, etc. ...) and downstream process (Tangential flow filtration (TFF)).

**Methods:** Experiments were conducted with 5 L Biostat B-DCU (Sartorius) fully instrumented. Three axenic cultures with *E. coli* WK6/pHEN6/NbF12-10 *versus* CH10-12 were investigated in pure oxidative culture, in order to produce biomass up to 10 gdcw/L with a control of growth rate by carbon substrate. The effect of time-temperature (28°C-25h and 37°C-4h) on nanobody expression was investigated. During the microfiltration process, the filters were used to concentrate the antibody fragments in extracellular. Filtration volume, differential pressure, and filtrate turbidity were recorded at different time intervals.

**Results:** During fermentation process, the growth kinetic is different during the batch, fed-batch and induction phase. During the oxidative batch phase, the mean growth rates have been defined: ( $\mu=0.12$  h<sup>-1</sup> and 0.05 h<sup>-1</sup> for CH10-12 at 37°C and 28°C respectively). For the same induction temperature (T°=37°C), the biomass concentration increase (13.9gdcw/L) conversely to a low NbF12-10 concentration (0,3mgP/L) in comparison with CH10-12. For CH10-12, the modulation of induction temperature from 28° C to 37°C improves protein production yields (0,83 and 1,47 mgP/L respectively). For the downstream process trial, we chosen Millistak+ HC F0HC, a filter grade designed specifically to follow a centrifugation step to improve the extracellular protein yield.

**Conclusion:** Based on our results, we demonstrated the impact of intrinsic (i.e. strains) and extrinsic factors (i.e. medium, glucose supply strategies, oxygen, temperature and induction time) on fed-batch fermentative expression of recombinant nanobodies (Nbs). In fact, the modulation of induction temperature from 28 to 37°C is a strategy applied to improve the protein production yields.

**Keywords:** NbF12-10, CH10-12, *E.Coli*, fed-batch bioreactor, yield and productivity.