Effect of oxygen carriers in microbial and plant cell cultures

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Dissolved oxygen concentration is one of the most limiting factors in aerobic cultures, due to the poor solubility of oxygen in aqueous media. In many processes, the microorganisms growth and production can be affected as a result of insufficient oxygen supply to the broths [1, 2]. To increase oxygen solubility, some methods can be used, such as the increment of aeration or agitation rates or decrease of the solution temperature [2]. A high air flow or intensive agitation rate can however leads to a phenomenon known as turbulent shear cell stress, which may have a negative effect on cell growth, particularly on vegetal or animal cells [3]. Thus another way to improve the oxygen concentration in a culture is by using a second liquid phase, commonly designated oxygen vector or carrier, in which oxygen presents a greater solubility than in water. These oxygen carriers can be saturated hydrocarbons, oils or perfluorochemicals, and their advantage is the increment of oxygen transfer to microorganisms [2].

In this study, the growth of the yeast *Saccharomyces cerevisiae*, the bacteria *Xanthomonas campestris* and *Cynara cardunculus* plant cells with addition of *n*-dodecane, soybean oil, polydimethylsiloxane or perfluorodecalin have been studied.

Experiments were carried out in 100ml Erlenmeyer flasks with 60 ml of YPD, medium described in Garcia-Ochoa *et al* [4] and B5 medium [5], respectively for, *S. cerevisiae*, *X. campestris* and *C. cardunculus*. Cultures were grown in duplicates at 150rpm and 29±1ºC, containing 10% (v/v) of vector. For each culture, it was determined the cell growth by dry weight, as well as the optical density for *S. cerevisiae* and *X. campestris*. Sugar contents in all cultures were quantified by HPLC analysis. The xanthan gum produced by *X. campestris* was quantified by dry weight, after precipitation with ethanol. The phenol content in *C. cardunculus* cultures was quantified by direct spectrophotometric method at 270nm.

The growth of *S. cerevisiae* presented a higher biomass density with perfluorodecalin next to the control culture (Figure 1). These results are concordant with the work developed by Pilarek & Szewczyk [3] where *S. cerevisiae* grown with perfluorodecalin shown an increase of 30 % in biomass. The presence of oxygen in *S. cerevisiae* cultures promotes the biomass production.

In the *X. campestris* culture, the *n*-dodecane presented the higher biomass dry weight and xanthan gum concentration, at 34h of culture. Studies performed with the bacteria *Pantoea agglomerans* with 1% (v/v) of *n*-dodecane, showed that this vector enhanced the oxygen mass transfer coefficient (K_La) of the culture as well as the biomass productivity [6].

The cellular growth of *C. cardunculus* culture are similar with the different vectors, with low levels of phenolic compounds, except for the culture grown with soybean oil that presented values slightly higher than the others cultures. Pilarek & Szewczyk [2] observed an increase in biomass production for tobacco cells grown with perfluorodecalin.

These results suggest that oxygen vectors tested in these different cultures, yeast, bacteria and plant cell culture are efficient liquid oxygen carriers and facilitate gas transfer in the broth culture. However, due to the difficulty of working with the oils, since they can interfere in the results and be metabolized by the cells, perfluorodecalin and *n*-dodecane seems to be the vectors which better improved the biomass production what may be attributed to higher oxygen availability in the culture.

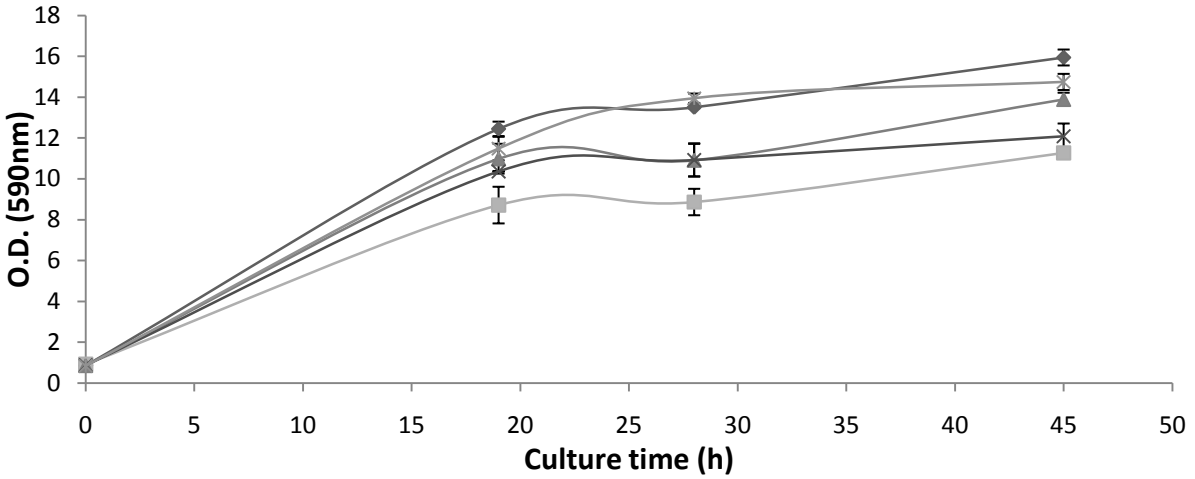

Figure 1. Optical densities, obtained at 590 nm, for *S. cerevisiae* growth culture, at 150 rpm and 29±1ºC, as a function of the culture time. Cultures with different oxygen vectors, —◆— perfluorodecalin, —■— soybean oil, —▲— polydimethylsiloxane, —✳— *n*-dodecane and —✳— control (with no vector).

This study shows a promising way to improve the oxygen transfer, in aerobic bioprocesses where this factor is limiting, and as other works have shown [7, 8], can contribute to a great enhancement of K_La. Antunes [6] work showed that at 1% (v/v) *n*-dodecane, K_La can achieve a value of 33.80 h^{-1}, compared to the 9.44 h^{-1} for water in the same conditions. Further studies with the oxygen vectors that presented better performance, with higher oxygen mass transfer, will be carried out in a mechanically stirred bioreactor and correlated with the broth rheology of different cultures.

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