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Study of heterogeneities in laboratory scale and prediction of mixing time evolution during the scale-up of an animal cells culture in a stirred bioreactor

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Nowadays, animal cell cultures are essential for the proteinic compound production. Besides, the size of the stirred tank bioreactor has never stopped to increase since the last decades. Given that animal cells have only a thin plasma membrane, they were considered, sometimes in a excessive way, as more sensitive than bacteria to mechanical constraints produced by liquid motions (velocity gradients, interactions with micro-eddies, collisions…) and by the air sparging (bubble rupture, coalescence and bursting…). Therefore, mild agitation conditions were usually chosen during the process adjustment of animal cell culture. But, these mild agitation conditions could involve more and more heterogeneities when the size of the bioreactor increases to reach industrial one. Nevertheless, these heterogeneities are already problematic at laboratory scale because the alkaline solution injected to regulate the pH generates high alkaline concentration near the injection point. In this area, cells could be instantly killed. The aim of this study is therefore double:

- Predict the evolution of mixing time with the size of the bioreactor (20L, 80L, 600L);
- Quantitatively describe the evolution of the concentration field after an alkaline injection in a laboratory scale stirred bioreactor (20 L).

These both studies were performed in stirred tank in geometrical similitude (2 baffles, hemispheric bottom, H/D=1; Y/D=1/3), filled with water and mixed successively by two kinds of axial impellers (Elephant Ear, propeller TTP) at typical rotational speed of animal cell culture.

To measure the mixing time in bioreactors with increasing size (20 L, 80L, 600L), a NaCl solution is injected at the top surface of the liquid. The conductivity evolution of the liquid is measured by a conductivity probe also placed at the surface of the liquid. The mixing time is determined by choosing a 95% homogeneity criterion ($\theta_{95\%}$). The Greenville correlation which depends on impeller kind, tank geometry and impeller rotational speed is fit on these experimental data. The agreement is quite good for each impeller.

To characterize the heterogeneities induced by alkaline injection in a laboratory scale stirred bioreactor, the temporal evolution of a tracer concentration field is recorded by PLIF technique in the vertical plane situated in the middle of the 20 L stirred tank. The evolutions are quantified in two ways. Firstly, the variance of each concentration field is plotted according to the time. The mixing time $\theta_{95\%}$ can be obtained from these plots. Furthermore, several agitation conditions can be directly compared on basis of the decreasing speed of the variance. Secondly, the amount percent of the plane characterised by a concentration larger than critical one is extracted from each concentration field. One thus obtains the evolution of that quantity according to the time. Moreover, a new mixing time can be defined as the time needed for that quantity to reach zero. Of course, this mixing time depends on the choice of the critical concentration.

Keywords. Animal cell culture, heterogeneity, mixing time, Planar laser Induced Fluorescence
Reference. Greenville,R.K, Ruszkowski,S. and Garred, E. (1995), 15th NAMF Mixing conference, Banff, Canada