# EFFECT OF THE OXYGEN TRANSFER RATE ON OXYGEN-LIMITED PRODUCTION OF PLASMID DNA BY ESCHERICHIA COLI

Alvaro R. Lara, Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa
alara@correo.cua.uam.mx
Karim E. Jaén, Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa
Olusegun Folarin, Department of Biochemical Engineering, University College London
Eli Keshavarz-Moore, Department of Biochemical Engineering, University College London
Jochen Büchs, RWTH Aachen University, AVT-Biochemical Engineering

Key Words: Oxygen limitation, microbioreactors, plasmid DNA, *Escherichia coli*, *Vitreoscilla* hemoglobin.

Oxygen limitation can increase the pDNA yield in cultures of *Escherichia coli*. Nevertheless, such effect has not been studied systematically. Namely, only cultures at low DOT have been performed, excluding important factors like the oxygen transfer rate (OTR). Moreover, to the best of our knowledge, there is no information regarding the impact of oxygen availability on the topology of the plasmid. The supercoiling of DNA requires energy and it is hypothesized that oxygen availability will affect the produced isoforms. In the present study, we performed fully aerobic and oxygen-limited cultures of *E. coli* bearing a high copy number plasmid. Cultures at $OTR_{max}$ values of 10, 14, 30, 45 (for oxygen-limited cultures) and 110 mmol $L^{-1}$ $h^{-1}$ (for aerobic cultures) were performed in microtiter plates with DOT, pH, biomass (measured as scattered light) and NADH fluorescence online monitoring. To further investigate the impact of oxygen limitation on pDNA topology, an *E. coli* strain constitutively expressing the *Vitreoscilla* hemoglobin (VHb) was used. VHb is known to improve aerobic respiration and consequently ATP generation at low oxygen availability. Our results show that the pDNA yields on biomass ($Y_{pDNA/X}$) were inversely proportional to the $OTR_{max}$ for both strains, and increased more than two-fold in cultures at the lowest $OTR_{max}$, compared to aerobic cultures. Expression of VHb resulted in lower $Y_{pDNA/X}$, compared to cultures of the parent strain. The strain expressing the VHb displayed higher specific growth rates at $OTR_{max}$ of 10, 14 and 30 mmol $L^{-1}$ $h^{-1}$, compared to the parent strain. However, at $OTR_{max}$ of 45 and 110 mmol $L^{-1}$ $h^{-1}$, the growth rate of the parent strain was higher. In general, the specific NADH fluorescence was lower in cultures of the engineered strain, which can be associated to a more oxidized intracellular state, in agreement with the proposed effect of VHb on the cellular metabolism. The pDNA supercoiled fraction (SCF) was maximum in cultures at $OTR_{max}$ of 30 mmol $L^{-1}$ $h^{-1}$, reaching 92.9 % for the wild type strain and 98.7 % for the strain expressing VHb, while no linearized pDNA was detected. This condition was replicated in a 1 L stirred tank bioreactor (STB) for W3110 *recA*$^{-}$, due to the higher productivity of this strain. The performance of cultures in the STB was very similar to that of cultures in the MTP concerning accumulated fermentative by-products, cell growth and pDNA production and SCF.

Altogether, these results show the existence of an optimal $OTR_{max}$ for oxygen-limited production of plasmid DNA. Furthermore, we demonstrate that studies in microtiter plates are excellent to predict culture performance of STB and to scale-up plasmid DNA production cultures.