# Influence of the nitrogen source and of the mitochondrial alternative oxidase (AOX) on the response to sulphur deficiency in *Chlamydomonas reinhardtii*

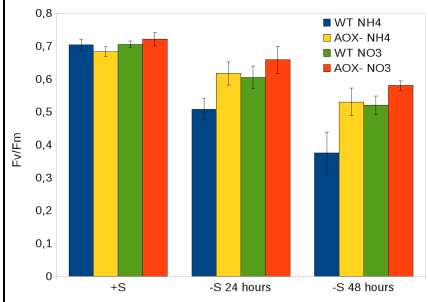
**Thomas de Marchin, Bart Ghysels, Fabrice Franck***

***Laboratory of Bioenergetics, University of Liège, Belgium***

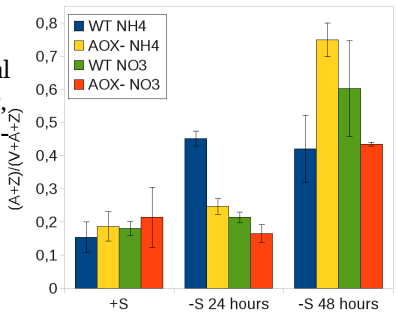
***contact: F.Franck@ulg.ac.be**

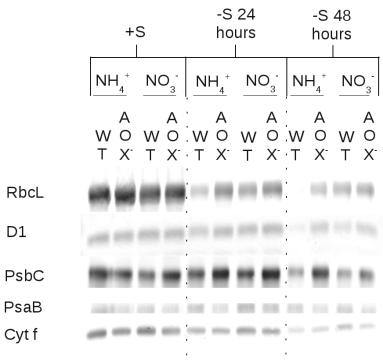
Hydrogen photoproduction by *Chlamydomonas reinhardtii* is usually studied in sulphur-deprived cells, in which photosynthetic activity is reduced and starch accumulates to high levels. Media used in standard protocols generally contain ammonium as nitrogen source. We show here that the nitrogen source (nitrate or ammonium) and the presence of the mitochondrial alternative oxidase (AOX, a nitrate-inducible enzyme[1]) strongly influence the photosynthetic response to sulphur deficiency.

The AOX-deficient mutant (AOX$^-$) used here (see Mathy et al.[2]) does not express the AOX1 gene. As this Western Blot experiment shows, AOX protein was not detected in this mutant whatever the conditions. In S-replete wild type, it was detected only on nitrate. The protein level increased during S-deficiency and after 48 hours it was detected both on nitrate and ammonium in wild type.

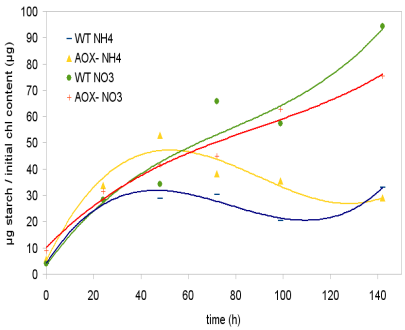

In standard (ammonium based) media, S-deficiency is known to cause a drop in PSII activity. As shown here (left), PSII photochemical efficiency (Fv/Fm) strongly decreased. However, this decrease was strongly attenuated in the AOX- mutant (both on nitrate and ammonium), as well as in wild type cultivated on nitrate. As a stress indicator, the xanthophyll de-epoxidation index (right) was found to increase strongly under S-deficiency. Here again, the effect was most pronounced for wild type on ammonium.




The amounts of photosynthetic proteins (RbcL of Rubisco, D1 and PsbC of PSII and to lesser extents PsaB of PSI and Cyt f) decrease during S-deficiency (left). Again, this effect was most evident for wild type cells on ammonium, especially for RbcL and D1 after 48 h.

Under S-deficiency, the persistence of photosynthetic activity on nitrate resulted in a remarkable starch accumulation compared to ammonium (right). On ammonium, starch accumulation was stronger in the AOX- mutant than in wild type.




The drop of photosynthetic activity and the accumulation of starch under S-deficiency are both important in the context of $H_2$ photoevolution by *Chlamydomonas reinhardtii*. Here we have shown that these parameters are strongly dependent on the nitrogen source, which might (at least partly) be explained by the interactions between nitrogen assimilation and photosynthetic electron transport. On the other hand, AOX deficiency attenuates the response to S-deficieny. This may arise from the upregulation of oxidative stress defence systems in AOX-deficient cells, as shown recently[2].

References:

1.Baurain D, Dinant M, Coosemans N, Matagne R (2003) Plant Physiol. 131: 1418-1430

2. Mathy G, Cardol P, Dinant M, Blomme A, Ghysels B, Cloes M, De Pauw E, Leprince P, Remacle C, Sluse-Goffart C, Franck F, Matagne R, Sluse F (2010) J. Proteome Res. 9 : 2825-2838