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BIOTECHNOLOGY OF HYDROGEN PRODUCTION WITH THE MICROALGA *Chlamydomonas reinhardtii* CULTURES IN LABORATORY AND OUTDOOR PHOTOBIOREACTORS

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Abstract

The increase in the cost of energy and the problem of global warming have fostered considerable international efforts to discover a sustainable way to produce energy with zero CO_2 emission. One friendly way is photobiological H_2 production using the microalga *C. reinhardtii*. A D1 protein mutant of *C. reinhardtii* has been screened in our laboratory for its H_2 evolution capacity. The D1 mutant (L159I-N230Y) carries a double amino acidic substitution. The first substitution involved the L159 leucine residue, which was replaced by isoleucine, while the second mutation involved the N230 residue asparagine, which was replaced by tyrosine. This mutant has proved to be 5 times more productive as compared to the CC124 strain, a reference strain commonly utilized for hydrogen experiments worldwide. A preliminary phenotypic characterization has identified some important features in L159I-N230Y, such as: (i) a reduced antenna size (i.e., a reduced amount of chlorophyll per cell), (ii) high photosynthesis rates, and (iii) higher D1 content per cell.

We have also investigated the possibility of using diluted pretreated olive-mill wastewaters (OMW), which contain organic acids and sugars, as a substrate on which to grow *Chlamydomonas*, to obtain suitable biomass to produce hydrogen. Growth of *C. reinhardtii* cells in a mixture of treated OMW diluted with TAP (Tris-Acetate- Phosphate), caused considerable changes in both the growth rate and the biochemical composition of the biomass. Because of the nitrogen limitation and the high organic compound content, the growth of cells on OMW-TAP was mainly addressed towards carbohydrate synthesis which contributed to hydrogen production. Indeed, these exhibited a greater production of hydrogen (150 ml H_2 l^{-1} culture), compared to the control cells (100 ml H_2 l^{-1} culture). The results offer a useful perspective for the utilization of wastewaters, with high COD (5000-10000 mg l^{-1} O_2), which constitute an environmental problem.

Finally, results of experiments conducted with sulfur-deprived cultures of *C. reinhardtii* in a 50-L and 110-L tubular photobioreactors (PBRs) are also presented. H_2 production experiments were carried out under both artificial and direct solar light. The final H_2 output attained with acclimated cultures under sunlight reached 0.93 L, in the 50-L PBR and reached 3 L in the 110 L PBR. These amounts corresponded to about 20% of what obtained in the laboratory.