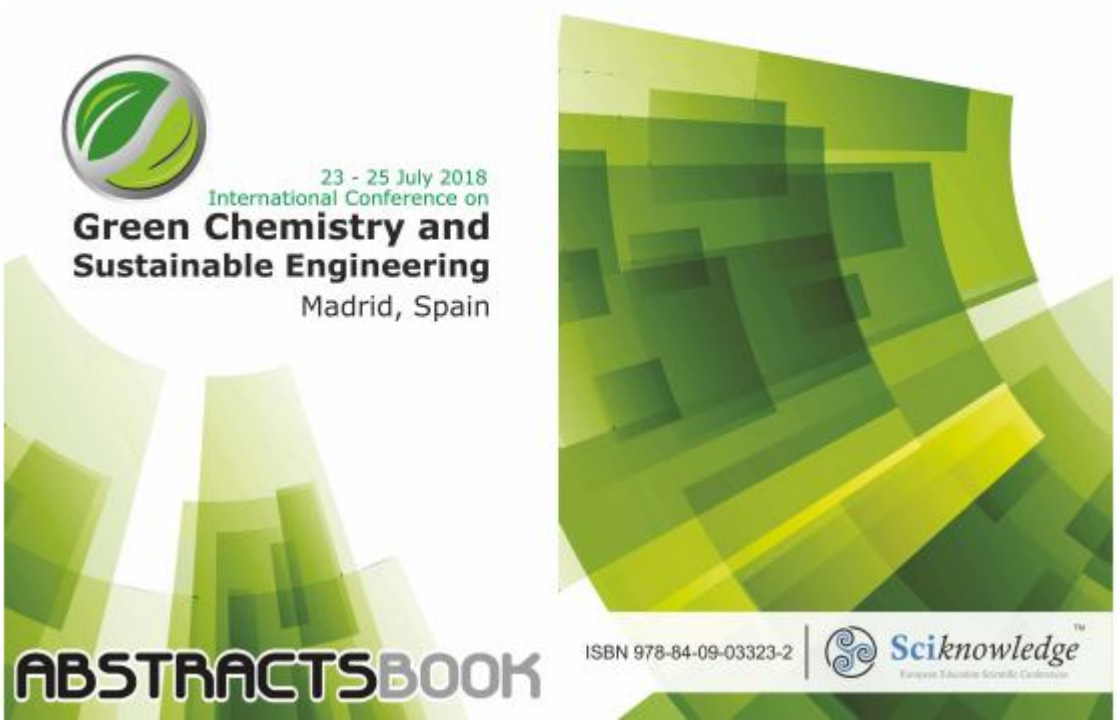

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Titanium dioxide nanoparticles under heat-shock negatively modulate the Crabtree Effect in *Saccharomyces cerevisiae*

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1. Introduction – Although nanomaterial is widely used in consumer products, few studies on its potential effects on eukaryote cells have been carried out and the mechanisms underlying its action are only partially understood since their unique physicochemical properties are such that its interaction on living organisms are thought to be quite different from that of bulk materials with the same composition. The energy metabolism of the eukaryotic cell may provide a good subject for such studies since in the literature the occurrence of drastic changes in glycolysis, citrate cycle and fermentative metabolism in tumour cells is frequently described. The Crabtree Effect, or the catabolic repression of respiratory metabolism by glucose, which has been detected in several yeast species, is similar to the aerobic glycolysis of tumour cells. As the level of titanium dioxide nanoparticles (TiO_2-NP) in the environment has increased in the last few decades, there is now an urgent need to test its possible effects on the energy metabolism of eukaryote cells. The aim of this study was to confirm the occurrence of this metabolic change in the *Saccharomyces cerevisiae* UE-ME_3 and also to test the capacity of TiO_2-NP < 100nm (5 µg/mL) under heat-shock over 200 min for modulating these metabolic pathways.

2. Experimental – TiO_2-NP (size <100 nm, Sigma) stock suspensions were prepared by sonication. Bioassays were performed in YPG medium (1 % yeast extract, 2% peptone, 3% glycerol). Culture flasks were inoculated using a fresh culture of wild-type *S. cerevisiae* UE-ME_3 and shaken at 150 rpm, at 28 ºC. At the exponential growth phase (OD=0.8) was added 2% glucose (YPGD medium) and/or 5 µg/mL TiO_2-NP. Yeasts cells were allowed to grow for 200 min at 28 or 40 ºC (heat-shock, HS). Cell viability at time t_0, t_{200} was determined by cfu. Post-12,000 *g* phosphate buffer supernatants were used for determining contents in ROS and MDA by means of fluorescence, and enzyme activities HXK, PDC, ADH, SOD1 and GPx by means of UV/Vis spectrophotometry. Post-12,000 *g* tris-sucrose buffer pellets were used for determining enzyme activities CS, SDH and NDE1. Stat analysis: ANOVA I, Duncan test.

3. Results and Discussion - The results showed that cell viability and HXK, PDC, ADH enzyme activities in the cells grown in YPGD medium (fermentative-respiratory) were higher than those detected in cells grown in YPG medium (respiratory). Furthermore, cells grown in YEPGD medium exhibited lower levels of CS, SDH, NDE1, GPx enzyme activities and MDA contents than those detected in yeasts grown in YPG medium. *S. cerevisiae* grown in YPGD medium and 5µg/mL TiO_2-NP under heat-shock showed a level of cell viability (%) lower than that found in the yeasts which used glycerol as a carbon source. Additionally, it was found that *S. cerevisiae* grown in YPGD and NP under heat-shock exhibited levels of HXK, PDC and ADH, GPX and SOD1 enzyme activity which were similar to or lower than those determined in the cells which were grown in exclusive respiratory mode. In contrast, it was found that yeast cells exposed to NP under heat shock exhibited ROS and MDA contents as well as levels of SDH and NDEI enzyme activity where were similar to or higher than those found in respiratory cells.

4. Conclusions - The findings confirm the UE-ME_3 strain as a Crabtree-positive yeast since the addition of glucose to respiratory cells caused an increase in cell viability, fermentative and glycolytic activities, repression of the citrate cycle and the respiratory chain, followed by a decrease in cell damage. The exposure of *S. cerevisiae* to TiO_2-NP < 100 nm under heat shock (28/40 °C), for 200 min prevented the glucose-dependent transition from respiratory to respiratory-fermentative metabolism, as evidenced by both the maintenance of ADH enzyme activity which was similar to that detected in respiratory cells and the very low levels of cell viability, glycolytic, fermentative, and antioxidant activity and very high levels of oxidative stress, cell damage and active respiratory metabolism.