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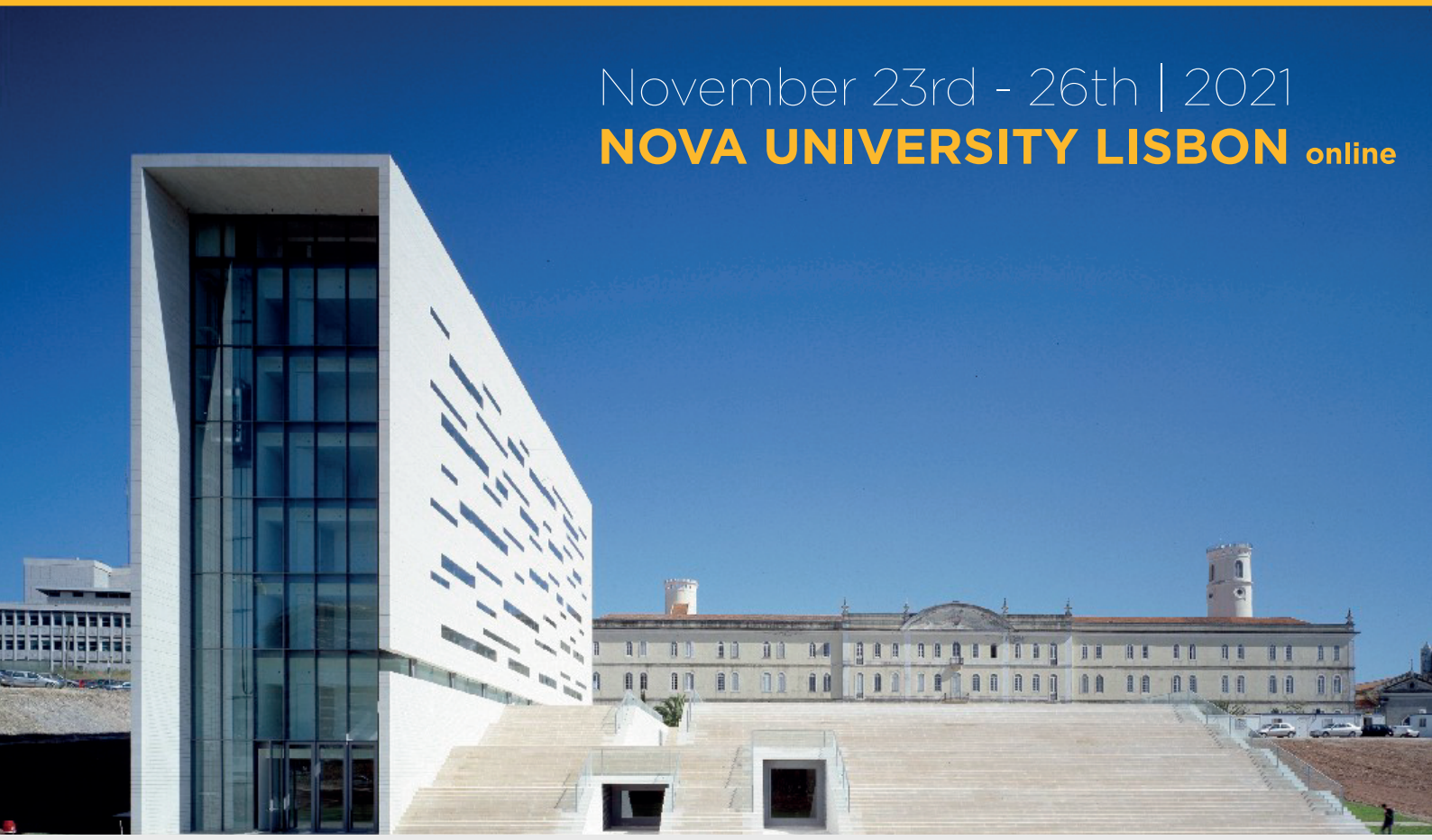

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102. Sustainable lysis of *Bacillus subtilis* biomass to recover the biopharmaceutical L-asparaginase

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The first-line biopharmaceutical used to treat Acute lymphoblastic leukemia (ALL), Oncaspar, is based on the enzyme L-asparaginase (ASNase), and has annual sales of ca. USD $100 million. In addition to other sources, genetically modified *Bacillus subtilis* is regarded as one of the most promising hosts for the ASNase production. The *Aliivibrio fischeri* ASNase type II, which has anti-tumour activity due its higher specific affinity for L-asparagine, expressed in *B. subtillis* is located in the periplasm. Therefore, cell lysis is required for the ASNase recovery. Nevertheless, typical cell lysis approaches, e.g. chemical methods with surfactants lead to some biocompatibility concerns and the need of extra purification steps. To overcome this drawback, in this work, ultrasound sonication (USS) conditions were studied to develop a greener and more biocompatible method for ASNase recovery from *B. subtilis* cell lysis. The USS cell lysis was optimized regarding the amplitude of USS pulse, number of lysis cycles and mass of cells/volume of solvent ratio. The identification and quantification of ASNase and major impurities present in the cell extract after lysis were investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion high-performance liquid chromatography (SE-HPLC). ASNase activity was determined by monitoring the hydrolysis of the substrate, L-asparagine. The results obtained show that the ideal conditions for *B. subtilis* cell lysis are an amplitude of USS pulse of 60%, 40 cycles of lysis and 10 mL of phosphate-buffered saline (PBS) per 1 g of cells. Overall, an optimized sustainable *B. subtilis* cell lysis method was developed, avoiding the use of surfactants and with low energy consumption.

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