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Expression of TrCel5A via *K. lactis* thus imparts stability properties which could be of significant benefit to this enzyme for industrial use, such as in biofuel production.

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ABILITY OF *GORDONIA ALKANIVORANS* STRAIN 1B FOR ENHANCED DESULFURIZATION OF DIBENZOTHIOPHENE AND ITS DERIVATIVES USING FRUCTOSE AS CARBON SOURCE

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In order to keep up the strict sulfur limits on fossil fuels and their derivatives, refineries commonly use a desulfurization method, which combines high temperatures and pressures with molecular hydrogen known as hydrodesulfurization (HDS). However, the effectiveness of HDS to desulfurize recalcitrant organic aromatic compounds such as dibenzothiophene (DBT) or its derivatives is low.

Biodesulfurization (BDS) has been described as a promising complementary technique to HDS. Using microorganisms, BDS is able of desulfurize several recalcitrant compounds usually present in fossil fuels at mild temperatures and pressures without hydrogen, making it a simple and eco-friendly process. In this context and based in the fructophilic behavior of the desulfurizing bacterium, *Gordonia alkanivorans* strain 1B, several recalcitrant sulfur sources were tested in BDS assays using fructose as carbon source. So, strain 1B was used in desulfurization assays testing 4-mDBT, 4,6-dmDBT and 4,6-deDBT, as sulfur source, in comparison with DBT. Growth and desulfurization kinetics using the different sulfur sources were evaluated and the desulfurization rates were determined by GC analysis of x-DBT consumed. The results showed that the strain 1B using fructose as carbon source was able to fully desulfurize all the sulfur compounds tested in less than 121 hours. For 4-mDBT, 4,6-dmDBT and 4,6-deDBT the maximal bacterial growth rates obtained were 0.072 h^{-1}, 0.069 h^{-1} and 0.095 h^{-1} with maximum desulfurization rates of 1.58, 4.84 and 4.30 µmol $g(DCW)^{-1}$ h^{-1}, respectively. In comparison with previous results obtained for m_{max} of strain 1B in glucose as carbon source and DBT as sulfur source (0.025 h^{-1}), all the m_{max} obtained in this study highlight once more the importance of use fructose as carbon source, independently of sulfur source. In addition, contrary to what has been described for other strains, the desulfurization rates obtained for the compounds with two alkyl groups were higher than for DBT (2.12 µmol $g(DCW)^{-1}$ h^{-1}). In fructose, the desulfurization of 4,6-dmDBT and 4,6-deDBT by strain 1B were more than 2-fold in comparison with that for DBT. These promising results indicate the high potential of use this bacterium towards fossil fuels BDS.

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OPTIMIZATION OF ANAEROBIC DIGESTION PROCESSES OF SOLID WASTE FROM WINE PRODUCTION

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The wine industry processes millions of tons of grapes each year, which leads to significant wastewater production. Through auxiliary processes (cleaning of vats, machines, pipes and floors), a mostly organic waste stream is created, which typically exceeds regulatory limits and must be treated. Usually treatment is accomplished via aerobic systems, which are costly to operate due to energy requirements of aeration equipment. The AD-WINE project, a Marie Curie Industry-Academia Partnership comprised of institutions from five European countries, aims to develop an anaerobic treatment system adapted specifically for the effluents from medium-sized wineries. The development of high-performance digesters and optimized treatment schemes will serve to lower energy and operation costs, reduce production of sludge and residues, and improve valorisation of by-products and biogas. Wine production is characterized by high variance in wastewater composition due to seasonal differences in organic load. During the vintage (harvest and initial processing) season, which lasts roughly three months, most of the total organic wastewater is produced. The non-vintage season is characterized by lower organic wastewater loads.

In order to obtain a single reactor which can be useful during both periods, two options were studied:

- To add microbes at the beginning of vintage period. Therefore, the number of microorganisms, which integrate the biologic system, would increase rapidly. Thus, it would be easier to prepare the reactor for the vintage period
- To store and pre-treat the organic solid wastes produced during the vintage period, in order to solubilize the COD from organic solid wastes. Then, out of vintage season, to treat simultaneously: the liquid fraction produced from pre-treatment of solid wastes and wastewater generated in the winery. This would increase the biogas production outside of the vintage period.

The second issue was studied at the Consortium for Applied Research in Biotechnology (CRAB) in Avezzano, Italy. The following tasks were performed:

1. Characterization of organic solid wastes generated through grape processing
2. Comparison of different pre-treatment methods for transferring the organic matter from solid wastes to a liquid zone (COD solubilized)
3. Determination of anaerobic biodegradability of the pre-treated liquid zone, including specific biogas formation (*Valorisation of biomass waste streams*)

Tests were carried out under different operating conditions and with varying time increments. Results will be presented with respect to the above-listed categories, including tables and graphs. Additionally, the work performed in this section of the AD-WINE project will be described in relation to the overall project evolution, including relevant conclusions and future work.