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Las siguientes Instituciones han financiado y auspiciado la organización del XII Congreso Argentino de Microbiología General:

/ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

/ Agencia Nacional de Promoción Científica y Técnológica (ANPCyT)

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/ International Society for Microbial Ecology (ISME)

/ Secretaría de Estado de innovación y Desarrollo Tecnológico (SIDETEC)

/ Centro de Innovación e Investigación para el Desarrollo Educativo, Productivo y Tecnológico (CIIDEPT)

/ Centro Científico Tecnológico-Tucumán (CCT-Tucumán)

/ Ente Tucumán Turismo

La Comisión Organizadora Local agradece muy especialmente la colaboración, trabajo ypermanente disposición de Daniela Russo y Diana Vullo.



















PRODUCTION OF A LIPASE ACTIVITY BY SOLID STATE FERMENTATION USING Aspergillus niger MYA 135: IMPACT OF DIFFERENT HUMECTANT MIXTURES

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The solid state fermentation is an economical alternative for production of industrial enzymes such as lipases mainly because this technology proposes the reuse of agro-industrial waste as well as adding value to those residues. Lipases (EC 3.1.1.3) are triacylglycerol hydrolases that catalyze the hydrolysis of triglycerides to free fatty acids and glycerol. In non-conventional systems these enzymes can also catalyze esterification, transesterification and interesterification reactions. The aim of this work was to evaluate the production of a lipase activity from Aspergillus niger MYA 135 by solid state fermentation using different humectant mixtures. As solid substrate, washed sugarcane bagasse was used. Humidity was adjusted to 90 % with the following mixtures containing (in g/l): 1) M1: Vinasse 5.0, molasses 5.0, milk serum 10.0, peptone 5.0, cerelose 5.0, waste frying oil 10.0, FeCl3 1.0, CaCl2 0.5. 2) M2: Sucrose 10.0, KH2PO4 1.0, NH4NO3 2.0, MgSO4 0.2, CuSO4 0.06, FeCl3 1, olive oil 20. 3) M3: Sucrose 10.0, KH2PO4 1.0, NH4NO3 2.0, MgSO4 0.2, CuSO4 0.06, yeast extract 1.0, peptone 5.0, olive oil 20. Humectant mixtures M1 and M2 were previously used as culture media for A. niger MYA 135 lipase production by submerged fermentation. Humectant mixture M3 was formulated from literature data. Reactors were inoculated with 105 conidia per gram of solid substrate and incubated at 30°C during 48 h. Then, the fermented substrate was dried at 45 °C and used as enzyme source. The hydrolytic activity was measured with p-nitrophenyl palmitate (C16) as substrate. The molar extinction coefficient of p-nitrophenol (p-NP) under the given assay conditions was 0.0073 µM-1 cm-1. One unit of enzyme activity (U) was defined as the amount of biocatalyst that released 1 µmol of p-NP per minute. Specific activity was expressed as Unit per gram of dry fermented substrate (U/gdfs). All experiments were performed in triplicate and analyzed with the Minitab software for Windows. Under our assays conditions, the specific lipase activity obtained from sugarcane bagasse supplemented with M1, M2 and M3 were 626.7, 978.1 and 252.8 U/gdfs, respectively. In addition, the performance of the biocatalysts produced by submerged fermentation using the same liquid mixtures was also discussed. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

BF-019

METAGENOMIC DIVERSITY DURING START UP STAGE OF ANAEROBIC DIGESTERS

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Anaerobic digestion constitutes a sustainable process widely used for organic waste management that has the advantage of generating biogas in addition to the stabilization of organic matter. The process of anaerobic digestion depends on the assembly of a complex microbial community, in which methanogenic archaea and syntrophic bacteria are the leading actors. It has been observed that for certain substrates, a suitable inoculum source may be critical for efficient biogas production. However, the availability of adequate inocula is severely limited at the local level, due to the very low degree of adoption of technologies based on anaerobic digestion. This work aims at understanding the adaptation of inocula arising from different sources to food waste. Four lab- scale anaerobic reactors (5 L) were operated during 91 days and fed daily with increasing concentrations of food waste. Reactor operational parameters, including biogas production, volatile solids (VS), alkalinity and volatile fatty acids concentration (VFA) were measured on regular basis. Metagenomic DNA was obtained from sludge samples taken weekly. Methanogenic archaea abundance was estimated using qPCR assays, whereas total microbial community analysis was conducted using amplicon sequencing with primers for the V3-V4 rRNA16S region. Specific biogas production (Biogas/VS) and volatile fatty acids (VFA) varied depending on the inoculum's source and feeding rate. Using USEARCH, OTUs were defined at 97%, obtaining 2690 OTUs that then were classified with rdp database. Under our experimental set up, adaptation to food waste was associated to the presence of acetoclastic methanogenic archaea from Methanosaeta genus, which were mapped with high abundance when biogas production was higher and identified as key taxa associated to process stability. This results underlies the critical role of the inoculum source for reactor start up. We are currently analyzing methanogenic archaea applying amplicon sequencing of the mcrA gene, which encodes methyl coenzyme-M reductase.