CELLULOLYTIC MICROORGANISMS FROM FORESTS SOILS, MANGROOVES, CARDBOARD TREATMENT WATER, THERMAL WATERS AND LEAVES

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Introduction. Cardboard treatment waters (CTW) were evaluated to determine COD, BOD, pH, TSS, dissolved solids and sediments. Experimental results were outside of permissible range of mexican standars. This kind of industry have cellulose as principal solid residue. Some cellulolytic microorganisms have been employed to decrease cellulose in cardboard waste water, but water quality with variable pH and temperature cause loss of microorganisms. Previous description suggest the necessity of stable cellulolytic microorganisms or enzymes. This work shows the isolation of cellulolytic microorganisms from CTW, forest leaves, soil and water from mangroves and thermal waters.

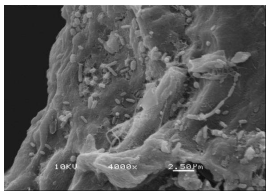
Objective of this work is evaluate the presence of cellulolytic microorganisms in zones whit several characteristics.

Methods. CTW was determined following Mexican Standards: pH (NMX-AA-003-1980); turbidity (NMX-AA-038-SCFI-2001); solids (NMX-AA-004-SCFI-2001 and NMX-AA-034-SCFI-2001); COD (NMX-AA-030-SCFI-2001). Cellulolytic microorganisms were growth and isolated in minimal medium NaCl (5.5); $(NH_4)_2 \cdot SO_4$ (2.5); $CaCl_2 \cdot 2H_2O$ (0.1); $MgSO_4 \cdot 7H_2O$ (0.1), nutritive agar (2%) and in jelly medium (g/L): K_2HPO_4 (5); $MgSO_4$ (2.5); cellulose (10); jelly (0.2); both medium (1) were added with congo red 1% (3 mL/L) used to select cellulolytic microorganisms producing hydrolysis zones in the plate and glucose when it was needed, pH and incubation temperature were 4.5-7.5 and 30-37 °C, respectively. Isolation of microorganisms were also selected by restricted growth on solid or liquid medium using Carboxy methyl cellulose (CMC), crystalline cellulose (CC), filter paper (FP) as only carbon source and cellulose activity was determined by DNS method described by Miller, 1959 using CMC, CC and PF as substrates. Colony morphology was realized in optical microscope with phase contrast imaging and Scanning Electron microscopy. In addition, mix cultures were also analyzed in this work.

Results. Quality of CTW had higher values in COD, TSS, caused by high cellulose concentration, values were out of range of Mexican Standard, table 1. Cellulolytic microorganisms were isolated from CTW, leaves, water and soils from mangroves, thermal waters and forest soils. Some selected microorganisms were those showing hydrolysis zones when the plate was added with congo red, other microorganisms were selected when they presented colonies of 1 mm of diameter, growing in plates with CMC, CC or FP as only carbon source. All positive strains (37 colonies) were growth in liquid medium and assayed by DNS method. Only six colonies are reported in this work (Fig. 1). Some strains did not grow in liquid medium, isolated strains and mix cultures growth in this conditions were assayed by DNS method, some isolated microorganisms showed no hydrolysis of cellulose, but consortia studied (from water mangroves, thermal water and forest soils) presented cellulolytic activity. Microorganisms analyzed in the optical microscope with contrast phase imaging showed the presence of coco, sporulated bacilli, sporangiospores. In Fig. 1 a region of leave from forest is covered of putatives cellulolytic microorganisms coco and rod-shaped cells was observed in the scanning electron microscope, cells were in several forms and lengths with 0.3-3 µm in length.

Table 1. Quality determination of Cardboard Treatment Water.

Parameter	Results	Mexican Standars
Total solids (mg/mL)	260000	125-150
Total suspended solids (mg/mL)	126000	125-150
Sediments (mg/L)	955	4-5
COD (mg/L)	1649	290-360
Ph	6.5	3-9
Turbidity	63.2	4



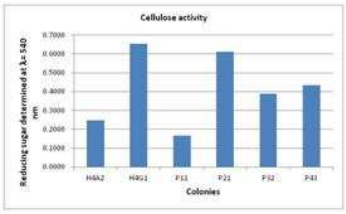


Fig.1. Microphotography showing putative cellulolytic microorganism over a leave and cellulose activity of microorganisms from leaves and CTW.

Conclusions. Cellulose activity was presented in all samples from different regions, were employed different assay conditions of pH and temperature and different substrates. Molecular identification will be assay by 16S rDNA.

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