Monitoring of lignin biodegradation using respirometric test and GC×GC-MS

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Introduction

Humic substances have an important role in soil fertility, plant nutrition and are considered to be involved in the stabilization of soil aggregates. They are divided into three components: humin, humic acids and fulvic acids. In nature, lignocellulose accounts for the major part of biomass and its degradation is one of the main contributors to the formation of humic substances. It is composed of polysaccharides (hemicelluloses and cellulose) and aromatic polymer (lignin). White and brown rot fungi are well known for their essential role in naturally occurring degradation of lignocellulosic biomass, which is enabled by secretion of extracellular ligninolytic enzymes. Composting is an aerobic process during which different waste products are partly mineralized and partly converted to humic substances. It has been claimed that during composting, lignin is the major contributor to the biosynthesis of humic acids. One of the most abundant forms of lignin is Kraft lignin, a byproduct of the alkaline sulfide treatment of lignocelluloses in the paper mill industry. The aim of this work was to investigate the potential of mixed culture of fungi to degrade kraft lignin. Degradation process was monitored with respirometric test to assess the CO_2 production and O_2 consumption and with comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC-MS), to identify the degradation products.

Materials and methods

Five different fungal strains isolated from soil were inoculated into liquid mineral medium containing kraft lignin (2.5 g/L) and glucose (1 g/L) as the only sources of carbon. Aerobic biodegradation of lignin was assessed using an open flow 12 chamber Micro-Oxymax respirometer (Columbus Instruments, USA). Degradation was monitored for 30 days, after which the degradation products were extracted with dichloromethane and analysed using GC×CG-MS (Shimadzu, Japan). Chromatograms were analyzed using ChromSquare 2.1 software (Chromaleont, Italy). Mass spectra were compared with NIST11, NIST11s and WILEY8 databases.

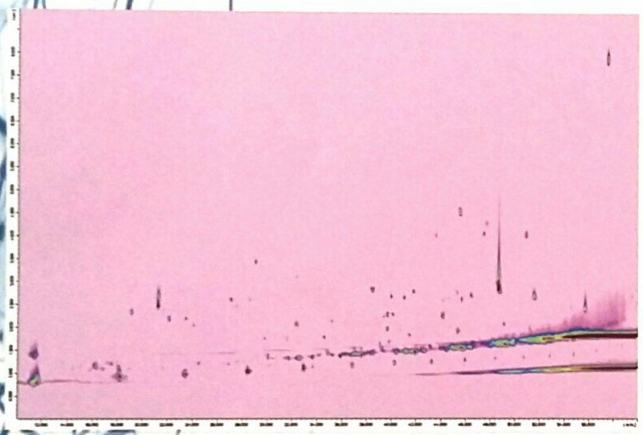

Figure 1. GCxGC-MS chromatogarm of kraft lignin

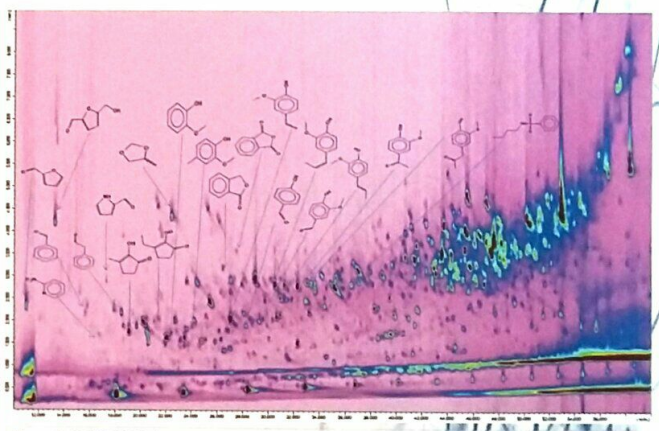

Figure 2. GCxGC-MS chromatogram of kraft lignin after degradation with mixed fungal consortia

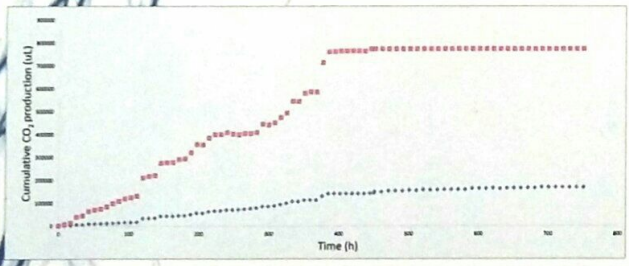

Figure 3. Cumulative CO_2 production in control sample (blue) and inoculated sample (pink)

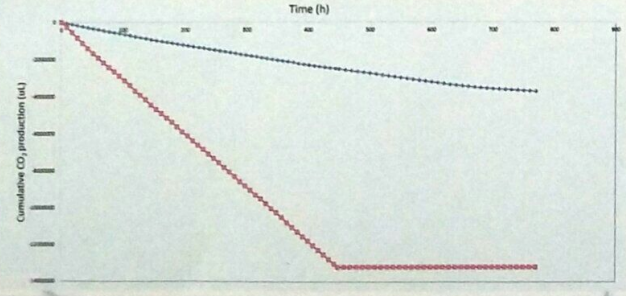

Figure 4. Cumulative O_2 depletion in control sample (blue) and inoculated sample (pink)

Results

The GC×GC-MS chromatograms of lignin before and after degradation sre shown in figures 1 and 2. The results indicate that kraft lignin sample inoculated with fungi contained several aromatic lignin-related compounds that were not present in control sample. The compounds identified in inoculated sample were mostly derivatives of p-coumaric and benzoic acid. High metabolic activity of fungal cells toward lignin was confirmed respirometrically. The CO_2 production rate was significantly higher compared to the control sample (Fig 3). Also, oxygen uptake has increased compared to the control sample (Fig 4).

Conclusion

This work demonstrates that fungal consortium can significantly degrade kraft lignin. Next, the activity of this consortium should be determined in soil in order to assess the degradation potential and the impact on soil quality.

Literature:

1. Smidt et al. *Ind. Crops Prod.* 27 (2008) 196-201.
2. Rahimi et al. *Nature* 515 (2014) 249-252.
3. Salvachua et al. *Green Chem.* 18 (2016) 6046-6062.

This study was supported through Project III 43004 by the Ministry of Education, Science and Technological Development of the Republic of Serbia.