

# Molecular microbial ecology of lignocellulose mobilisation as a carbon source in mine drainage wastewater treatment

Anna M Clarke<sup>1\*</sup>, Ralph Kirby<sup>2</sup> and Peter D Rose<sup>1</sup>

<sup>1</sup>Environmental Biotechnology Research Unit (EBRU), Rhodes University, Grahamstown, South Africa

<sup>2</sup>Department of Life Sciences, National Yang-Ming University, Taipei, Taiwan

## Abstract

The community structure of complex microbial consortia which develop in lignocellulose packed passive treatment systems for acid mine drainage remediation were investigated. An understanding of interactions between these populations is important in determining mechanisms by which such systems operate. A degrading packed bed reactor was packed with lignocellulositic material as a sole carbon source and fed continuously with simulated acid mine drainage. Samples were collected every two months at different depths of the reactor to isolate the total genomic DNA and PCR amplify section of 16S rDNA gene. PCR primers, GM5F and 907R incorporating GC clamp were used to amplify 586-bp region of 16S rDNA gene. Denaturing gradient gel electrophoresis (DGGE) indicated clearly a highly differentiated pattern of r-DNA – derived amplicates between different depths of the bioreactor. Predominant DGGE bands were further excised, reamplified, cloned and sequenced. Sequencing analysis revealed phylogenetic affiliation of specific bacterial populations in different depths of the bioreactor.

## Introduction

Previous studies undertaken by Rhodes University and Pulles, Howard and de Lange Inc. (PHD) in the use of lignocellulose packed reactor in passive treatment of acid mine drainage (AMD) had shown that certain factors play an important role in their sustained operation over the long time.

Little is known, however, why under certain conditions the performance of these reactors may decline after a period of several months.

This study was part of an Innovation Fund Project in which these problems were addressed and the performance of the microbial consortia shown to play an important role in sustaining operational performance.

The novel degrading packed bed reactor was developed based on these insights and the IMPI passive treatment process has been successfully implemented by PHD.

The carbon source provided for microbiota in this study was lignocellulositic material in the form of pine chips and grass as these are easily available in the country and thus make the process economically viable. Lignocelluloses are some of the most abundant biological polymers on earth. However due to the complexity of its components and chemical structure of lignin in particular its enzymatic degradation is known to be very difficult (Bumpus, 1989; Burland and Edward, 1999; Crawford and Crawford, 1976; Crawford, 1981; Crawford et al., 1983). Except for a few lignin solubilising actinomycetes (Crawford and Crawford, 1976; Crawford, 1981; Crawford et al., 1983), lignin degradation by microorganisms is known to be mainly the domain of aerobic fungi (Achi, 1994; Blondeau, 1989; Bumpus, 1989; Dehorter and Blondeau, 1992). Little is known about and the degradation of lignin in biosulphidogenic environment (Parek et al., 2001) however, several studies suggest that the contribution of anaerobic

organisms to degradation of lignin has been underestimated and should be reevaluated (Kim et al., 1997). Recent findings point to anaerobic degradation of aromatic compounds (Burland and Edward, 1999; Meckenstock et al., 2000) We suspected that strong involvement of microorganisms belonging to different physiological groups and their collaboration may govern the degradation process and the aim of this project was to look for distinct population patterns.

## Materials and methods

### Sample collection

Samples were collected from a DPBR. Specimens for DNA extraction were collected directly into a sterile 50 ml Falcon tubes (Laboratory & Scientific Equipment Co.) and kept on ice or at 4°C until processed (usually within 4 days).

### DNA extraction and purification

Prior to extraction samples were pelleted by centrifugation, washed once with 2 x buffer A (200 mM Tris [pH 8.0], 50 mM EDTA, 200 mM NaCl, 2 mM sodium citrate, 10 mM CaCl<sub>2</sub>) and one part 50% glycerol (Bond, P.L. Appl.Env.2000), and then resuspended in 0.5 ml of 2 x buffer A in 2 ml microcentrifuge tube. It was necessary to include this washing step as low pH of the specimen may cause the hydrolysis of DNA, and high concentration of metals could contaminate the extracted DNA and further have inhibitory effect on subsequent PCR. Polyadenylic acid (200 µg/ml), and lysozyme (3 mg/ml) were added to the suspension and incubated for 40 minutes at 37°C. Samples were subjected to four cycles of freezing in liquid nitrogen and heating for one minute at 80°C. Proteinase K (2 mg/ml) and SDS (10% wt/vol) were added to the mixture, and this was incubated overnight at 37°C. Cell lysates were extracted with phenol-chloroform-isoamyl alcohol (24:24:1). Nucleic acids were precipitated with 2.5 volumes of 96% rectified ethanol overnight at -20°C, pelleted by centrifugation and resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

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\* To whom all correspondence should be addressed.

☎+2746 6222656; fax: +2746 6222656;; e-mail: [ebru@ru.ac.za](mailto:ebru@ru.ac.za)