

# Degradation of low molecular weight organic acids in high pH solutions – Scientific Report

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A full report will be transcribed for publication once the experiments have been completed in their entirety. This document is a contextual summary of the ESF-MOLTER funded visit to Mid Sweden University (February 2010).

## 1. Introduction

Terrestrial (plant and soil) turnover of carbon is the dominant flux in the global carbon cycle (transferring 20 times more carbon than anthropogenic emissions into the atmosphere (Dupre et al., 2003)). There are multiple pathways for carbon transfer from plant to soil (root respiration, decaying biomass, sloughing off cells etc). However, the exudation of low molecular weight organic acid from plant roots is considered an important mechanism in this system (Kuzyakov and Domanski, 2000). Organic acids are oxidised chemically or by microorganisms through the Krebs cycle producing a range of low molecular weight organic compounds (citrate, malate, fumarate etc), water and CO<sub>2</sub>. Quantification and turnover rate of organic acids in the environment is an emergent field of study, which has direct implications for global carbon cycle modelling and nutrient plant dynamics (Jones et al., 2003; Ryan et al., 2001).

Exudation of organic acids (and protons) into the soil is a nutrient acquisition mechanism used by plants particularly on poor or high pH soils, by lowering pH and forming organic complexes with metals (Ca, Mn, Zn etc) (Jones et al., 2003). Research has investigated the dynamics of organic acids on calcareous soils (pH <8.5) (Strom et al., 2005; Strom et al., 2001). Understandably, research has yet to investigate breakdown in high pH soils (pH >10.5) given the usual range of natural soils (pH 6-8). However, recent research at Newcastle University has justified the case for high pH organic acid degradation research as part of work investigating how soils can be engineered to capture carbon.

Engineering the soil carbon sink is a project led by Prof. David Manning at Newcastle University which investigates the use of calcium rich minerals (silicates) as soil amendments to chemically capture atmospheric carbon as stable calcium carbonate minerals (Manning, 2008), and recent results suggest that brownfield soils in urban environments act as substantial carbon sinks capable of accommodating as much as 435MtC globally (Renforth et al., 2009). This project has begun to focus on calcium rich materials specific to the construction industry, particularly demolition waste containing calcium silicate hydrate minerals and calcium hydroxide. Dissolution of calcium hydroxide in solution buffers the pH to >11 which favours the precipitation of calcium carbonate provided the components of calcium carbonate (calcium and dissolved carbon dioxide) are available. With this in mind, it is important to investigate the contribution of degraded organic acids to the dissolved carbon dioxide pool, as a consequence of their decomposition.

The funding from MOLTER was used to collaborate with Prof Ulla Lundstrom and colleagues at Mid Sweden University who have developed a novel method for speciation of low molecular weight organic acids using liquid chromatography coupled with electrospray ionisation mass spectrometry 'LC-MS/MS' (Bylund et al., 2007). Liquid chromatography is used to separate isobaric compounds, the effluent of which is channelled into a mass spectrometer for compound identification (analysing the main fragments from generated in the collision cell). The output of this technique is the quantification of individual organic acid compounds within the sample solution to a greater resolution than was previously possible. The application of LC-MS/MS to environmental analysis is globally unique and may begin to shed light on organic anion speciation information missing from hydrochemistry and mineral weathering research.

## 2. Aims and Objectives

The aim of this study was to investigate the breakdown pathway of citric acid in high pH solutions. This was achieved by undertaking a series of microcosm experiments designed by delivering the following tasks:

1. Collect soil with micro-organisms capable of operating at alkaline pH levels (harvesting and identifying the community in high pH soils and waters at the former Consett steelworks site, Co Durham, UK see Renforth et al, (2009)).
2. Using the microorganisms in task 1, degrade citric acid in sterile and biotic microcosms buffered to pH > 10 using calcium hydroxide, in containers open and closed to the atmosphere.
3. During the microcosm trial, measure the organic and inorganic carbon content of the solution. Measure the organic and inorganic carbon content of the solid phase pre and post trial.
4. Use ion chromatography techniques developed by Prof. Ulla Lundstrom and colleagues at Mid Sweden University to determine the organic acid species in solution over the time period of the experiments.
5. Measure pH change of solution during the microcosm trial.

## 3. Methodology

### 3.1 Site Description

Soil was collected from the Hownsgill Valley near Consett County Durham, UK (54 50 20 N, 1 51 29W). Approximately 50 Mt of slag were deposited at the site after the closure of the adjacent steelworks in 1980 (Harber and Forth, 2001). A pond and wetland have developed in the drainage regime of the deposited slag (Figure 1) and subsequent analysis of the water is presented in Table 1.

Attribute	Value
pH	7-12.5
Electronic conductivity	1164-3747 $\mu\text{S cm}^{-1}$
Total Alkalinity	432mg/l
Ca	195mg/l
Mg	1.6mg/l
K	11mg/l
Na	13mg/l
Cl	34mg/l
SO <sub>4</sub>	45mg/l

Table 1: Adapted from Mayes et al, (2006) and Mayes et al, (2008).

Wet soil samples were centrifuged and the supernatant was analysed for organic and inorganic carbon. The soils were air dried and sieved to passing 2mm.



Figure 1: Hyper alkaline pond in the drainage regime of the former steel works, Consett, Co Durham.

### 3.2 *Microcosm Experiments*

Using the soil collected from the Hownsgill Valley, a microcosm trial was conducted to investigate the degradation of citrate in high pH solutions.

Some of the soil was sterilised using 32,000mg kg<sup>-1</sup> mercuric chloride for 24 hours prior to the experiment in accordance with the method identified in (Trevors, 1996). Soil was also sterilised in an autoclave (1h, 121°C 100 kPa). It is probable that background concentrations of organic acids are present in the soil. Therefore, some of the soil was washed in calcium hydroxide solution for 24 hours before the experiment

<b>Substrate</b>	<b>Solution</b>
Field moist soil	13mM Citric acid + 14mMol of Calcium Hydroxide
Field moist soil sterilised in an autoclave	
Field moist soil sterilised using mercuric chloride	14mMol Calcium Hydroxide
Field moist soil washed in XM calcium hydroxide solution	
Field moist soil sterilised in an autoclave washed in XM calcium hydroxide solution	13mM Citric acid
Field moist soil sterilised using mercuric chloride washed in XM calcium hydroxide solution	Deionised water control
No material control	

Table 2: Experimental matrix

1g of field moist substrate was transferred into each 30ml polyethylene centrifuge tube sterilised in an autoclave at 121 C at 100 kPa for 1hour. Each centrifuge tube was dosed with a solution composed of a mixture of citric acid, calcium hydroxide, and deionised water. A total of 28 vials were used to test the matrix in Table 2.

The vials were shaken on a reciprocating shaker set at 200rpm for 15min to ensure adequate mixing of the soil and solution. The samples were centrifuged at 12000rpm for 5min and 2.5ml aliquots were removed from each sample, diluted to 7.5ml and passed through a 0.2µm cellulose nitrate filter. The vials were transferred to a water bath set at 40°C for 24 hours. 2.5ml samples were removed after 12 hours and 24 hours, using the same method as described above.

This method was repeated twice, however, whole samples were sacrificed at 12 and 24 hours for organic and inorganic carbon analysis of the solution and solid.

### 3.3 Solution Analysis

Organic carbon and inorganic carbon was analysed using a Shimadzu TOC5050A Total Carbon Analyser (Newcastle University). 1ml splits of the aliquots were diluted with 0.5M Octane sulphonic acid, sonicated for 40 minutes and analysed for acetate accumulation using a Dionex ISC-1000 at (Newcastle University) fitted with an IonPac® ICE-AS1 column. Organic acids were measured using liquid chromatography couple with quadrapole mass spectrometry (Mid Sweden University, Sweden- Figure 2) as described in Bylund et al, (2007). pH measurements were taken using a Jenway 3020 pH meter at the start and end of the experiment.

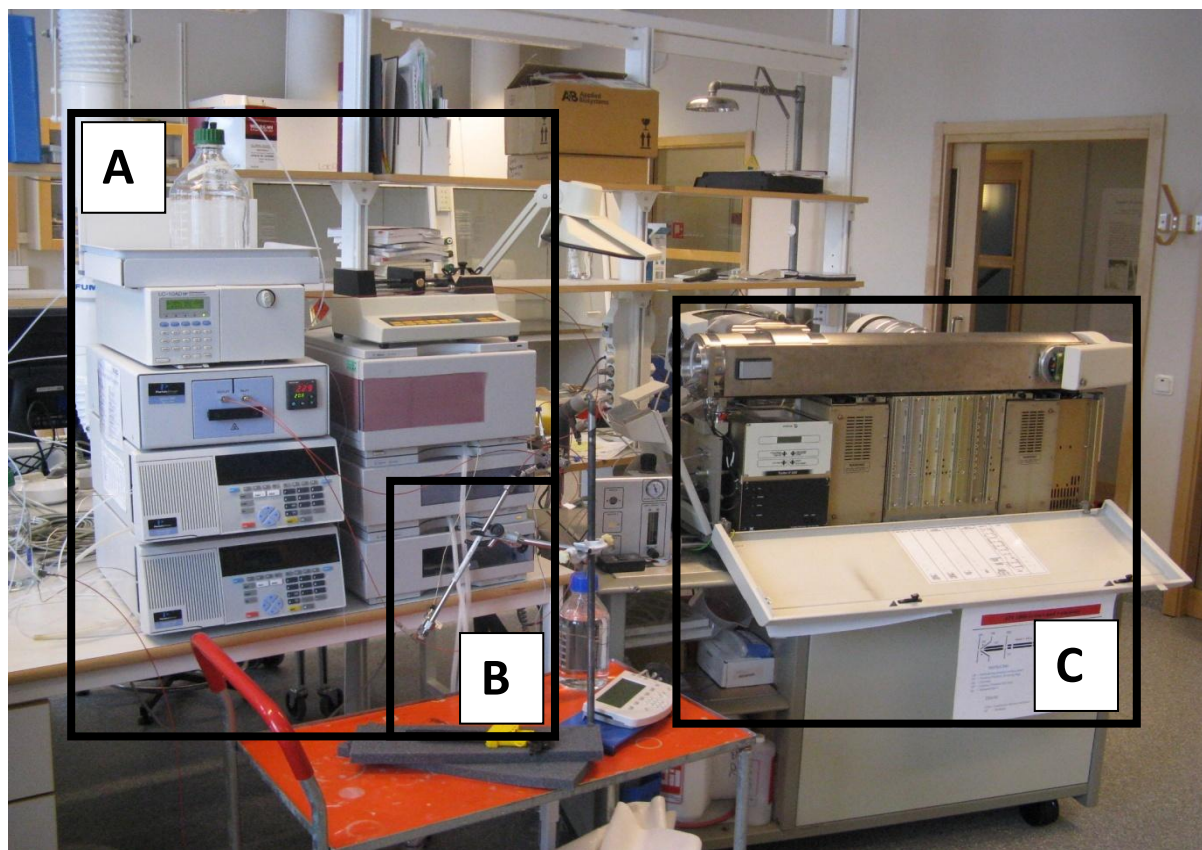


Figure 2: LC/MS system at Mid Sweden University (A) Liquid chromatographer - Shimadzu LC-10AD pump and Agilent 1100 autoinjector (B) Supelcogel C610-H column (C) API3000 mass spectrometer

### 3.4 Solid Analysis

The soil sample was analysed for organic carbon using thermo gravimetric analysis (Netzsch Simultaneous Thermal Analyzer STA 449C – Newcastle University) and inorganic carbon was analysed using an Eijkelcamp calcimeter to BS 7755-3.10:1995 (Newcastle University).

### 4.0 Furtherwork

Repetition of the experiment is required prior to publication of results. Furthermore, analysis of the microbial community in the soil would be undertaken, but is not essential

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