Application of *Citrus sinensis* Solid Waste as a Pseudo-Catalyst for Free Cyanide Conversion under Alkaline Conditions

Bruno Alexandre Quistorp Santos, Seteno Karabo Obed Ntwampe,*
James Hamuel Doughari, and Gift Muchatibaya

In this study, *Citrus sinensis* (*C. sinensis*) solid waste was used to catalyze the conversion of free cyanide (F-CN) under alkaline conditions; conditions which represent most industrial wastewater containing F-CN. Acid hydrolysis of the solid waste increased the catalytic conversion of F-CN by 3.86 compared to the unhydrolysed solid waste. The conversion of F-CN using unhydrolysed and hydrolysed solid waste increased linearly with an increase in pH and temperature. The maximum catalytic conversion of a 100 mg F-CN/L solution containing 0.1% (w/v) of unhydrolysed and hydrolysed *C. sinensis* solid waste was 17.82% and 62.48%, respectively, at a pH of 12 and a temperature of 50 °C. The catalytic process was largely dependent on the availability of activated hydroxyl groups in the solid waste. As most wastewater contains heavy metals, it was determined that the presence of metallic species (Ni, Zn, and Cu) reduced the conversion of F-CN as the metallic ions attached to the hydroxyl groups. The observed reduction was 26.35% when 10 mg/L of heavy metals were present in the F-CN solution containing the hydrolysed solid waste at a pH of 12 and 40 °C.

Keywords: Citrus sinensis; Free cyanide; Catalysis; Wastewater treatment

Contact information: Faculty of Applied Sciences, Department of Agriculture and Food Sciences: Biotechnology, Cape Peninsula University of Technology, PO Box 652, Cape Town 8000, South Africa.
** Corresponding author: ntwampes@cput.ac.za*

INTRODUCTION

The release of cyanides into the environment *via* a variety of disposal methods, particularly as wastewater, has been shown to be detrimental to the environment. Cyanide and its complexes have been shown to have bio-accumulative properties which result in ecological deterioration (Patil and Paknikar 2000). Cyanide-based electroplating operations produce wastewater that contains significant quantities of F-CN and free heavy metals, particularly Ni, Zn, and Cu, and a lesser degree of heavy metal cyanide complexes derived from the rinsing of the plated workpieces (Cushnie and CAI Resources Inc. 1994).

F-CN is the simplest and most toxic form of cyanide, and it is formed by the dissociation of cyanide compounds/complexes used in these cyanide based electroplating operations (Nesbitt 1996; Rao *et al*. 2010). For the conversion of F-CN, physical/ chemical methods are widely used, but the currently used methods are capital intensive and further produce by-products that contribute to environmental contamination to a greater extent than bioremediation processes (Nesbitt 1996; Santos *et al*. 2013).

However, bioremediation processes tend to be more sensitive to fluctuations in contaminant concentrations. Thus, the use of a suitable process which is designed to reduce the impact of fluctuations on the activity of microorganisms used in the bioremediation process is desirable. Similarly, when the concentration of F-CN exceeds the microorganism(s) tolerance level, the designed biodegradation process will be ineffective (Santos *et al*. 2013).

Solid waste generation in South Africa is problematic, as the majority of landfill sites are reaching maximum capacity. Approximately $427x10^6$ tonnes of solid waste is generated in South Africa every year, of which 40% (w/w) is organic waste (Greben and Oelofse 2009). Similarly, approximately $31.2x10^6$ tonnes of citrus fruits are processed globally every year. This generates approximately $15.6x10^6$ tonnes of solid citrus waste, of which 75% is *C. sinensis* solid waste, making it the largest contributor to agro-waste generated worldwide (Li *et al*. 2008; Jarrell 2012).

Some of this solid waste can be potentially bioaugmented in biological processes, particularly for contaminant biodegradation. Overall, there is a need to develop a sustainable and environmentally friendly process for the conversion of F-CN using bioresources such as waste from the agricultural sector. *C. sinensis* solid waste has shown potential as a biosorbent for the removal of toxic contaminates such as heavy metals and dyes (Li *et al*. 2008; Boota *et al*. 2009; Asgher and Bhatti 2012a,b; Bhatti *et al*. 2012).

This study demonstrates the feasibility of using *C. sinensis* solid waste as a pseudo-catalyst for the conversion of F-CN to ammonium and formate. The results highlight the importance of developing an environmentally friendly catalytic process using the waste in particular, to minimize the influence of F-CN fluctuations prior to a bioremediation process in which a suitable microorganism is used.

EXPERIMENTAL

Citrus sinensis Waste Preparation

Unhydrolysed and hydrolysed Citrus sinensis solid waste

For unhydrolysed *C. sinensis* solid waste, 60 g of powdered *C. sinensis* solid waste (≤ 100 μm) was added to a 1 L Schott bottle, and a 1 L solution was made using sterile distilled water without autoclaving.

For hydrolysed *C. sinensis* solid waste, 60 g of powdered *C. sinensis* solid waste (≤ 100 μm), 800 mL distilled water, and 5 mL H_2SO_4 (98%), were mixed, and a 1 L solution was made by adding distilled water. The solution was autoclaved at 116 °C for 13 min and cooled to room temperature (Talebnia *et al*. 2008); thereafter, the pH was adjusted to 4.5 using a 1 M NaOH solution.

The mixture was filtered through a No. 1 Whatman filter paper using a Brüchner funnel under vacuum. The filter cake was then dried at 80 °C for 24 h and milled to a fine powder (≤ 100 μm).

Benzylation of Citrus sinensis solid waste

Potassium hydroxide (16 g) was dissolved in 32 mL of distilled water in a 250-mL flask. A mass of 8 g of the hydrolysed *C. sinensis* solid waste was added to the flask, and the solution was mixed by continuous stirring using a Labmaster isopad (LMUL/ER/ 500 ML) magnetic stirrer and heater under a fume hood. The flask was fitted with a reflux condenser, and the mixture was heated to a gentle boil, which was followed by the

addition of bromobenzene (12 mL) through the condenser to the boiling solution. The solution was gently boiled for an additional 10 min before being transferred to a 250-mL beaker in order to cool to room temperature. The pH of the mixture was then adjusted to 4.5 using HCl (32%) and transferred to an ice bath.

The mixture was filtered through a No. 1 Whatman filter paper using in a Brüchner funnel under vacuum, after which the filter cake was dried for 24 h at room temperature and then stored in a desiccant. This method was used to reduce hydroxyl functional groups in the hydrolysed *C. sinensis* solid waste.

Experimental Procedure

Effect of pH and temperature on free cyanide conversion

A mass of 0.05 g of the unhydrolysed, hydrolysed *C. sinensis* solid waste was added to a 100-mL Schott flask containing 50 mL of a buffered 100 mg F-CN/L solution, constituting a 0.1% (w/v) concentration of the waste in solution. The cyanide solution was prepared by adding 0.250 g KCN to 800 mL of phosphate buffer solutions with the pH values stipulated in Table 1.

Table 1. Experimental Map for pH and Temperature Variable Optimisation

Run	Temperature (˚C)	pH
1	35.00	9.50
2	35.00	12.00
3	50.00	9.50
4	24.39	11.27
5	45.61	7.73
6	20.00	9.50
7	35.00	9.50
8	24.39	7.73
9	35.00	7.00
10	35.00	9.50
11	35.00	9.50
12	35.00	9.50
13	45.61	11.27

Distilled water was added to the solution to make a 1-L solution with a corresponding F-CN concentration of 100 mg/L. The flasks were incubated in a shaker at 150 rpm at the required temperature (see Table 1) for 48 hours. The benzylated *C. sinensis* solid waste without hydroxyl functional groups was used as a control.

Effect of heavy metals on free cyanide conversion

A heavy metal-cyanide solution was prepared by adding 0.050 g $Ni(NO_3)_2.6H_2O$, 0.037 g $Cu(NO_3)_2.2.5H2O$, and 0.040 g $Zn(NO_3)_2.4H_2O$ to the 100 mg F-CN/L phosphate buffered solutions, with the final concentration of the heavy metals in solution being 10 mg/L. The influence of heavy metals on the catalytic conversion of F-CN using the hydrolysed *C. sinensis* solid waste was determined at a pH of 12 and 40 °C for 48 h using a rotary shaker at 150 rpm, as these conditions were determined to be operational conditions for which the maximum conversion of F-CN using the hydrolysed *C. sinensis* solid waste was achieved.

Analytical methods

Merck cyanide (CN- 09701) and ammonium (N-NH_4^+ 00683) test kits were used to quantify the residual F-CN and NH_4^+ concentrations in solution.

The formate ($CHOO^-$) concentration was quantified according to methods developed by Sleat and Mah (1984) using a Jenway 6715 UV/Visible spectrophotometer at appropriate settings.

The functional groups were analysed using a Perkin Elmer Fourier Transform Infrared (FTIR) Spectrum 1000 spectrophotometer (absorbance range: 4000 to 400 cm^{-1}) and Perkin Elmer Inc. spectrum software (Version 5.3.1).

RESULTS AND DISCUSSION

Effect of pH and temperature

Both unhydrolysed and hydrolysed *C. sinensis* solid waste were able to catalyse the conversion of F-CN to NH_4^+ and $CHOO^-$ with a linear increase in the rate of catalysis with respect to temperature and pH, as shown in Fig. 1. Although it is known that temperature is one of the most common and critical factors influencing the rate of reaction, some of the catalytic kinetics were observed to be largely influenced by the pH of the solution. The maximum F-CN conversion was achieved at a pH of 12 and a temperature of 50 °C, which corresponded to a conversion efficiency of approximately 63%.

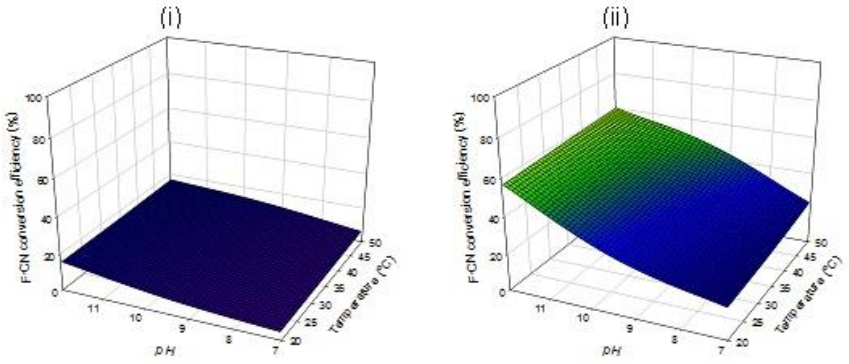

Fig. 1. F-CN conversion using (i) unhydrolysed and (ii) hydrolysed *C. sinensis* solid waste

The F-CN conversion increased linearly as the pH and temperature were increased. The free hydroxyl functional groups present in agro-waste, particularly citrus waste, were determined to facilitate F-CN conversion. This functional group is associated with heavy metal binding (Eccles 2000), as it behaves as a weak acid, especially at alkaline pH. Therefore, it deprotonates to produce an alkoxide in the presence of a strong base, such as F-CN, more readily as the pH is increased. This proton thus hydrolytically facilitates the destabilisation of the carbon-nitrogen triple bond in the cyanide and initiates its decomposition. Similarly, the increase in temperature results in an increased rate of reaction since the substrate, in this case F-CN, has more free energy, causing increased movement and collisions involving the solid waste, in this case the *C. sinensis*, which facilitates the reaction.

Furthermore, this process was observed to readily occur when the hydrolysed *C. sinensis* solid waste was used, which resulted in a significantly higher conversion when

compared to the unhydrolysed *C. sinensis* solid waste. The hydrolysis of the waste results in the release of components in the waste, thus increasing the surface area of the waste by making it more porous—a process that facilitates the availability of the hydroxyl functional groups, which facilitate the deprotonation of the cyanide group.

Factors affecting the catalysis of F-CN

The FTIR spectra (Fig. 2) depict a number of spectral peaks that indicate the types of functional groups present in the *C. sinensis* waste. The spectral peaks appearing at wave lengths of 3700-3600, 3600-3200, 1745, 1320-1210, 1250-950, and 1120 cm^{-1} are characteristic of O-H groups associated with free hydroxyl and phenolic groups, O-H stretch in H-bonded hydroxyl of alcohol and phenol groups, C=O stretch of 5-membered ketone group, C-O stretch of acetyl group, and C-O stretch of ether group, respectively.

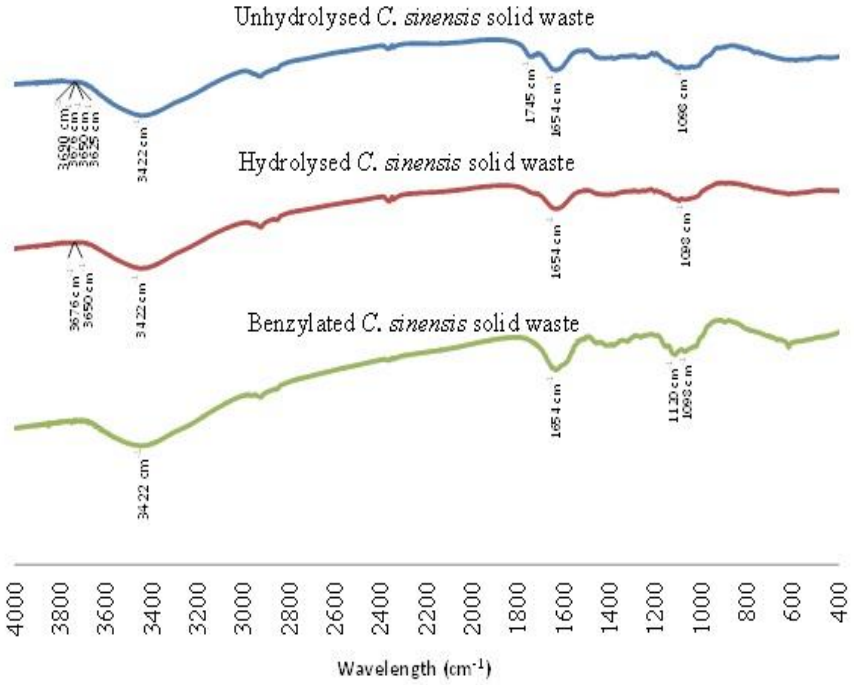

Fig. 2. FTIR of unhydrolysed, hydrolysed, and benzylated *C. sinensis* solid waste

The reduction of the free hydroxyl, 5-membered ketone, and acetyl functional groups present in the hydrolysed *C. sinensis* solid waste was attributed to their liberation from the waste into solution due to acid hydrolysis. One of the key factors that could greatly affect the conversion of cyanides is the presence of species having a greater affinity to the free hydroxyl functional groups. A common example is the presence of heavy metals such as Zn, Ni, and Cu, which have been shown to have an affinity to attach to free sites on hydroxyl functional groups and are commonly found in electroplating wastewater (Cushnie and CAI Resources Inc. 1994; Eccles 2000; Wan Hgah and Hanafiah 2008). For this study, it was shown that an F-CN conversion of 62.48% was achievable in metal-free 100 mg F-CN/L solutions at 40 °C and pH 12 after 48 h using the hydrolysed *C. sinensis* solid waste. However, the conversion was reduced to 43.80% in solutions containing 10 mg/L of heavy metals such as Zn, Ni, and Cu, under the same conditions.

The results just described indicate a 26.35% reduction in F-CN conversion due to the binding of heavy metals to the hydroxyl functional groups responsible for the deprotonation effect on F-CN. It was hypothesised that the benzylation of the hydrolysed *C. sinensis* solid waste should result in the formation of ether groups from the hydroxyl groups, thus minimizing F-CN conversion. It was observed that negligible F-CN conversion was detected when the benzylated *C. sinensis* solid waste was used – an indication of the catalytic properties, thus usability, of the hydrolysed *C. sinensis* solid waste in a pre-treatment step prior to a F–CN biodegradation process for wastewaters containing a higher concentration of F-CN.

CONCLUSIONS

1. The use of biomaterials characterized by high free hydroxyl content, such as *C. sinensis* solid waste, has shown its potential as an environmentally friendly pseudo-catalyst for F-CN conversion since such materials are (1) cheap, (2) relatively abundant, and (3) less chemical-intensive compared to conventional methods.
2. Hydrolysis of the *C. sinensis* wastes by chemical, thermal, biochemical, or a combination of these methods should be evaluated to assess their potential.
3. However, acid hydrolysis of the *C. sinensis* waste using acid and thermal hydrolysis produces an extract rich in hydrolyzed sugar monomers, which can be used to as a supplement for a post-bioremediation system.
4. Similarly, this process can be used as a pre-treatment process for F-CN post-biodegradation systems, in particular to counteract the fluctuations of F-CN present in the wastewater.
5. However, pH and temperature can significantly affect the catalytic conversion of F-CN, which is favoured in a linear trend with increasing pH and temperature.
6. Although the presence of heavy metals inhibited the catalytic conversion of F-CN, this phenomenon can be controlled by the acid regeneration of the *C. sinensis* solid waste.

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