TECHNICAL BRIEF

August 2016
The WRC operates in terms of the Water Research Act (Act 34 of 1971) and its mandate is to support water research and development as well as the building of a sustainable water research capacity in South Africa.

Bioremediation of wastewater

A recently-published Water Research Commission (WRC) study has improved South African knowledge on the bioremediation of fruit processing waste streams.

Background

The total fruit production in South Africa is about 5.4 million tons per annum, of which 2.4 million tons are processed. Processing of fruit ranges from canning, juicing, the making of preservatives to drying, among others.

During process, a substantial amount of agri-industrial wastewaters, high in organics, and therefore high chemical oxygen demands (CODs) are generated. The citrus fruit industry alone generates more than 10 000 ℓ of wastewater per ton of citrus fruit processed.

After citrus, grapes and apples are the dominant fruit crops in South Africa, with more than 1.5 million tons of grapes processed in 2011/12 and more than 0.24 million tons of apples processed in the same year.

The olive market is also emerging strongly in South Africa, with an annual production of 3 000 t of table olives and 700 000 ℓ a year olive oil, most of which is currently being produced in the Western Cape.

Understanding fruit processing waste streams

The following challenges are being experienced in terms of managing fruit and olive mill wastes:

- Agri-industrial wastewaters typically have low, variable concentrations of solutes and particulate matter, and are not suited to fermentation to generate bio-ethanol or biogas, unless very efficient lignocellulosic carbon conversion is achieved or by the addition of solid wastes.
- Fruit waste streams from deciduous fruits are viscous liquids containing tiny fibrous particles and swollen materials, which hardly settle and make centrifugation or filtration nearly impossible.
- The residues associated with agri-industrial wastewater are often lignocellulosic, and to provide simple metabolisable carbohydrates, lignocellulosic materials must be depolymerised. The problematic complexity of achieving this efficiently is well documented.

It is important to understand the composition of fruit wastewaters before suitable disposal and treatment can be implemented. Lignocellulose is one of the major carbohydrates found in fruit processing wastewater and there has and continues to be extensive research carried out on the topic of lignocellulose degradation.

Sugars and phenolic compounds are also commonly found in

fruit processing water. The processing of fruits in the canning, juicing, winery and distillery industries also produces a certain amount of solid residue, known as pomace, which has a high moisture and sugar content and can be added to the wastewater streams.

The aims of this WRC project were to, among others, develop an improved understanding of fruit bio-based waste streams in South Africa; develop a conceptual model for the bioconversion of bio-residues in bio-based waste streams; and identify successful strains and enzymes for the bioconversion of bio-residues in waste streams. The study also sought to characterise the enzymes and their applications in waste stream reclamation and lignocellulosic conversion.

Results of the study

Proof-of-concept experiments were performed to determine the suitability of recombinant ***Aspergillus niger*** strains for producing useful enzymes on three bio-based waste streams (apple pomace, grape waste and potato waste). The ability of the recombinant strains to produce enzymes under these cultivation conditions was assessed.

Analytical tests were performed to determine the properties of the waste samples and how these properties were altered due to the growth of the fungal strains.

Potato waste proved the best bio-based waste stream for growth of the recombinant ***A. niger*** strains and the heterologous expression of endoglucanase, endomannanase and endoxylanases. In addition, bioethanol production using ***S. cerevisiae*** was also achieved using potato wastes for the fermentation feedstock. The wastewater, together with the potato waste can also be used for bioethanol production through fermentation and the addition of amylase enzymes.

Trametes pubescens was capable of growing, and producing laccase, on apple and potato wastes without the addition of other nutrients or inducers. The laccase obtained was similar to a purified ***T. pubescens*** laccase that was produced under optimised media, environment and inducer conditions.

Due to the diverse potential applications of laccases, the ease of growth of ***T. pubescens*** and the lack of additional supplementation, this could be a very valuable value-added product for the biorefinery and warrants further investigation. Overall, the results showed that the strains identified for high-value enzyme production could successfully produce these enzymes using bio-based waste streams in the cultivation medium.

Biochemical characterisation of the enzymes was performed to further assess their fitness-for-use in waste stream reclamation and lignocellulosic conversion. The three enzymes, endo-ß-1,4-glucanase, endo-ß-1,4-mannanase and endo-ß-1,4-xylanase, showed great potential for being used as key enzymes for lignocellulose degradation.

The enzymes are stable at 50C for at least 24 hours and maintain strong activity at pH 5; conditions often maintained and utilised in bio-refineries.

The multi-functionality of the enzymes for different substrates such as carboxymethyl cellulose, locust bean gum and Beechwood xylan indicate that they will play important roles in synergistically degrading the cellulosic and hemicellulosic components of lignocellulosic biomass.

The biochemical characteristics of the three enzymes indicated that they are very suitable for application in the biorefinery sectors of the bioeconomy.

This project, therefore, demonstrated the bioremediation of lignocellulosic wastewater by recombinant ***A. niger*** D15 strains by reducing of organic content, and the concomitant cheap and high production of commercial valuable enzymes on inexpensive and abundant bio-based waste streams.

Further reading:

To obtain the report, *Integrated bioremediation and beneficiation of bio-based waste streams* (**Report No. 2225/1/16**), contact Publications at Tel: (012) 330-0340; Fax: (012) 331-2565; Email: orders@wrc.org.za or Visit: www.wrc.org.za to download a free copy.