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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.

TECHNICAL BRIEF

Agri-industrial water treatment

Improving water efficiency in fruit processing industry

A newly-completed Water Research Commission (WRC) study developed a new system to treat agri-industrial wastewater using enzymatic processes.

Background

The fruit industry is a multibillion dollar industry that is growing rapidly in all regions of the world. The production of juice consumes a large amount of fresh fruit and water, while generating large quantities of recalcitrant material called pomace.

Up to 25% by wet mass of citrus fruit production is pomace. The industry does not have a particular use for the pomace and, as a result, it is considered waste.

Fruit wastewater has a poor water quality with a chemical oxygen demand (COD) of up to 10 000 mg/L. Fruit wastewater is normally released into the sewerage system, which can result in clogging. If it is introduced directly into rivers it and lead to eutrophication of water bodies.

Objectives of the WRC study

Most fruits wastes are lignocellulosic in nature, which pose specific problems for hydrolysis as an enzymatic treatment, as such substrates are particularly recalcitrant. The proposed process was postulated as a successful and cost-effective solution to the treatment of these wastes and involved the enzymatic treatment of agricultural wastes using suitable combinations of cellulases and oxidases (ligninases) in an effective ratio.

The major aim of the Tunable Immobilised Lignocellulosic Enzyme (TILE) system was to treat agri-industrial wastewater using enzymatic processes in order to generate clean water, and at the same time, produce value-added products from such waste.

The study focused on apple pomace and apple derived wastes from industries in the Western Cape.

Methodology

Commercial enzyme mixtures presented a relatively cheap source of enzymes due to the fact that they are not purified and therefore present a lower processing cost. Activities of the individual mixtures, and mixtures in combination with each other were determined.

Synergy studies assisted in the optimisation of ratios of these enzyme mixtures to achieve optimal hydrolysis. Suitable enzymes and enzyme mixtures were assayed to determine optimal pH and temperature conditions. Stability of enzymes was an important parameter as this would reduce the overall cost of enzymes by allowing enzymes to be reused through several hydrolysis cycles.

Apart from the optimal ratios of enzymes, the optimal enzyme concentrations, substrate concentrations and enzyme/substrate ratios were also determined for optimal hydrolysis. The change in the degree of synergy over time for the selected enzymes was also determined.

This was important to achieve maximum hydrolysis and also for bioreactor design. Bubble batch reactors (1- 2L) and 20 L stirred tank reactor systems were set up in order to monitor the release of the products and to follow the reaction kinetics over time within these bioreactors.

In order to determine the hydrolysis yield of specific sugars, the substrate used in reactions was also analysed to identify the chemical composition, allowing further optimisation

of enzyme mixtures. This was combined with advanced analytical techniques, such as high performance liquid chromatography (HPLC) to determine the specific sugars produced, which also assisted in the optimisation of the enzyme combinations.

Main results

The most appropriate enzymes, based on previous studies related to the composition of the fruit wastes and wastewater, availability and cost, were selected for the desired conversion of the apple pomace substrate. The specific commercial enzyme mixtures Celluclast 1.5 L and Viscozyme were especially efficient, and synergy studies with another commercial enzyme mixture, Biocip Membrane, were performed.

Synergy studies assisted in optimisation of the ratios of the enzyme mixtures to achieve optimal hydrolysis, pH and temperature optimal, as well as temperature stability studies were obtained and important areas of overlapping enzyme activities were also identified.

Immobilisation of Viscozyme L and Celluclast 1.5 L posed a major challenge. A consolidated bioreactor system with both immobilised lignisases and hemi(cellulases) was established. However, the requirements of the glycoside hydrolases versus the laccase enzymes were very different, and these enzymes may have to be utilised in different reactors.

Based on the challenges and costs experienced with using an immobilised system, we changed to using a free enzyme system. In addition, the commercial enzymes showed a very high degree of stability during the hydrolysis reaction, further supporting the choice of using a free enzyme system.

Room temperature could be used for the TILE bioreactor and this assisted in lowering the operational costs. An alternative mixing method was explored for the enzyme bioreactors – mechanical mixing instead of air-sparging. Spiking of the bioreactors with new substrate resulted in a subsequent increase in the amount of sugars released and high substrate loadings resulted in high sugar yields. Viscozyme L and Celluclast 1.5 L were very stable (even after 400 h) and were not limiting to the hydrolysis of apple pomace.

Due to the stability of these enzymes and low formation of inhibitory products like cellobiose, Viscozyme L and Celluclast 1.5 L could be used in a fed-batch bioreactor system without immobilisation. A yield of 35 g/L glucose was possible after 400 h hydrolysis using high substrate loading.

Besides glucose, other products such as galacturonic acid and arabinose were released in high amounts from the hydrolysis of apple pomace. These products can be used for value addition, making the whole TILE system more cost-effective.

Using a one-litre batch reactor, 4.2 g/L glucose and 16.8 g/L reducing sugars were released, which corresponded to a 75% yield. This was an indication that the conditions employed were optimal for sugar release by the enzymes.

The release of reducing sugars and galacturonic acid from apple pomace was faster than that of glucose, suggesting that the pectin and hemicellulose component of the pomace was hydrolysed first.

Using experimental data of varying temperature, initial pH, enzyme concentration, substrate concentration, an artificial neural network (ANN) was successfully constructed to model and predict glucose and reducing sugars release.

Initially it was found that TPlac (in-house produced ***Trametes pubescens*** laccase) could potentially enhance the amount of sugars released from the lignocellulosic substrate with the use of Viscozyme L and Celluclast 1. 5 L, however, the use of laccases was effectively negated by the addition of the third enzyme cocktail.

When using all three enzyme cocktails in an optimise ratio, the addition of laccases did not improve the generation of sugars from the substrate and were subsequently eliminated during the reactor trials.

With regards to bioreactor performance and tenability, enzyme cocktails and conditions efficiently reduced the bulk solids of two industrial apple pomace substrates, industrial peach pomace and industrial pear pomace.

Sugar release was minimal from the AFP-Decanter substrate and the process did not reduce the find solid nature of the substrate. This showed that this process was most efficient for treatment of pomace (after pressing) and not the fine solids generated during classification of fruit juices.

The COD results showed an increase in COD content after treatment, reflecting the release of organics (including sugars) due to the physical breakdown (during stirring) and the enzymatic degradation of the lignocellulosic pomace substrates. In general, treatment of apple (AFP-B and CFP) and pear pomaces resulted in similar reduced sugar (and COD) generation; peach pomace yielded only slightly lower reduced sugar (and COD) generation.

The project showed that apple pomace solids can be efficiently reduced within 24 h in a 20 L stirred tank reactor system using a mix of commercial cellulose cocktails.

Besides the reduction in bulk solids, the treatment generated an effluent rich in sugars that can be directly fermented (i.e. no dilution required) for ethanol production or used as a substrate for the production of commercially and industrially important fungal enzymes (e.g. laccase produced by *T. pubescens*).

Furthermore, the COD results show that the enzymatic treatment was complete within 24 hours and further treatment of the remaining solids in the current system was unnecessary.

Conclusions

The study therefore confirmed that, via optimising enzyme synergy in the bioreactor, the TILE system was able to hydrolyse apple pomase substrate loadings of up to 20%. Overall, this system has been shown to be flexible and robust as it could be used to effectively reduce the bulk solids and release sugars from different pomace substrates, i.e. the TILE system was 'tuneable' to various fruit waste types.

Further reading:
To obtain the report, *A tunable lignocellulosic enzyme system for treatment of industrial wastewaters* (**Report No. 2009/1/15**) 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.